NIXTAMALIZATION OF A SUPPLEMENTARY PORRIDGE FOR CHILDREN: EFFECT ON PHYSICO-CHEMICAL CHARACTERISTICS, NUTRIENT INTAKE, AFLATOXIN CONTENTS AND AFLATOXIN EXPOSURE

BY

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DECLARATION

This dissertation is my original work and, to the best of my knowledge, has not been presented for an award in any other institution

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Table of contents

DECLARATION i

ACKNOWLEDGEMENTS ii

LIST OF FIGURES v

LIST OF TABLES vi

LIST OF ACRONYMS vii

OPERATIONAL DEFINITIONS viii

ABSTRACT ix

CHAPTER ONE: INTRODUCTION 1

1.1 BACKGROUND INFORMATION 1

1.2 PROBLEM STATEMENT 3

1.3 JUSTIFICATION 3

1.4 OBJECTIVES 4

1.4.1 Overall Objective 4

1.4.2 Specific Objectives 4

1.5 HYPOTHESES 5

CHAPTER TWO: LITERATURE REVIEW 6

2.1 PRODUCTION AND CONSUMPTION OF MAIZE AND SOYBEAN IN MALAWI 6

2.2 THE STATE OF MALNUTRITION IN MALAWI 7

2.3 FOODS FOR FEEDING YOUNG CHILDREN IN MALAWI 7

2.4 LIKUNI PHALA PORRIDGE 8

2.5 NATURE AND OCCURRENCE OF AFLATOXINS 9

2.6 THE FATE OF AFLATOXINS DURING PROCESSING OF MAIZE AND THE PREPARATION OF MAIZE FOODS 11

2.7 AFLATOXIN CONTAMINATION OF MAIZE IN MALAWI 12

2.8 TOXICITY OF AFLATOXINS IN HUMANS AND ANIMALS 14

2.9 HEALTH IMPLICATIONS OF AFLATOXIN INGESTION IN CHILDREN 14

2.10 METHODS OF REDUCING AFLATOXIN LEVELS IN MAIZE AND MAIZE FOODS 16

2.10.1 Physical methods 17

2.10.2 Chemical methods 17

2.11 METHODS OF ANALYZING AFLATOXIN IN FOOD 18

2.11.1 Chromatographic methods 19

2.11.2 Spectroscopic methods 21

2.11.3 Immunochemical methods 21

CHAPTER THREE: STUDY DESIGN AND METHODOLOGY 24

3.1 STUDY DESIGN 24

3.2 METHODOLOGY 24

3.2.1 Study Setting 24

3.2.2 Phase 1: Collection and Analysis of *Likuni phala* Samples from Processors..... 26

3.2.3 Phase 2: Cooking of *Likuni phala*..... 27

3.2.4 Analytical Methods..... 28

3.2.5 Contribution to the protein, energy and calcium intake by young children..... 31

3.2.6 Determination of aflatoxin contents and exposure 31

3.2.7 Sensory evaluation..... 32

3.3 DATA ANALYSIS..... 33

CHAPTER FOUR: RESULTS AND DISCUSSION..... 34

4.1 AFLATOXIN CONTENTS OF LIKUNI PHALA FLOUR FROM MANUFACTURERS 34

4.2 NIXTAMALIZATION OF LIKUNI PHALA PORRIDGE AND EFFECT ON AFLATOXIN CONTENTS..... 36

4.3 SENSORY EVALUATION OF NIXTAMALIZED PORRIDGE..... 38

4.4 POTENTIAL EXPOSURE OF CHILDREN TO AFLATOXINS 41

4.5 PROXIMATE COMPOSITION, ENERGY AND CALCIUM..... 44

4.5.1 Moisture Contents..... 44

4.5.2 Crude Protein 45

4.5.3 Crude Fat..... 45

4.5.4 Crude Fibre 46

4.5.5 Total Ash..... 47

4.5.6 Calcium 47

4.5.7 Soluble Carbohydrates 48

4.5.8 Energy contents of the porridge 49

4.6 CONTRIBUTION OF THE PORRIDGE TO CHILDREN NUTRIENT INTAKES OF PROTEIN, ENERGY AND CALCIUM 50

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION 51

5.1 CONCLUSIONS 51

5.2 RECOMMENDATIONS 51

REFERENCES 53

APPENDIX 61

LIST OF FIGURES

Figure 1: Flow chart diagram for production of Likuni phala 9

Figure 2: Chemical structures of aflatoxins 10

Figure 3: Map of the Southern Region of Malawi showing the districts where the sampled factories were located 25

LIST OF TABLES

Table 1: Mean total aflatoxin contents of *Likuni Phala* flour by factory 35

Table 2: Changes in aflatoxin contents during nixtamalization of the porridge..... 37

Table 3: Mean sensory attribute scores for nixtamalized porridge at different lime additions 38

Table 4: Aflatoxin exposure to children by age..... 41

Table 5: Proximate composition, energy and calcium contents of nixtamalized porridge at different lime concentrations 44

Table 6: Nixtamalized porridge contribution to children's RDA of protein, energy and calcium according to age 50

LIST OF ACRONYMS

AOAC: Association of Official Analytical Chemists

HAZ: Height for Age

IYCN: Infant and Young Child Nutrition

MTL: Maximum Tolerable Limit

PEM: Protein Energy Malnutrition

RDA: Recommended Dietary Allowance

USAID: United States Agency for International Development

UV: Ultra-violet

WAZ: Weight for Age

OPERATIONAL DEFINITIONS

Aflatoxin: One of the carcinogenic mycotoxins produced by some fungi under stressful conditions as their secondary metabolites.

Aflatoxin exposure: The amount of aflatoxin ingested expressed per kilogram body weight per day.

Likuni Phala: A maize-soy flour blend rich in energy ideally used for the preparation of supplementary feeding porridge for young children in Malawi.

Nixtamalization: The cooking of a food with alkali.

Provisional Maximum Tolerable Daily Intake: The daily amount of a toxin that can be ingested by humans without causing adverse health effects.

ABSTRACT

Prevalence of Protein Energy Malnutrition (PEM) in under five year old children in Malawi is one of the highest in the world at 30% underweight, 49% stunting and 70% wasting. This prompted the development of a maize-soybean flour blend for porridge preparation called *Likuni Phala* to help alleviate the problem. The product is manufactured by local industries, some of whom distribute for retail to the general public and some distribute to rehabilitation institutions involved in child feeding and rehabilitation.

Maize is a staple food for Malawi but consumption is challenged by the high levels of aflatoxin. Because of this, it is expected that there will be carryover aflatoxin to *Likuni phala* during processing but limited information is available. Nixtamalization or alkali cooking of maize and maize products has been found to effect beneficial properties including reduction of aflatoxin contents.

This study was therefore designed to assess the levels of aflatoxin contamination of *Likuni phala* porridge flour and the efficacy of cooking the porridge with lime (nixtamalization) to reduce the aflatoxin contamination to tolerable levels. Three samples of the product were each taken at two week intervals from three manufacturers in three different districts of the Southern Region of Malawi. Two factories were of the cottage type and one was large. The samples were analysed for total aflatoxin, then cooked into porridges with addition of lime at levels ranging from 0.1% to 0.8% based on dry product. The porridges were analysed for total aflatoxin, proximate composition and subjected to sensory evaluation. Potential exposure to aflatoxin and the dietary contribution to intakes of protein, energy and calcium on young children were also calculated.

Results showed that aflatoxin levels were lower than the tolerance of 10µg/kg in the samples from the two cottage industries where the average was 0.4µg/kg and 0.7µg/kg, but all the three samples from the large factory contained aflatoxin well above the tolerance for total aflatoxin with an average of 20.5µg/kg. On cooking the samples with addition of lime, the porridges were acceptable up to 0.4% lime addition, and the aflatoxin levels reduced to way below the tolerance levels even with the lowest level of lime addition of 0.1%. The potential aflatoxin exposure to children was correspondingly reduced. Results of proximate composition of the porridges cooked with addition of lime showed increases in total ash from 2.07% to 2.45%, calcium from 123mg/100g to 242mg/100g and fibre levels from 3.15% to 5.4% and decreases in crude fat levels from 5.72% to 2.38%. Nixtamalization slightly improved the intake of protein and calcium by the children.

The study concluded that *Likuni phala* generally contains higher levels of aflatoxin than the tolerance limit and these levels are not reduced significantly by cooking using the recommended method. However, cooking with addition of lime up to 0.4% resulted in porridges that were acceptable; with aflatoxin levels well below the tolerable levels and therefore low aflatoxin exposure to young children in addition to improvements in selected nutrients' intake.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND INFORMATION

Malawi is one of the countries in the world with poor health and nutritional indicators among children (Thakwalakwa *et al*., 2009). The country has one of the highest prevalence of Protein Energy Malnutrition (PEM) of under five children in the world; with 30% underweight, 49% stunted and 7% wasted (NSO, 2005). A commercial product to help alleviate the problem was developed as a maize-soy blend flour called *Likuni Phala*. The product is formulated from locally grown maize (*Zea mais*) and soy beans (*Glycine max*) flours in the ratio of 4:1. The product is culturally and organoleptically acceptable by the communities in Malawi (LaGrone *et al.*, 2012) as most of the country's diet is cereal and legume based. *Likuni Phala* is designed to be rich in energy and protein to serve as supplemental food for the children as well as serving in specialized cases as rehabilitation food for PEM cases (LaGrone *et al.*, 2012).

The product is manufactured by two types of factories. The cottage type factories which usually produce on a small scale for sale in their localities and also distribution to rehabilitation institutions and hospitals and the large factories process and package in retail plastic packages for distribution to retail outlets.

Likuni Phala is processed from locally grown maize and soybean. The maize has been reported to be contaminated with aflatoxins. Aflatoxin contamination of maize in Malawi is high and studies have shown that locally stored maize contain aflatoxin levels beyond the acceptable threshold levels (Matumba *et al.*, 2009; Matumba *et al*., 2016) with highest levels of up to 140µg/kg being reported in the Southern Region (Mwalwayo and Thole, 2016). The

contamination is likely to be carried over to the processed and prepared foods and this adversely affects the health of the consumer.

Nixtamalization or alkali cooking has been used in South America since long time ago for preparation of tortillas (Méndez-Albores *et al*., 2004). The method has been found to lower the aflatoxin levels in the final product as consumed and also has the following other nutritional advantages according to Méndez-Albores *et al*., 2004 and Afoakwa *et al.*, 2007:

1. Releases niacin or Vitamin B3 from the bound form niacinogen to make it more available
2. Produces resistant starch good for diabetics
3. Transforms protein functionality to acquire gluten characteristics
4. If lime or calcium hydroxide is used, it enriches the product with calcium, important for growing children

Alkali cooking though not practiced in Malawi, is not a new phenomenon in Africa. It is practised in the Rift Valley region of Kenya by the Kalenjin community where infusions of alkaline plant ashes are used for decortication of maize kernels before cooking (Imungi, 2013). The Kisiis of Western Kenya also use alkaline herbal salts for cooking a maize and beans mixture called *githeri.* The maize kernels cooked this way change colour to light yellow and are said to be much tastier than those cooked with plain water (Imungi, 2013). Many other communities in Kenya also cook maize porridge with soda ash and the porridge cooked this way turns yellow and is also said to be tastier than that cooked without the alkali (Imungi, 2013).

Very little information is available on the aflatoxin contents of processed or prepared foods in Malawi, and no information is documented on the levels of contamination of *Likuni phala*. This study assessed the extent of aflatoxin contamination of *Likuni Phala* and established the extent of reduction of aflatoxin in the porridge when cooked with the addition of lime or calcium hydroxide [$Ca(OH)_2$]. The contribution of the nixtamalized porridge to the intakes of protein, energy and calcium as well as the aflatoxin exposure in young children were also evaluated.

1.2 PROBLEM STATEMENT

Maize is a staple food of Malawi and is the major component of the two constituents of *Likuni Phala*. Aflatoxin contamination of maize is highly prevalent in Malawi with levels as high as 140ppb reported in stored maize. The processing of *Likuni Phala* is unlikely to reduce the aflatoxin levels to the acceptable levels. The levels of carryover aflatoxins in the product have not been well established and neither have the effect of the recommended cooking method of the porridge on residual aflatoxin levels. It is possible therefore that the children are exposed to aflatoxin levels above the tolerance limits, from consumption of the porridge.

The method of cooking with alkali proposed has only been sparingly applied in Malawi for cooking of maize foods, and not with *Likuni phala*. Therefore the effect of the alkali cooking on the physico-chemical characteristics, nutritional quality and sensory characteristics of the porridge are not known.

1.3 JUSTIFICATION

This research was designed to establish the residual aflatoxin levels in the cooked maize-soy blend porridge, *Likuni Phala*. The product was chosen because of its wider use as a

supplementary food to reduce the high prevalence of PEM in Malawi. Establishment of cooking regimen that reduce the aflatoxin contamination to tolerable levels would guarantee the quality, safety and effectiveness of utilization of the product. This will give the Government and stakeholders the confidence to enforce the use of the product in treatment of acute malnutrition and continued use as complementary food.

The effectiveness of the reduction of aflatoxin levels by cooking the porridge with Calcium hydroxide will also guide the Malawi Government in formulating a policy to add the chemical to the product during processing or cooking.

1.4 OBJECTIVES

1.4.1 Overall Objective

The overall objective of the study was to assess the extent of aflatoxin contamination of *Likuni phala* porridge flour and the effect of cooking with alkali on the porridge.

1.4.2 Specific Objectives

1. To determine the levels of aflatoxin contamination of *Likuni phala* with reference to the tolerance limits
2. To determine the change in aflatoxin levels and exposure during cooking of the porridge with addition of lime
3. To determine the acceptance of the porridge cooked with addition of lime
4. To determine the proximate composition of the porridge cooked with lime and the contribution of the porridge to intake of protein, energy and calcium by children

1.5 HYPOTHESES

1. *Likuni phala* does not contain aflatoxin levels above the tolerances
2. Cooking with lime will reduce the levels of aflatoxin to the tolerable levels in the porridge as consumed
3. Cooking the porridge with addition of lime will not change the acceptability of the porridge
4. Cooking with addition of lime does not change the proximate composition and nutrient contents of the porridge.

CHAPTER TWO: LITERATURE REVIEW

2.1 PRODUCTION AND CONSUMPTION OF MAIZE AND SOYBEAN IN MALAWI

Malawi has a sub-tropical climate with 3 seasons: hot wet (December-April), cool dry (May-August) and hot dry (September-November). Production of staple crops like maize and soy is done during the hot wet season and harvesting is done around the months of May (Donald *et al*, 2015).

Maize is by far the most important food staple in Malawi grown country wide (Donald *et al.*, 2015). Production of maize in Malawi is about 2.4 million metric tons a year (Minot, 2010). The actual harvest in a given year depends on the type of weather experienced. Generally, maize prices in Malawi are determined largely by domestic supply and demand (Minot, 2010).

Soybean is also widely produced in Malawi and is one of the most important crops for food and income, animal feed, export earnings and also for the improvement of soil fertility. However, the major production areas are in the central and part of the northern regions of the country which account for 80% of the total soybean production in the country (Nzima & Dzanja, 2015).

Harvesting is the first step in the grain supply chain and is a critical step in deciding the overall crop safety and quality. In Malawi, crop harvesting is generally performed manually using hand cutting tools such as sickles and knives (Kumar & Kalita, 2017). Local storage of grain is done in indigenous storage structures which are made of locally available materials like grass, wood and mud without any scientific design. This kind of storage cannot guarantee to protect crops against pests and contamination, for example with aflatoxins, for a long time (Kumar and Kalita 2017).

In Malawi, food security is mostly perceived in terms of the availability of maize since it is the staple crop. Maize production is reported to make up more than 60% of the total food production in the country (Mazunda & Droppelmann, 2012). As reported by Ecker & Qaim (2011) on the maize consumption in Malawi; by 2010, maize accounted for 45% of the total food quantity, 60% of energy and 45% of protein consumption. The estimated per capita consumption of maize was then reported to be 382 grams which translated to a total national maize consumption of 1.79 million tons (Mazunda & Droppelman, 2012). Maize is usually processed into flour and cooked into porridge and *nsima*; a thick paste made with maize flour and water. This means that diets in Malawi are poorly diversified as only the energy dense foods like maize dominate the diet.

2.2 THE STATE OF MALNUTRITION IN MALAWI

According to the Malawi National Statistical Office. (2005), 30% children in Malawi are underweight, 49% are stunted, 7% are wasted and 63% are reportedly anaemic. Infant mortality in Malawi was reported to be at 42 deaths per 1000 live births (NSO, 2016). Malnutrition, especially inadequate protein and energy intake, has been found to account for half of the child deaths in Malawi (UNICEF, 2007). Over the years, the challenge observed in Malawi has been on dietary diversification due to the dominance of starchy foods and poor consumption of pulses, fruits, fats and non-starchy vegetables (Mazunda & Droppelmann, 2012).

2.3 FOODS FOR FEEDING YOUNG CHILDREN IN MALAWI

In Malawi, most of the complementary foods are made from grains or cereal and prepared in the form of porridge where a legume, milk or milk products, meat, and oil may be added to enrich it (Mazunda & Droppelmann, 2012). According to the Malawi National Statistical Office (NSO)

(2005), 91% of breastfeeding children aged between 6-9 months are fed semi-solid or solid foods. Most children under the age of 5 years old receive foods made from grains, predominantly maize (73%), while 50% are fed fruits and vegetables, 16% are fed foods made from legumes, and 11% receive foods made from roots and tubers. Almost half (48%) of the children are given fruits and vegetables rich in vitamin A, while over 20% are given meat, fish, poultry, or eggs.

2.4 LIKUNI PHALA PORRIDGE

Since malnutrition in Malawi has been of concern for decades, *Likuni Phala* has been promoted by the Government of Malawi as a complimentary food for a long time (Kalimbira *et al.,* 2004). The flour mix was developed in 1966 by Catholic nuns in a village called *Likuni* just outside the capital city, Lilongwe. The flour mix is rich in energy and macronutrients; it provides 396 Kcal of energy, 16g protein and 7.7g fat per 100g (Kalimbira *et al*., 2004). These figures have been found to surpass whole and refined maize flours which are widely consumed in Malawi and also used for preparation of weaning porridges (Ministry of Health, 1992).

When compared with cassava based flour mixes used for complementary feeding, a study by Kalimbira *et al* (2004) found that *Likuni Phala* had the highest calorific value which was attributed to the high energy content of soy beans (407Kcal/100g) as evident to their high fat and protein content. Furthermore, whole maize grain contains high energy (363Kcal/100g) than cassava flour (342Kcal/100g) (Kalimbira *et al.,* 2004). Hence the product is justified as one of the common complementary foods and also it is used to treat acute malnutrition.

The processing method of *Likuni phala* is standardized by all manufacturers and is shown in Figure 1. The final product contains maize and dehulled soybean in the ration of 4:1.

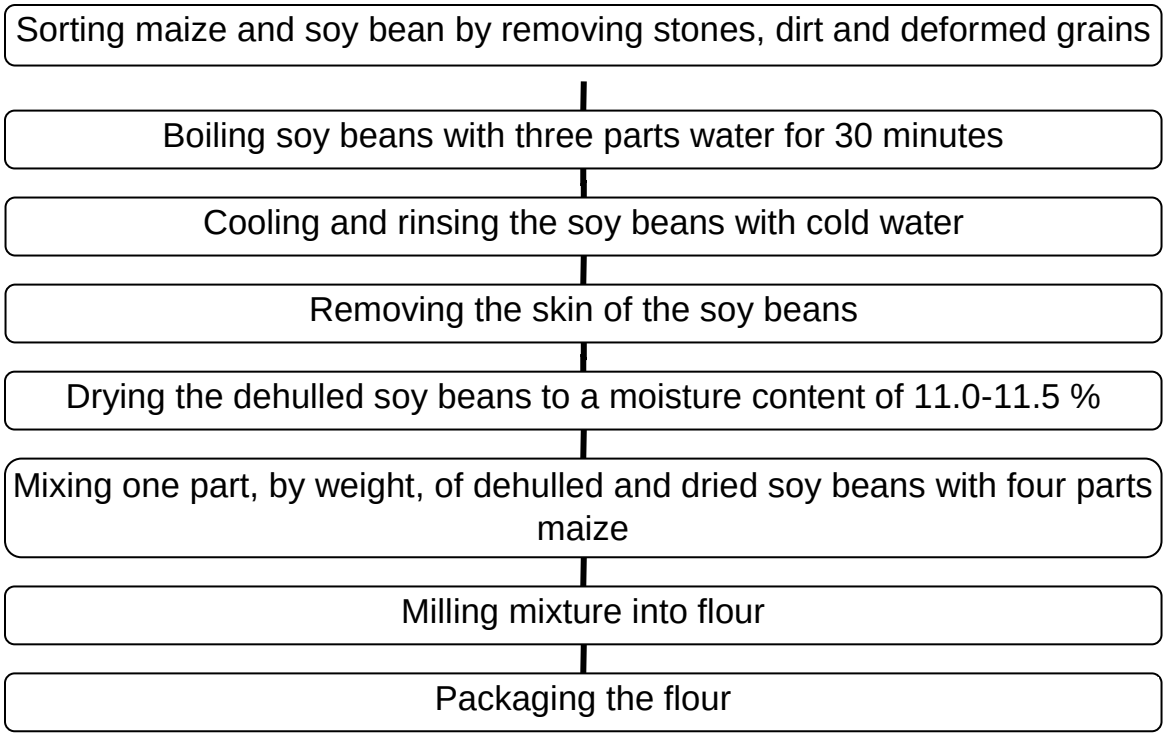

Figure 1: Flow chart diagram for production of Likuni phala

**adopted from Malawi Government's maternal, infant and young child nutrition in Malawi: Community nutrition workers recipe book (2011).*

2.5 NATURE AND OCCURRENCE OF AFLATOXINS

Aflatoxins are secondary metabolites produced by fungi mainly the Aspergillus species like *Aspergillus parasiticus* and *Aspergillus flavus*. These fungi are found in many countries, especially in tropical and subtropical regions, where the temperature and humidity conditions are optimal for the growth of moulds and the production of toxin (Bosco & Mollea, 2012). The contamination of crops with aflatoxin starts in the fields and intensifies in storage if the grains are not properly handled (Matumba *et al*., 2009). Aflatoxins occur in various forms like B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2) (Yao *et al*., 2015); the letters representing them are assigned because of their colour when exposed to ultra violet light. AFB1 has been identified to be the most prevalent and most virulent (Turner *et al*., 2009). It is considered as one of the most

potent naturally occurring carcinogen with a Group 1 human carcinogen designation (Streit *et al*., 2012) targeting the liver and lungs (Turner *et al*., 2009).

Aflatoxin M1 and M2 (AFM1, AFM2) are hydroxylated metabolites of AFB1 and AFB2 respectively (Yao *et al*., 2005). They are also called milk-AFs as they're present in the mammalian milk after the consumption of food and feed contaminated with AFB1 and AFB2 (Bosco & Mollea, 2012). AFM1, is the major metabolite of AFB1 that has been shown to have a significant oral toxicity which is considered to be nearly as potent as AFB1 (Dhanasekaran, 2011).

The chemical structures of the various types of aflatoxins are shown in Figure 2:

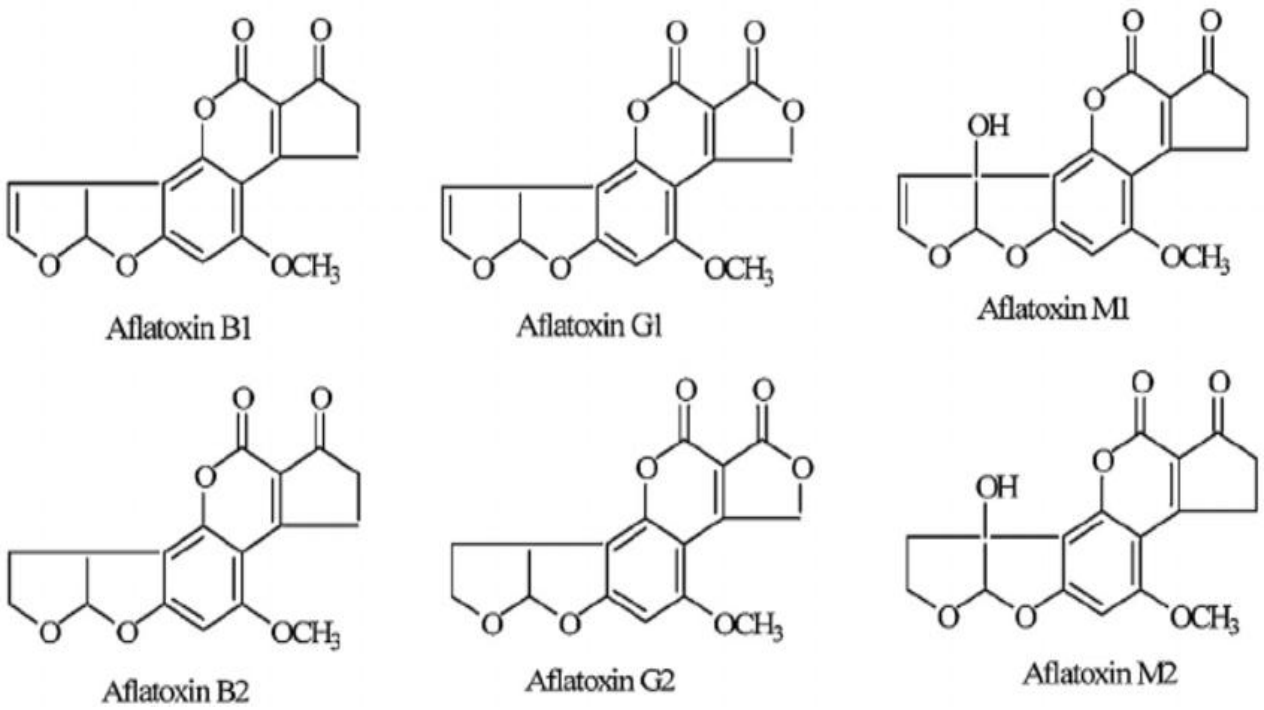

Figure 2: Chemical structures of aflatoxins

2.6 THE FATE OF AFLATOXINS DURING PROCESSING OF MAIZE AND THE PREPARATION OF MAIZE FOODS

Generally, aflatoxins have been reported to be chemically stable at processing temperatures and are not destroyed completely by boiling in water, autoclaving and a variety of food processing procedures (Fandohana *et al*., 2005). However, their stability in food during processing is a factor of many things like the nature of the process, the food matrix, and moisture content of the food, additives and mode and level of contamination (Scott, 2016). All these can affect their decomposition or loss. Generally, aflatoxins are moderately stable during roasting and are carried on to the finished product (Scott, 2016).

As reported by Scott, (2016), physical cleaning methods of maize usually carried out prior to food preparation like dry cleaning, wet cleaning and density separation have been found to be generally ineffective in lowering aflatoxin B1 contents of naturally contaminated maize.

Aflatoxins are stable during normal heating temperatures at home during food preparation. According to Oluwafemi (2004), after subjecting cereals to various processing: heating maize grain for 30 minutes at 100^{o}C resulted in aflatoxin values higher than untreated sample. With an increase in heating time to 120 minutes, there was a reduction in aflatoxin levels from 600µg/kg to 384µg/kg at grain moisture content of 7.6%. With an increase in heating to 250^{o}C for 30 minutes, aflatoxin reduction was reported to be as high as 99.5% however, the grains were charred. According to Betina (1989), aflatoxins have high decomposition temperatures ranging from 237-306^{o}C. This shows that normal cooking like boiling and roasting which still maintains food's edible state cannot be reliable in the reduction of aflatoxin levels.

Some of the methods recommended for mycotoxin reduction in grains pre-cooking include hulling, soaking, sun drying and milling and treatment with ammoniac solutions. A study by Matumba *et al*. (2009) found that, the processes of hulling maize in combination with other methods including milling, soaking and sun drying achieved a combined AFB1 reduction of 88.1%. The most efficient process was soaking, then hulling and lastly drying. The study therefore deduced that this reduction may not necessarily translate to reduced risk due to high consumption of the cereals in Malawi. Effective sorting to remove the grains that visually show the infection also reduces aflatoxin levels through the removal of visibly mouldy grains (Matumba *et al.*, 2009). The tolerance levels for aflatoxin in maize are 5µg/kg for AFB1 and 10µg/kg for total aflatoxins (FAO, 2004).

2.7 AFLATOXIN CONTAMINATION OF MAIZE IN MALAWI

Aflatoxin exposure has been proven to be hazardous to health (Yao *et al*., 2015) and exposure is particularly high for low-income populations in the tropics that consume relatively large quantities of staples such as maize and peanuts (Matumba *et al*., 2009). High aflatoxin exposure causes acute illness and death and chronic exposure has been linked to liver cancer. Liver cancer is estimated to cause as many as 26,000 deaths annually in Sub-Saharan Africa (Unnevehr & Grace, 2013). These problems can be devastating in children especially if they are exposed to the toxin at a very young age.

Studies have shown the associations between exposure to aflatoxins and Reye's syndrome, kwashiorkor, and acute hepatitis. Recent studies have linked aflatoxin exposure to impaired growth in terms of stunting (Egal *et al.*, 2005). The liver dysfunction leads to apparent

kwashiorkor due to inability to digest protein even in cases of sufficient consumption, which is the cause of impaired growth, mostly stunting (Gong *et al*., 2002).

Aflatoxin contamination is both a food safety and economic issue with implications in both crop loss and health-related costs. In addition, climate change and global food security have been seen to have a direct impact on aflatoxin contamination (Brown & Bhatnagar, 2015).

No serious mycotoxicosis outbreak has been reported so far in Malawi, but the favourable tropical climatic conditions, outbreaks of mycotoxicosis in neighbouring countries and knowledge of pre- and postharvest practices strongly suggest that Malawians are consuming mycotoxin-contaminated foods and exposed to chronic toxicity (Mwalwayo & Thole, 2016).

Studies have found that maize in Malawi is contaminated with high levels of aflatoxins above the tolerable limit. A study by Mwalwayo and Thole, (2016) found maize samples from the southern Malawi to have higher levels of aflatoxins and fumonisins as compared to northern and central regions. The maximum detected amount of aflatoxins reported in the study was 140µg/kg. About 20% of the sampled maize exceeded the tolerable maximum limit for aflatoxins in Malawi. Aflatoxins and fumonisins were found to co-occur with contamination levels exceeding 100 µg/kg.

Matumba *et al.* (2009) reported high levels of aflatoxins in central Malawi. AFB1 was detected in 45.3% of the sampled maize which was stored at household level and 12.3% of the maize exceeding the median AFB1 Maximum Tolerable Limit (MTL) of 5µg/kg according to FAO (2004). Highest contamination was AFB1 content of 16.9 µg/kg.

2.8 TOXICITY OF AFLATOXINS IN HUMANS AND ANIMALS

In an animal and human cells study by McKean *et al*. (2006)**,** higher doses of AFB1 (10.0 and 4.64 mg/kg bw) caused acute toxic symptoms immediately post-treatment. Mortality in treated animals occurred within 48 hour post-treatment and within 72 hours 100% mortality (5/5) was observed in animals treated with 10 mg/kg bw AFB1. Mortality reached 100% in animals treated with 4.64 mg/kg bw AFB1 at 96 hour post-treatment. Twenty percent mortality was observed in animals treated with 2.15 mg/kg bw AFB1 during the one-week study period. The Lethal dose (LD50), which is the amount of an ingested substance that kills 50% of a test sample, was determined to be 2.71 mg/kg AFB1 with the 95% confidence limit (CL) from 2.0 to 3.7 mg/kg bw.

Acute aflatoxin exposure in humans has been well documented in countries like Kenya where the first reported aflatoxicosis outbreak occurred in 1981. Multiple aflatoxicosis outbreaks have been documented since 2004, resulting in nearly 500 acute illnesses and 200 deaths (Yard *et al*., 2013). Chronic toxicity due to intake of sub-lethal doses of aflatoxin B1 has carcinogenic properties and is linked to causing liver cancer over time (Owaga *et al*., 2011).

2.9 HEALTH IMPLICATIONS OF AFLATOXIN INGESTION IN CHILDREN

It has been established that in tropical climates and developing countries, people are exposed to aflatoxin contamination as early as in utero and all the way through growth (Gong *et al*., 2002).

A study by Gong *et al*. (2002) on young children from Benin and Togo, observed that children who were still partially breast fed registered lower exposure to aflatoxins and this reflected lower toxin levels in milk compared to weaning and family foods. The study showed that impairment of growth in children happens when they are being introduced to solid foods when there is co-

exposure to aflatoxin and the occurrence of varying infections due to poor hygiene and other pre disposing factors (Gong *et al.*, 2002). In the study, aflatoxin-albumin adducts were detected in 99% of the samples. The concentrations increased with age and it peaked at 3 years old. The mean concentration for children aged 3 years and below was over 2 times higher in fully weaned children than those who were still partially breastfed. An association was therefore found between weaning status and aflatoxin-albumin concentration (Gong *et al*., 2002)

Egal *et al*., (2005) conducted another study in Benin and Togo on the exposure of aflatoxins in young children from maize and ground nuts. The study concluded that maize was the major source of aflatoxin exposure. This can be related to Malawi as maize is also a staple that is widely consumed and parts of Malawi also share the tropical climate as Benin and Togo which are predisposing factors to mycotoxin formation. Groundnut flour is also used in Malawi during the preparation of children's supplementary food like porridge.

The presence of AFB1 in body fluids can also be used to determine extent of exposure to the toxin and therefore its presence in body fluids like urine of children is a potential risk. In children aflatoxin exposure has been shown to lead to stunted growth, delayed development, liver dysfunction, and liver cancer (Owaga *et al*., 2011). Liver dysfunction in children has been associated with kwashiorkor due to the body's inability to metabolise protein (Yard *et al*., 2013).

A study by Tchana *et al*., (2010) on Cameroonian children detected AFB1 in urine of malnourished children and there seemed to be a link between malnutrition and the presence of AFB1 in the urine. The study suggested the possibility of the presence of the toxin being a reflection of food habits of the mothers whose knowledge, attitudes and practices with respect to food may vary considerably. A fumonisins exposure assessment for infants consuming maize-

based foods in Tanzania by Kimanya *et al*., (2009) showed that infants were at a very high risk of up to 24% of going above the Provisional Maximum Tolerable Daily Intake.

Consumption of mycotoxin contaminated food at low doses over time leads to some health concerns for children. Studies have established an association between aflatoxin consumption and stunting (Turner *et al*., 2007). Turner *et al*., (2007) conducted a study to establish the link between in utero aflatoxin exposure to growth status and reported that exposure in humans can also occur through the placenta. The study assessed the effect of in utero aflatoxin exposure on growth in the first year of life where maternal aflatoxin albumin adduct was compared with WAZ and HAZ scores. High maternal aflatoxin albumin adduct was strongly related to lower levels of weight for age in the sample. The study did not have a clear picture of the mechanism of growth faltering but attributed to the possibility of it being a consequence of inhibition of protein synthesis which is caused by the disruption of RNA synthesis attributed to aflatoxin exposure.

2.10 METHODS OF REDUCING AFLATOXIN LEVELS IN MAIZE AND MAIZE FOODS

The control of aflatoxins has been seen to be complex because the moulds responsible for their production are affected by a wide range of factors (Yao *et al.*, 2015). Good management practices in crop production and methods like drying, handling, and storage are recommended for aflatoxin reduction though they are not always sufficient (Unnevehr & Grace, 2013). Several mycotoxin decontamination techniques for grains have been reported and can be categorized into physical and chemical methods.

2.10.1 Physical methods

Physical decontamination remains the preferred way because it does not involve introduction of chemicals that may be of concern to consumers (Matumba *et al.,* 2015). The most common physical decontamination technique in cereals and grains is sorting through the removal of visibly defective grains (Matumba *et al,* 2015). Other methods which can be carried out are floatation and washing of non-floating grains and dehulling (Matumba *et al.,* 2015)

As reported by Matumba *et al*., (2015), although hand sorting seems to be successful in mycotoxin reduction as it is widely recommended for use in export grains, it seems to be underutilized locally. One of the reasons could be that the negative effects of mycotoxins are still not known by most consumers. As noted in many local settings, the grains which are visibly deformed may not be thrown away but may just be made into flour which can be used for porridge to feed the family or otherwise fed to domestic animals. The inadequate sorting would therefore imply that local supply of grains should have high levels of mycotoxins which translate to contaminated cereal based foods.

2.10.2 Chemical methods

Lime treatment of foods has been studied as one of the methods that help in the reduction of aflatoxins in food products. Alkaline cooking of food especially maize originated in Mexico where it is used in the making of a traditional food called tortilla in a process called nixtamalization (Méndez-Albores *et al.,* 2004). In this process, maize is soaked in an alkali solution and it brings about beneficial properties like increased flavour, aroma, and

hemicellulose dissolution in addition to the reduction of mycotoxins (Méndez-Albores *et al*., 2004).

Aflatoxins have been found to be unstable under alkaline conditions (Karlovsky *et al*., 2016). Bases degrade aflatoxins through a reversible reaction where the lactone ring is opened up (Karlovsky *et al*., 2016). The reaction therefore needs to be allowed to proceed up to completion for it to be irreversible (Park *et al.*, 1988). The degradation is achieved by using alkaline solutions like sodium hydroxide ($NaOH_2$) and calcium hydroxide $Ca(OH)_2$ (Karlovsky *et al*., 2016).

Degradation of aflatoxins using ammonia has been studied and has been proved to be effective (Park *et al.* 1988). The reaction yields two major breakdown products (aflatoxin D1 and D2) which lose the lactone ring and maintain the difuran moiety (Park *et al.,* 1988). Park *et al*. (1988), reported that ammoniation can decrease aflatoxin levels in maize by more than 75% and according to Chelkowski *et al.,* (1981) it can completely decompose Ochratoxin A (OTA) in maize, wheat and barley and reduce aflatoxin concentration by more than 99%.

In a study by Méndez-Albores *et al*., (2004) on the reduction of aflatoxins through nixtamalization of corn flour through nixtamalization in Mexico, corn flour was treated with 3% lime (which is a traditional preparation method used in Mexico for making tortillas). It was found that nixtamalization decreased the levels of AFB1 by 94% from 495 to 28.5 mg/kg.

2.11 METHODS OF ANALYZING AFLATOXIN IN FOOD

The detection of aflatoxins begins with an extraction step. Aflatoxins are generally soluble in polar protic solvents including methanol, acetone and chloroform. A number of studies have

singled out methanol as a preferred solvent for use in immunoassay techniques because it has a less negative effect on antibodies (Wacoo *et al.*, 2014).

2.11.1 Chromatographic methods

The analysis is based on the physical interaction between a mobile phase and a stationery phase and the components to be separated are distributed between the two phases (Wacoo *et al.*,, 2014). During the analysis, the sample to be analysed is dissolved in the mobile phase which can be a gas, liquid and sometimes supercritical fluids and applied as a spot on the stationery phase (Wacoo *et al.*, 2014). Common methods in chromatography are Gas chromatography (GC), liquid chromatography (LC), High performance liquid chromatography (HPLC) and Thin-layer chromatography (TLC) and LC and HPLC are the commonly used methods (Espinosa-calderón *et al.*, 2009).

a. Liquid chromatography

The method is mostly combined to fluorescence detection stage (FLD), UV absorption and amperometric detection. LC coupled with fluorescence stage uses the aflatoxins fluorescence properties to identify them (Espinosa-calderón *et al*., 2009). This implies that improvement of sensitivity of this test is based on the improvement of the fluorescence properties. Fluorescence is mostly improved through the use of pre-column derivatization with trifluoretic acid and post-column derivatization with iodine or bromine (Elizalde-González, 1998).

At first, GC was the only method used for separation but its use was limited to a small set of biological molecules (Espinosa-calderón *et al*., 2009). LC was observed to provide good sensitivity, high dynamic range, versatility and soft ionization conditions able to provide access to the molecular mass of intact biological molecules (Espinosa-calderón *et al.*, 2009).

b. High Performance Liquid Chromatography

HPLC is the most popular chromatographic technique and widely used (Wacoo *et al.*, 2014). It is combined with UV absorption, fluorescence, mass spectrometry and amperometric detectors. The technique uses a stationary phase confined to a glass or a plastic tube and a mobile phase consisting of aqueous or organic solvents, which flow through the solid adsorbent (Wacoo *et al.*, 2014).

As reported by Wacoo *et al.* (2014), the natural fluorescence of AFB1 and AFG1 may not be high enough to reach the required detection limit on HPLC and because of this, chemical derivatization using acid or halogens can be used to improve the sensitivity.

The method gives fast and accurate results and a sensitivity of detection of as low as 0.1 ng/Kg has been achieved. As observed by Elizalde-González *et al.* (1998) after analysing aflatoxins B1, B2, G1 and G2 using HPLC and amperometric detection, it was able to detect 5 ng of all the 4 aflatoxin types. HPLC is therefore recommended for detection and quantification of the less toxic aflatoxin B2 which are present in cereals.

The disadvantages of the method is the need of rigorous sample purification using immune-affinity columns and the tedious pre- and post-column derivatization processes which are needed to improve the detection limits of aflatoxins B1 and G1 (Wacoo *et al.*, 2014).

2.11.2 Spectroscopic methods

a. Fluorescence spectrophotometry

This technique uses the absorption in the UV-visible region which is essential for unravelling the molecular structure of compounds (Wacoo *et al.*, 2014). For some molecules, the process of absorption is followed by emission of light of different wavelengths and such molecules are said to be fluorescent which is one of the properties of aflatoxins (Wacoo *et al*., 2014). The method can quantify aflatoxin from 5 to 5000 µg/kg in a small period of less than 5 minutes.

One of the disadvantages of the method is that it also requires derivatization to improve the fluorescence of aflatoxins. The limit of detection with fluorescence is also a bit higher compared to 4 µg/Kg which was set for European standards (Wacoo *et al.*, 2014).

b. Frontier Infrared Spectroscopy

The method relies on the variation in molecular vibrations upon irradiation with infrared radiations and the vibrations by the bonds within the molecule are measured (Wacoo *et al.*, 2014). The various bonds of organic molecules vibrate at different frequencies considering that the atomic size, bond length, and bond strength vary greatly from molecule to molecule which affects the rate at which the molecules absorb infrared radiation (Wacoo *et al.*, 2014)

2.11.3 Immunochemical methods

These methods work on the principle of specificity of binding between antibodies and antigens including receptors and ligands (Sargent & Sadik, 1999). Formation of the complexes between antibody-antigen and receptor-ligand is quantified through following the change in absorbance of photons of light energy spectrophotometrically (Wacoo *et al*., 2014).

a. Radioimmunoassay (RIA)

This was the first immunochemical technique developed and it relies on the principle of competitive binding between a radioactive-labelled antigen and a nonradioactive antigen (Wacoo *et al*., 2014). The radioactive-labelled antigen competes with unlabelled nonradioactive antigen for a fixed number of antibody or antigen binding sites on the same antibody (Berson &Yalow, 1968).

As reported by Langone and van Vunakis (1976) in detection of aflatoxin B1 in peanuts, a detection limit of 1µg/kg was achieved. The major advantage of RIA is the ability to perform multiple analyses simultaneously with high levels of sensitivity and specificity (Wacoo *et al.*, 2014).

However, RIA has disadvantages which limit its use in aflatoxin analysis; it requires an antigen in a pure state, the potential health hazards regarding the use of a radioactive isotope and the problems associated with the storage and disposing of the radioactive waste (Tseng *et al.*, 1989). This led to the development of safer non-radioactive alternatives like ELISA.

b. Enzyme-Linked Immunosorbent Assay (ELISA)

This technique labels the antigens or the antibodies with enzymes and it's the common method of choice for aflatoxin detection of a wide range of agricultural products in research and regulatory bodies for quality assessment and proficiency testing (Wacoo *et al*., 2014).

ELISA technique also relies on the specificity of antibodies for antigens and the sensitivity of the assay is increased by labelling either the antibodies or the antigens with an enzyme that can be easily assayed by use of specific substrates (Babu, 2010).

The major advantage of ELISA kits is that they are cheap and easy to use, do not require extensive sample clean-up, they are not associated with health hazards unlike RIA and their sensitivity and specificity even in the presence of impurities (Wacoo *et al.*, 2014).

Immunochemical methods are widely preferred to chromatographic and spectrophotometric methods.

CHAPTER THREE: STUDY DESIGN AND METHODOLOGY

3.1 STUDY DESIGN

The study adopted a cross-sectional design with analytical component and was carried out in two phases.

Phase 1 – Sampling of *Likuni Phala* from three local processors in the Southern region of Malawi and analysing the samples to determine the levels of aflatoxins.

Phase 2: Simulation of cooking to include: modification of the recommended cooking method by addition of lime with a view to reduce the aflatoxin content to tolerable levels in the porridge.

3.2 METHODOLOGY

3.2.1 Study Setting

The study was conducted in the southern region of Malawi in the districts of Zomba, Blantyre and Thyolo. Samples of *Likuni phala* were collected from three factories, one in each district. The factories in Zomba and Thyolo were of the cottage type and they manufacture the flour for sale only in their locality and supply some to institutions like hospitals, maternal and child health clinics and rehabilitation centres. The factory in Blantyre is large size and processes and packages in retail packs sorely to supply to supermarkets and other retail outlets in the country.

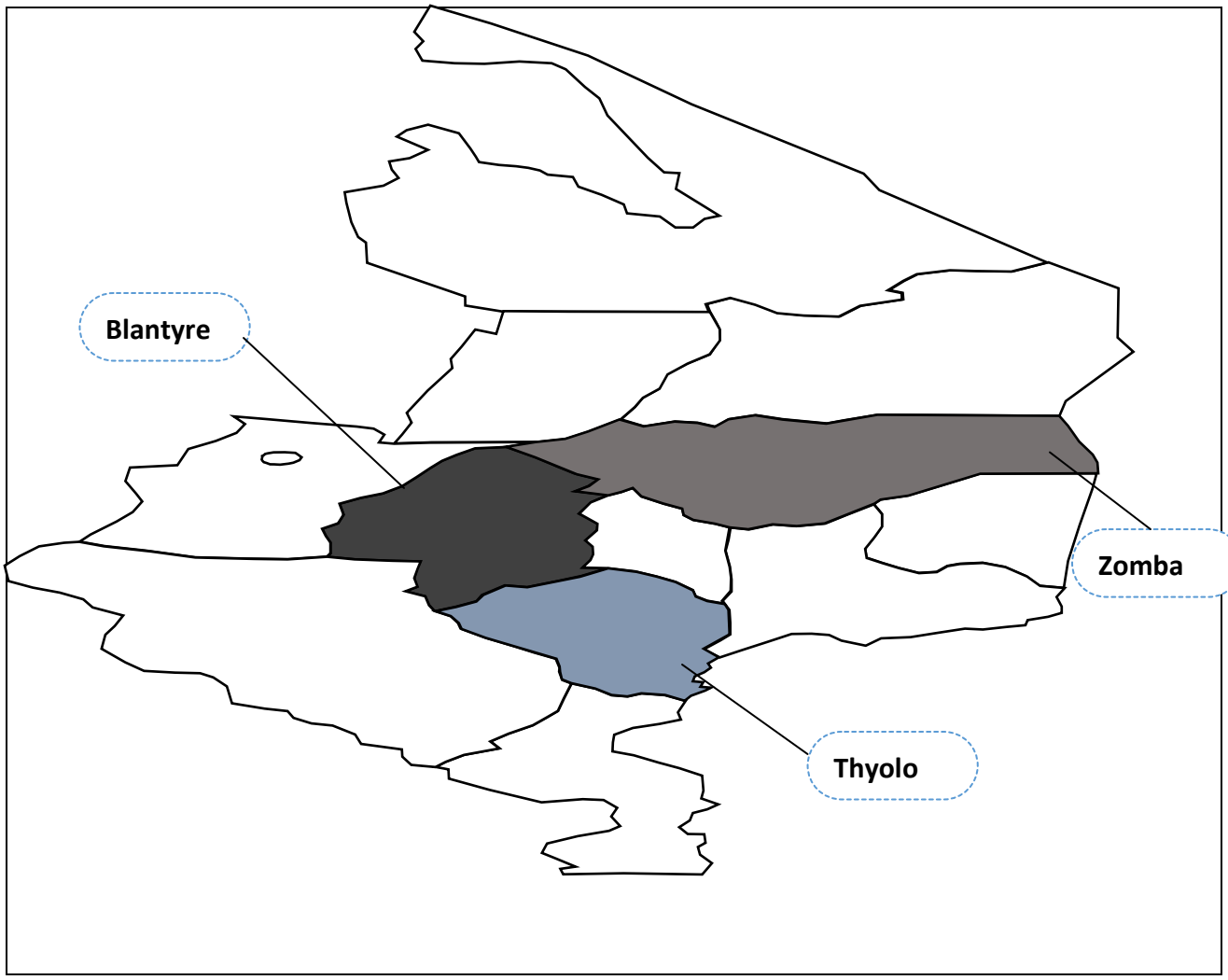

Figure 3: Map of the Southern Region of Malawi showing the districts where the sampled factories were located

The Southern region of Malawi covers an area of 31,753 km² and by 2008, the population was 5,876,784 in its 13 districts (Republic of Malawi and The World Bank, 2006). Livelihood activities are more diverse in the region where non-agricultural livelihood activities are common. Poverty is also high compared to the central and northern regions (Republic of Malawi and The World Bank, 2006). Because agriculture activities in this region are not as extensive as the

central region, much of the population engage in low status, salaried work and small household businesses (Orr & Orr, 2002).

Blantyre city is the centre of finance and commerce in Malawi and the major city in the southern region with an estimated 1,068,681 inhabitants by 2015. Blantyre district covers an area of 2012km^2 (Republic of Malawi and The World Bank, 2006).

Zomba is the fourth largest city in Malawi and the centre for the tobacco and dairy farms of the region. It also produces rice, maize, softwoods and fish from Lake Chirwa. The district covers an area of 2580km^2 with a population of 583,167 by 2009 (Zomba District Assembly, 2009).

Thyolo district is one of the major tea producing districts in Malawi covering an area of 1,715 km² and has a population of 458,976 (Republic of Malawi and The World Bank, 2006).

All districts share the same maize season with the entire country. Staple crops like maize are grown in the hot wet season between the months of December and April (Donald *et al*, 2015).

3.2.2 Phase 1: Collection and Analysis of *Likuni phala* Samples from Processors

Three processors of *Likuni Phala*, one from each sampled districts, were purposively sampled according to whether they were in production and their willingness to participate in the study. Five kilograms of flour were sampled from each processor at a time and placed in Kraft paper bags. The samples were randomly collected from the warehouses using simple random sampling where every fifth (5th) bag of product was identified for sampling and a 1 Kilogram sample taken from each bag until 5 bags were sampled.

Samples were collected at three random times with a two week sampling interval in between collection to have a total of 9 samples for analysis.

Samples were then brought to the laboratory at Chitedze Agricultural Research Station in Lilongwe, Malawi for laboratory analyses.

3.2.3 Phase 2: Cooking of *Likuni phala*

3.2.3.1 Cooking without lime addition

Cooking of the porridge was simulated in the laboratory using the recipe as outlined by the USAID's IYCN project recipe book for Malawian Children (Malawi Government, 2011) as follows:

Four tablespoons of flour and 1½ cups of cold distilled water were mixed in a cooking pan and placed over heat. The mixture was heated to the boil while stirring. A pinch of salt and 2 level teaspoons of sugar were added, mixed well to dissolve and the heat lowered. The porridge was simmered for five minutes, allowed to cool at room temperature to about 40^{o}C and subjected to analysis.

3.2.3.2 Cooking with lime in the management of aflatoxins

Nixtamalization, cooking in alkali using Calcium hydroxide $Ca(OH)_2$, was used in the reduction of aflatoxin during cooking. Samples of *Likuni phala* flour with higher than the threshold levels of aflatoxins of 10µg/kg total aflatoxins were used for these trials. The porridge was cooked with alkali addition at different concentrations of lime [$Ca(OH)_2$] on the basis of flour at 0.1%, 0.2%, 0.4%, 0.6% and 0.8% and cooked according to the recommended recipe already described

(Malawi Government, 2011). The porridges prepared in this manner were then analysed for aflatoxin, proximate composition and subjected to sensory evaluation.

3.2.4 Analytical Methods

Proximate analysis

Likuni phala porridges were subjected to proximate analyses including moisture, crude protein, crude fat, crude fibre, total ash, soluble carbohydrates, calcium and total energy calculations.

3.2.4.1 Determination of moisture

Moisture was determined using the oven drying method (AOAC, 2002). Two grams of sample were weighed accurately, placed in a crucible and dried for five hours at 105ºC in a thermostatically controlled air-oven (Jermaks, TS-8136). The sample was then cooled to room temperature in a desiccator and the loss in weight was calculated as percent moisture content.

3.2.4.2 Determination of crude protein

Crude protein was determined as total nitrogen using Kjeldahl method (AOAC, 2002) where about two grams of sample were accurately weighed and placed in a digestion flask. Kjeldahl catalyst tablet, a glass bead and 30ml concentrated sulphuric acid were added. The digestion tubes were put in a digester and then boiled briskly until the solution was clear.

The digestion mixture was then transferred into a 250ml volumetric flask. The digestion tubes were rinsed with few millimetres of distilled water and the rinse added to the flask. The contents of the volumetric flask were mixed and the volume was made to the 250ml mark.

A 10ml aliquot was then transferred to a distillation tube with 10ml of 40% sodium hydroxide solution. In an Erlenmeyer flask, 10ml of 2% Boric acid and 5 drops of methyl red and

bromocresol green as indicator were added. The sample was then steam distilled for 5 minutes using a distillation unit (Kjeltec System Distilling unit; SN 25809) and the indicator in the Erlenmeyer flask flashed to green. The solution was then titrated until it turned to the original pink or wine red colour using 0.01N Hydrochloric acid. The titre was used to calculate the percentage nitrogen, which was multiplied with an empirical factor of 6.25 to convert to protein content.

3.2.4.3 Determination of crude fat

Crude fat was determined using AOCS Official procedure Am 5-04 (AOCS, 2011) using petroleum ether. About 1.5g of sample was weighed and placed in a pre-weighed filter bag which was heat sealed and closed within 4mm of the top to encapsulate the sample. The sample was then placed in a drying oven at 102±2 ºC for three hours, cooled in a desiccant pouch and weighed. The filter bags with the samples were then put into the bag holder and placed in the extractor. Samples were then dried for 15-30 minutes in the oven at 102±2 ºC, cooled in the Desiccant Pouch and weighed. The weight of the residue was calculated as percent crude fat.

3.2.4.4 Determination of crude fibre

Crude fibre was determined using AOAC procedure (AOAC, 2002) by determining the inorganic residue remaining after digesting with 0.255 N sulphuric acid (H_2SO_4) and 0.313 N sodium hydroxide (NaOH). Compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin. Crude fibre was calculated as the loss in weight of inorganic matter.

3.2.4.5 Determination of total ash

Total ash was determined using the dry ashing method (AOAC, 2002) where about two grams of sample were weighed accurately and placed in pre-dried, cooled and tared crucibles. The samples were then incarnated in a furnace at 550 ºC until the residue became completely grey-white. Furnace temperature was then reduced to 180 ºC and crucibles transferred to a desiccator, cooled and weighed. The ash content was calculated as a percentage of the sample.

3.2.4.6 Determination of calcium

Calcium was determined using atomic absorption spectroscopy (AOAC, 1995). Approximately one gram of dried and ground sample was accurately weighed and ashed into glazed, high-form porcelain crucible for 2 hours in a muffle furnace at 500^{o}C. The ash was allowed to cool and 10 drops of deionised water followed by 3-4 ml of concentrated nitric acid were added. Excess nitric acid was evaporated by placing the sample on a hot plate set at 100 - 120^{o}C. The sample was returned to the furnace and ashed for an additional one hour and after being cooled, the ash was dissolved in 10 ml hydrochloric acid and transferred quantitatively to 50 ml volumetric flask. The releasing agent in form of lanthanum was added to counteract chemical interferences that depress calcium absorbance.

Analysis for calcium was carried out using atomic absorption spectroscopy. The burner height was manually adjusted on the instrument to ensure maximum absorption.

3.2.4.7 Determination of soluble carbohydrates

Soluble carbohydrates were determined as difference by AOAC methods (AOAC, 2002) by subtracting the sum of the moisture, crude fat, crude protein, total ash and crude fibre from 100% to give the soluble carbohydrate as a percent.

3.2.4.8 Calculation of Energy

Energy was calculated as kilocalories (Kcal) using conversion factors according to FAO (2003) as follows:

Total energy in Kcal = (crude protein ×4) + (soluble carbohydrates ×4) + (crude fat ×9)

3.2.5 Contribution to the protein, energy and calcium intake by young children

Two age groups of children (6-9months and 30months) were selected as representative groups during the calculation of the contribution of the porridge to nutrient intakes of the children as percent Recommended Dietary Allowance (RDA) for each nutrient.

The RDAs for various nutrients used were adapted from the Expert Group on Vitamins and Minerals (2003) according to the various age groups as follows:

6-9 months old children: 540mg calcium per day, 108Kcal/kg body weight energy per day; translating to 766Kcal/day for Malawian children whose weight is 7.1kg (Maleta *et al*, 2003) and 2g/kg body weight protein per day; translating to 14.2g/day for Malawian children.

30 months old children: 800mg calcium per day, 1300Kcal energy per day and 23g protein per day.

3.2.6 Determination of aflatoxin contents and exposure

3.2.6.1 Aflatoxin contents

Flour samples collected from processors and nixtamalized porridge cooked at the various lime concentrations were analysed for aflatoxin levels using a VICAM fluorimeter S/N: EX03150 as per manufacturer's manual. The aflatoxin levels were expressed as ppb (µg/kg).

3.2.6.2 Potential aflatoxin exposure assessment

Potential aflatoxin exposure to the children in ng/kg body weight/ day was calculated using the formula by The World Health Organization (WHO, 2008) as follows:

$$\text{Dietary exposure (ng/kg bw/day)} = \frac{\text{Food consumption (kg/day)} \times \text{Aflatoxin concentration (µg/kg)}}{\text{Body weight (kg)}} \times 1000$$

3.2.7 Sensory evaluation

Six porridge samples were prepared with the flour which had above the tolerable levels of total aflatoxins with varying concentrations of lime (0%, 0.1%, 0.2%, 0.4%, 0.6% and 0.8%) added. The porridges were prepared following the standard preparation procedure (Malawi Government, 2011), cooled to about 40^{o}C and subjected to sensory evaluation by 16 randomly selected females who were familiar with the product. The panellists were presented with coded samples and requested to indicate their liking for each product on colour, appearance, odour, taste, mouthfeel and overall acceptance based on the hedonic rating scale as shown:

1=Dislike very much

2=Dislike slightly

3=Dislike moderately

4=Neither like nor dislike

5=Like slightly

6=Like moderately

7=Like very much

3.3 DATA ANALYSIS

Data collected was analysed using GenStat® Discovery 15th Edition software. Descriptive statistics (means and standard deviations) were generated and a 95% confidence interval ($P \leq 0.05$) used.

Data on the aflatoxin levels in the flour blend, sensory evaluation and proximate composition of the porridge were subjected to one-way Analysis of Variance (ANOVA) to test for variability. Variable means for measurements showing significant differences in the ANOVA were compared using the Least Significant Difference (LSD).

Data on aflatoxin levels of *Likuni Phala* porridge nixtamalized with varying lime concentrations were subjected to two-way ANOVA and LSD was used to separate different means.

Means were perceived to be significantly different by LSD if $P < 0.05$.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 AFLATOXIN CONTENTS OF LIKUNI PHALA FLOUR FROM MANUFACTURERS

Results of total aflatoxin contents of samples collected from the three manufacturers are presented in Table 1. The factories were all using the same standard processing procedure and the results are presented based on the products as is. The average moisture contents for all products ranged between 11%-12% even as indicated in the package labels.

The factories in the districts of Zomba (DS) and that in Thyolo (MK) were of cottage type and were processing mainly for supply to specialized institutions like hospitals and rehabilitation centers, while the factory in Blantyre district (RL) was large and was processing for distribution to the market for access by consumers.

Table 1: Mean total aflatoxin contents of *Likuni Phala* flour by factory

Sample	Mean Total aflatoxin (ppb)
DS1	0.0 ± 0.0^{d}
DS2	0.4 ± 0.1^{d}
DS3	0.3 ± 0.4^{d}
Mean for DS	0.4±0.3
RL1	11.9 ± 0.7^{c}
RL2	16.5 ± 0.0^{b}
RL3	33.0 ± 5.0^{a}
Mean for RL	20.5±2.0
MK1	0.0 ± 0.0^{d}
MK2	1.8 ± 0.0^{d}
MK3	0.2 ± 0.1^{d}
Mean for MK	0.7±0.0

*Mean ± SD (n=3). Means along the column with the same superscript are not significantly different at $p<0.05$.
(DS represents samples from Zomba factory, RL represents samples from Blantyre factory and MK represents samples from Thyolo factory)

The aflatoxin contents of all the three samples from the large manufacturer RL were above the tolerable level for total aflatoxin of 10 ppb. The values ranged between 11.9 – 33 ppb, with average of 20.5 ppb. The levels of aflatoxin in samples from the two cottage factories DS and MK on the other hand were all lower than the tolerance levels. The large manufacturer processes for the open market, while the cottage industries mainly process for supply to specific institutions like hospitals and nutritional rehabilitation centres. The two categories of manufacturers indicated that they usually work with the Malawi Bureau of Standards (MBS) for quality control. Furthermore, the three districts in which the factories are lie in the same agro-ecological zone of the country and therefore the maize seasons are the same. The reason for the

differences between the aflatoxin contents of the products from the cottage and the large processor is probably due to the less stringent quality control by the latter than the former manufacturers. The specifications are likely to be better enhanced by the specified institutions than by the general market because the latter have to depend on the national regulatory body.

Other studies which have analysed children's food in various African countries have also found them to be high in aflatoxins and this poses a big risk in the health of the children (South African Medical Research Council, 2001; Egal *et al*., 2005; Kang'ethe *et al*., 2017).

A study by Egal *et al*. (2005) on the dietary exposure to aflatoxin from maize and groundnut in under five year old children from Benin and Togo concluded that maize was an important source of aflatoxin exposure among the children. This was attributed to the fact that maize being a staple, is consumed in larger quantities compared to other crops. Even in mixes with other foods, maize forms the basal and therefore main component. In Malawi, the case is similar where 73% of children are given foods prepared from grains, predominantly maize (NSO, 2005).

4.2 NIXTAMALIZATION OF LIKUNI PHALA PORRIDGE AND EFFECT ON AFLATOXIN CONTENTS

The products from the large manufacturer (RL) which had above the limit total aflatoxin contents were used to carry out nixtamalization (alkali cooking) studies to assess how the cooking affects aflatoxin contents. Table 2 shows the total aflatoxin contents of the porridges cooked with addition of lime at different concentrations and the corresponding percentage decrease in aflatoxins.

Table 2: Changes in aflatoxin contents during nixtamalization of the porridge

% Lime	Total aflatoxin (ppb)		
	RL1	RL2	RL3
0	6.9 ± 0.6^{c}	9.8 ± 0.0^{b}	19.2 ± 5.0^{a}
0.1	4.2 ± 0.1^{defg} (39.1)	5.1 ± 0.3^{de} (48.0)	4.6 ± 0.1^{def} (75.9)
0.2	3.4 ± 0.0^{defgh} (50.7)	$4.0.\pm0.1^{defg}$ (59.2)	6.0 ± 0.1^{d} (68.8)
0.4	1.8 ± 0.1^{gh} (73.9)	3.2 ± 0.1^{egh} (67.3)	1.4 ± 0.3^{h} (92.7)
0.6	2.3 ± 0.1^{fgh} (66.7)	4.2 ± 0.1^{defg} (57.1)	2.9 ± 0.0^{egh} (84.9)
0.8	2.0 ± 0.1^{gh} (71.0)	2.1 ± 0.0^{fgh} (78.6)	2.2 ± 0.1^{fgh} (88.5)

*Mean ±SD (n = 3). Values with same letter superscript along the columns are not significantly different at $p<0.05$,
(Values in parenthesis represent percent reduction in aflatoxin from the initial porridge formulation)

As shown in Table 2, on cooking without lime, the aflatoxin content of one of the samples RL3 was still way above the tolerance levels. The other two samples RL1 and RL2 reduced to slightly below the tolerance levels, probably due to dilution and slight oxidative degradation during cooking in the open pan. On addition of lime, the aflatoxin contents dropped dramatically initially even at the lowest level of 0.1% of the lime addition which resulted in an average of 54.3% reduction, then decreased steadily up to 0.8% lime addition which resulted in an average of 79.4% reduction.

Aflatoxins are reportedly unstable under alkaline conditions (Karlovsky *et al*., 2016) using alkaline solutions like $Ca(OH)_2$. The results obtained are comparable to those of Méndez-Albores *et al*. (2004), who reported a reduction of aflatoxin during nixtamalization of corn flour for tortillas in Mexico. Addition of 3% lime during the cooking resulted in reduction of aflatoxin B1 by 94% from 495ppb to 28.3 ppb. From the results, and these of the present study, it's seen that

the destruction of aflatoxin is not dependent on the initial concentration as long as the added alkali is adequate for the amount of product.

Park *et al*. (1988) studied degradation of aflatoxins using ammonia and it also proved to be effective. The mechanism of degradation of aflatoxin with alkali involve conversion to two major breakdown products aflatoxin D1 and D2 which lose the lactone ring but maintain the difuran moiety but loses the potency as a toxin (Park *et al.,* 1988). It was also reported that ammoniation can decrease aflatoxin levels in maize by more than 75% and according to Chelkowski *et al.* (1981) it can reduce aflatoxin concentration in maize by more than 99%.

4.3 SENSORY EVALUATION OF NIXTAMALIZED PORRIDGE

The mean scores for each attribute of the porridges tested are shown in Table 3.

Table 3: Mean sensory attribute scores for nixtamalized porridge at different lime additions

	Attributes					
% Lime	**Colour**	**Appearance**	**Odour**	**Taste**	**Mouthfeel**	**Overall acceptance**
0	5.44±1.59^{a}	4.75±2.18^{bc}	5.94±0.77^{a}	5.13±1.54^{a}	4.38±1.20^{b}	5.44±1.15^{a}
0.1	5.75±1.07^{a}	6.13±0.96^{a}	5.75±1.18^{a}	4.88±1.63^{a}	5.06±1.44^{ab}	5.06±1.39^{ab}
0.2	5.81±1.28^{a}	5.81±0.83^{ab}	5.94±0.93^{a}	5.31±1.25^{a}	5.63±0.96^{a}	5.5±1.21^{a}
0.4	5.25±1.24^{a}	4.94±1.65^{bc}	4.5±2.033^{b}	5.38±1.63^{a}	5.56±1.44^{a}	5.38±1.36^{a}
0.6	4.00±1.97^{b}	4.31±1.96^{c}	3.88±2.31^{b}	4.25±2.08^{a}	4.38±2.00^{b}	4.00±2.07^{b}
0.8	4.06±2.24^{b}	4.25±2.21^{c}	3.88±2.21^{b}	4.19±2.11^{a}	4.44±1.90^{b}	4.06±2.18^{b}

*Mean ± SD (n = 16) Values with same letter superscripts within the column are not significantly different at $p<0.05$

On appearance, the porridge cooked with 0.1% lime addition was most preferred. Porridges cooked with 0%, 0.1%, 0.2% and 0.4% lime addition were, however, most preferred for their

colour with the porridge cooked with 0.2% lime having the highest score. Porridges cooked with 0%, 0.1% and 0.2% were most preferred for their odour and the porridges cooked with addition of 0.4% lime were sparingly acceptable for the attribute. The porridges cooked with addition of 0.6% and 0.8% scored below 4 in acceptability based on their odour although the scores were not significantly different from that of the 0.4% lime added porridge. Porridges cooked with 0.2% and 0.4% lime addition were most preferred in terms of mouthfeel. Based on taste, there was no significant difference among all lime concentrations. On overall acceptance, porridges cooked with 0%, 0.2% and 0.4% lime addition were most acceptable, although all the porridges were acceptable.

Considering all attributes, porridges cooked with 0.6% and 0.8% lime addition were least preferred by the panellists with values slightly below the "neither like nor dislike" though not significantly different from the porridge cooked with 0.4% lime addition. Porridges cooked with 0.1% and 0.2% lime addition were preferred more on all attributes.

Generally, all the means of the attributes tested of the porridge cooked with addition of alkali from 0.1% - 0.4% were clearly acceptable and not statistically different from that of the control sample. This shows that the porridges could be prepared with addition of lime up to 0.4% without a significant change ($p<0.05$) in acceptability. Although it was evident that cooking the porridges with lime at even the least lime addition of 0.1% reduced aflatoxin levels substantially to below the tolerance, it would be recommended to add lime up to 0.4% to take advantage of the added calcium.

Nixtamalization is known to change the colour and aroma of maize based foods which is perceived as desirable to those who practise it (Imungi, 2013). But as the case in Malawi, it is a new phenomenon and it is not practised.

Steele (2017) reported on the effects of nixtamalization of maize in Malawi where nixtamalized maize flour was used to cook porridge. Consumers preferred the porridge cooked with a mixture of non-nixtamalized and nixtamalized maize flour on a 1:1 ratio than the one cooked with nixtamalized flour alone. This would imply that if up to 50% of the alkali used was applied, the porridge from the nixtamalized maize alone would be acceptable. This showed that with nixtamalization being a new concept in Malawi, consumers are still open to incorporate it in their cooking of maize based foods. This might explain the preference of low percentage of lime added in the cooking of the porridge observed in this study.

With reference to the nixtamalization and sensory evaluation results (in Table 2 and Table 3 respectively), addition of 0.4% yielded maximum benefit. The fall in aflatoxin content of the porridge with addition of 0.4% lime, the highest addition of lime considered acceptable by panellists, ranged from 67% to 93%, with an average of about 78%. The aflatoxin content on the other hand ranged between 1.4 ppb to 3.2 ppb, with average of 2.13 ppb. This average aflatoxin content fell well below the tolerance for aflatoxin B1, the most lethal of the toxins.

However it must be noted that even the addition of 0.1% of lime in cooking of the porridge, the aflatoxin contents were drastically reduced by 76%, from 19.2 ppb to 4.6 ppb in RL3. The level of 0.4% alkali addition, the highest that elicited clear acceptability was selected in order to take nutritional advantage of the extra calcium, a vital nutrient for growing children's bone and teeth health.

4.4 POTENTIAL EXPOSURE OF CHILDREN TO AFLATOXINS

Potential aflatoxin exposure calculations were based on the porridges cooked without lime addition and their respective variants that were cooked with addition of 0.4% lime, the highest level of lime addition acceptable in the sensory evaluation. The calculations were done for two age groups of Malawian children; 6-9 months and 30 months; whose weights and daily porridge consumption rates were known from national figures: Body weight of rural children age 6-9 months, mean of 7.1kg and those at 30 months mean weight of 10.8kg (Maleta, *et al*. 2003) and daily porridge consumption rate of 150g by the former children (Malawi Government, 2011) and 239.5g by the latter children (Mphwanthe *et al*., 2016). The results are shown in Table 4.

Table 4: Aflatoxin exposure to children by age

Age of Child	**Aflatoxin exposure by children's' age and sample (ng/kg bw/day)**			**Mean exposure (ng/kg bw/day)**
	RL1	RL2	RL3	
No added lime				
6-9 months	145.8	207.0	405.6	252.6
30 months	153.3	217.8	426.6	265.9
Lime at 0.4%				
6-9 months	38.0	67.6	29.6	45.1
30 months	40.0	71.1	31.1	47.4

The aflatoxin content of the porridge cooked without lime was 19.1ppb. The exposure to the children of age 6 – 9 months was lowered from this porridge by 82%, while for 30 month old

children the exposure was lowered by the same percentage to the exposure of the porridge cooked with addition of 0.4% lime. Mean exposure to the children from the two porridges was always slightly higher for the children 30 months than for those between 6 – 9 months, probably because of the correspondingly higher consumption of the porridge and not the proportionate increase in body weight.

In a study by Dhanasekaran (2011), on aflatoxins and aflatoxicosis in humans, acute aflatoxicosis was characterized by an average daily intake per adult person of 2-6 mg of aflatoxins. Acute aflatoxicosis causes serious hepatic-related sickness, leading sometimes to death (Owaga *et al*, 2011). This calculated on the basis of a 70kg adult would reduce to exposure of 37,142ng/kg body weight. For the type of children in the study, this would reduce to about 4,245 ng/kg body weight, almost 100 times less than the mean exposure from the porridge cooked with addition of 0.4% lime. At this level of exposure, only chronic toxicity, (due to prolonged exposure at the sub-lethal dose), would be of concern. Chronic toxicity is associated with cancer mainly of the liver (Owaga *et al.*, 2011). Additionally in children, continued exposure to aflatoxin from diets has been reported to cause liver dysfunction, leading to apparent *kwashiorko*r due to inability to digest protein (Tchana *et al*., 2010), and cause impaired growth, mostly stunting (Gong *et al*., 2002, Egal *et al*., 2005). Further studies, maybe involving biomarkers are, however, required to ascertain to what extent at these exposures liver dysfunction can occur.

Kang'ethe *et al*. (2017) studied the aflatoxin exposure of under five year old children in Makueni County of the Eastern Province of Kenya and reported exposure levels as high as 490ng/kg bw/day attributed to the consumption of maize meal alone before cooking. Normally in Kenya,

maize meal is cooked into *ugali* (a stiff paste cooked with boiling water) or porridge and these exposure levels were not calculated based on the aflatoxin contents of the maize meal food as eaten. If this had been done, the exposure levels would probably have been slightly reduced due to the reduction of the aflatoxin levels during open air cooking, as was noted with the cooking of the *Likuni phala* porridge in this study.

The children in the county also elicited higher levels of underweight and stunting compared to those in Nandi County in the North Rift of Kenya which was also studied and showed lower levels of aflatoxin exposure. These results therefore support the possible relationship between aflatoxin exposure and poor growth indicators in children like stunting. Aflatoxin exposure poses a potential risk of growth impairment in children as it inhibits protein synthesis (Tchana *et al*., 2010).

In addition to aflatoxin exposure through contaminated foods, children who are breastfeeding are exposed to even higher levels of aflatoxin through the consumption of AFM1 found in breastmilk of their mothers. Tchana *et al*. (2010) detected AFM1 in milk from breastfeeding mothers in Cameroun and concluded that children are exposed regularly to the aflatoxin as they breastfeed. AFM1 is as potent as AFB1.

A review by Williams *et al*. (2004) reported that there could be a high probability of the top six WHO risk factors which are responsible for 43.6% of the DALYs in countries where short life span is prevalent to be controlled by aflatoxin exposure reduction. Human adults seem to have a better tolerance to aflatoxins than children because in the reported acute poisonings, it is usually children who die (Williams *et al*., 2004). This could be related to the ration of the aflatoxin

ingested with the ration of food and the body weight. In this study, it can therefore be presumed that the aflatoxin exposure in children aged 6-30 months shall lie between the ranges presented.

4.5 PROXIMATE COMPOSITION, ENERGY AND CALCIUM

Table 5 shows the proximate composition of nixtamalized porridge with various percentages of lime. The table also shows the energy and calcium contents of the porridges

Table 5: Proximate composition, energy and calcium contents of nixtamalized porridge at different lime concentrations

% Lime	Moisture (%)	Crude protein (N×6.25) (%)	Crude fat (%)	Crude fibre (%)	Total ash (%)	Calcium (mg/100g)	Soluble carbohy-drates (%)	Energy (Kcal/ 100g)
0	78.32±0.26^{a}	6.88±0.45^{a}	5.72±0.44^{a}	3.15±0.07^{f}	2.07±0.13^{c}	123±17.0^{b}	3.86±0.34^{c}	94.44
0.1	67.83±2.23^{a}	7.04±0.22^{a}	4.62±0.03^{b}	3.31±0.01^{e}	2.12±0.1^{bc}	129±17.0^{b}	15.08±0.65^{a}	130.06
0.2	75.21±4.99^{a}	7.04±0.22^{a}	3.77±0.14^{bc}	4.55±0.04^{d}	2.14±0.01^{bc}	138±14.8^{b}	7.29±1.35^{b}	91.25
0.4	77.25±3.57^{a}	7.19±0.44^{a}	4.3±0.93^{b}	5.2±0.00^{c}	2.31±0.08^{ab}	137±5.7^{b}	3.75±1.27^{d}	82.46
0.6	77.75±1.92^{a}	7.66±0.22^{a}	2.81±0.12^{cd}	5.74±0.06^{a}	2.41±0.06^{a}	147±9.9^{b}	3.63±0.60^{e}	70.45
0.8	78.52±1.78^{a}	7.97±0.23^{a}	2.38±0.04^{d}	5.4±0.00^{b}	2.45±0.08^{a}	242±9.2^{a}	3.28±0.53^{f}	66.42

*mean ±SD (n=2) Values with same letter superscript within the column are not significantly different at $p<0.05$

4.5.1 Moisture Contents

The moisture contents of the porridge ranged from 67.83%-78.52%. The values showed no clear pattern and they were not significantly different from each other.

Lime has however been reported to increase the absorption of water by maize grains as evident in a study by Sefa-Dedah *et al* (2004) where maize grains absorbed more water with increased lime concentration from 0% to 0.5%. Similar results were also reported by Pappa *et al* (2010)

during the nixtamalization of maize for tortilla processing where moisture content slightly increased after cooking maize with 0.4%, 0.8% and 1.2% of lime. The increase could be attributed to the gelatinization of the maize starch during cooking, making hydration easier and faster.

4.5.2 Crude Protein

Crude protein values ranged from 6.88% to 7.97%. Values slightly increased with increased addition of lime but there was no significant difference ($p<0.05$) between them. A number of studies have reported on the effect of alkali cooking on the protein content of various products like millet flour, sorghum flour and maize flour where protein was found to increase significantly after alkali treatment (Wanjekeche, Wakasa, & Mureithi, 2003; Sefa-Dede *et al*., 2004; Gutiérrez-Dorado *et al.*, 2008; Ocheme *et al*, 2010; Boniface & Gladys, 2011). The increase in protein was attributed to a concentration effect (Boniface & Gladys, 2011) where nixtamalization may cause the removal of soluble starch and thereby lead to an increase in the relative percentage of proteins (Owusu-kwarteng & Owusu-kwarteng, 2013). This indicates that alkali cooking of the porridge can help improve the intake of protein in the diets and help in reducing PEM in the children.

4.5.3 Crude Fat

Crude fat values were decreasing with increased concentration of lime and ranged from 5.72% to 2.38%. Even the lowest concentration of lime of 0.1% resulted in a significant decrease in fat content.

The nixtamalization significantly ($p<0.05$) decreased the fat content of the porridge. Similar results were obtained by Ocheme *et al*. (2010) during the nixtamalization of millet flour. The

reduction may be explained by looking at the elevated temperatures and the $Ca2^{+}$ ions which are known to lead to oxidation and the degradation of fat. Charley and Weaver (1998), Owusu-kwarteng & Owusu-kwarteng (2013), Wanjekeche *et al*., (2003) and Ocheme *et al*. (2010) also attributed the decrease to volatilization of fat during cooking. It is also possible that the cooking with alkali saponified some of the fat resulting in products that were fat insoluble and lower extractable lipids with the fat soluble content. Saponification releases the fatty acids from the glycerol which is not fat soluble. The apparent extractable triglycerides therefore reduce.

4.5.4 Crude Fibre

Addition of lime and the subsequent increase in concentration increased the fibre content of the porridge. Values ranged from 3.15% to 5.74%. Porridge cooked with 0.6% lime had the highest significant increase, and each increase in lime resulted in significant increase in each subsequent porridge. As noted by Ocheme *et al*. (2010), this could be attributed to the interaction between $Ca2^{+}$ and OH^{-} ions and the constituents of the flour. This interaction could have led to production of indigestible products, which translated into an increase in the crude fibre content.

Other studies have noted a decrease in fibre after the nixtamalization processes that involved steeping and washing (Owusu-kwarteng & Owusu-kwarteng, 2013). In these cases, the decrease in fibre content was attributed to the removal of the pericarp of the grains during nixtamalization (Bressani *et al*., 1990; Paredes-López & Saharopulos, 2000).

Fibre plays a crucial role in the diet of both children and adults. It has been reported that children who consume higher amounts of fibre also have a high intake of diets that are nutrient dense and they have a high chance of meeting the recommended daily allowances for major nutrients. (Anderson *et al*., 2009).

An increase in fibre is beneficial to children as fibre facilitates the ease of movement of waste in the bowel to prevent constipation, maintenance of normal blood glucose and lipid values and also the reduction of risk to future chronic illnesses like cancer and type 2 diabetes (Ocheme *et al*., 2010; Anderson *et al.*, 2009). In a study conducted by Morais *et al*. (1999) of Brazilian children with chronic constipation; it was shown that their dietary fibre intake was significantly lower compared to those children with normal intestinal habits where their difference in intake was 2.9g/day.

4.5.5 Total Ash

Values were increasing with increase in lime addition. Values ranged from 2.07% to 2.45%. Addition of 0.6% and 0.8% lime resulted in the highest significant increase of total ash content in the porridge. The increase can be explained due to the calcium in the lime when it was added to the porridge.

Similar results were reported by Sefa-dedeh *et al*. (2004) in Ghana during the nixtamalization of maize which was later milled into flour. Most studies have reported increases in ash contents of food products after nixtamalization (FAO, 1992; Sefa-dedeh *et al*., 2004). The increase is attributed to the addition of lime as the calcium in it forms part of total ash contents.

4.5.6 Calcium

Values were increasing with increased concentration of lime. Values ranged from 123mg/100g at 0.1% lime concentration to 242 mg/100g at 0.8% lime concentration. Porridge with 0.8% added lime had the highest significant increase. At the acceptable level of 0.4% lime, the calcium content was 137mg/100g still significantly different from the porridge with 0% lime. Increase in calcium is related to the increase in ash content as calcium is part of the minerals that make up

total ash content. The increase in ash and calcium is in agreement with reports by Ocheme *et al*. (2010) and Pappa *et al*. (2010).

In a study by Boniface and Gladys (2011**)** on the effect of alkali soaking and cooking on the proximate properties of sorghum flour nixtamalized using the traditional method of boiling, steeping, washing, drying and milling into flour, the calcium content was found to be lowered in the flour. The lowering of the calcium in the flour was attributed to the nixtamalization process where the seed coat of the grain is removed. The seed coat is well known to have a high concentration of inorganic material. The decrease was also attributed to the leaching out of the water soluble inorganic elements from the kernels into the soaking and/or cooking water.

In the current study, lime was added directly into the cooking mixture so that the calcium was quantitatively recovered in the porridge therefore increased with the increase in concentration of lime.

The increase in calcium concentration is desirable as children require calcium during growth. An association has been established between consumption of calcium rich diets and reduced instances of diseases like osteoporosis (Muñoz-Chávez *et al*., 1995). Although osteoporosis is known to be caused by a wide range of factors, calcium intake may help in the prevention (Trejo-Gonzalez *et al*., 1982).

4.5.7 Soluble Carbohydrates

Soluble carbohydrates were calculated by difference, the overall observation was that carbohydrate contents reduced with the increase in lime addition. A different pattern was therefore observed for samples with 0.1% and 0.2% lime which was attributed to the low

moisture content of the two samples which affected the final result. Porridge cooked with 0.8% lime recorded the lowest carbohydrate content.

Boniface & Gladys. (2011) also reported a variation in carbohydrate content of sorghum flour nixtamalized with lime. The study also calculated carbohydrates by difference and the variation was attributed to the increases and decreases that take place in the other parameters as a result of the cooking process. In this study, the major source of variation seemed to be in the moisture content of the various treatments.

A study by Bello-Perez *et al*. (2014) on the effect of nixtamalization with calcium carbonate on the indigestible carbohydrate content of corn tortilla also reported a decrease in soluble carbohydrate content after nixtamalization. The decrease was attributed to high temperatures which were thought to effect pirodextrinization of starch and non-starch polysaccharides with increases in indigestible products. Similar results were also reported by Laurentin *et al*. (2003).

Contradicting results were observed by Wanjekeche *et al*. (2003) where magadi soda (sodium carbonate) was used in the alkali treatment of mucuna beans where carbohydrate contents were shown to increase. The study also found that alkali treatment reduced the fibre and ash contents which might have implied that carbohydrates also increased.

4.5.8 Energy contents of the porridge

The energy contents ranged from 66.42Kcal/100g to 130.06Kcal/100g. Generally, energy contents of the porridge reduced after nixtamalization treatment though a different pattern was observed at 0.1% lime addition. This increase was attributed to the decrease in moisture content of the porridge at 0.1% lime addition which affected the soluble carbohydrates contents as observed in similar studies (Boniface & Gladys, 2011; Bello-Perez *et al*., 2014).

4.6 CONTRIBUTION OF THE PORRIDGE TO CHILDREN NUTRIENT INTAKES OF PROTEIN, ENERGY AND CALCIUM

The Recommended Dietary Allowance (RDA) for children 6-9months and 30 months old are 540mg and 800mg per day respectively for calcium, 108Kcal/kg and 1300Kcal per day respectively for energy and 2g/kg body weight and 23g per day respectively for protein (Expert Group on Vitamins and Minerals, 2003).

Assuming that children are given nixtamalized porridge, improvements can be made on the percentage of RDAs met by consuming the porridge compared to non-nixtamalized porridge in terms of calcium and protein intakes as indicated on Table 6. Nixtamalized porridge on the other hand was found to be lower in energy contribution compared to non-nixtamalized porridge.

Table 6: Nixtamalized porridge contribution to children's RDA of protein, energy and calcium according to age

	%protein contribution (RDA)		**%energy contribution (RDA)**		**%calcium contribution (RDA)**	
Age / Lime	6-9Months	30 Months	6-9Months	30Months	6-9Months	30Months
0%	72.7	71.6	18.5	17.4	34.1	36.8
0.1%	74.4	73.3	25.4	24	35.8	38.6
0.2%	74.4	73.3	17.9	16.8	38.3	41.3
0.4%	76	74	16.1	15.2	38.1	41.0

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION

5.1 CONCLUSIONS

The *Likuni phala* flour that is processed especially by the large factory which is sold on the large market has levels of aflatoxin above the tolerance levels of aflatoxin B1 and total aflatoxin. The product from the cottage type factories had levels of aflatoxin slightly below the tolerances for total aflatoxin.

Cooking the porridge with addition of lime reduced the levels of aflatoxin to below the tolerances for both aflatoxin B1 and total aflatoxin. This was the case even with addition of the lowest level of lime. Cooking porridge with lime also reduced the potential exposure of the children to aflatoxin substantially.

Nixtamalized porridge was acceptable to consumers in the attributes tested only up to 0.4% lime addition. Beyond this level of lime, the odour of the porridge was unappealing.

Nixtamalization resulted in changes in the proximate composition of the porridge where crude fat, soluble carbohydrates and energy contents were reduced. On the other hand, crude fibre, total ash and calcium contents increased with the increase in lime concentration in the porridge.

Cooking with lime slightly increased the contribution to the dietary intake of protein and calcium but slightly decreased the contribution to the dietary intake of energy from the porridge by the children.

5.2 RECOMMENDATIONS

Further studies on nixtamalization be carried out with other maize foods like boiled kernels, porridges and *nsima/ugali* (a stiff paste prepared with boiling water and maize flour) to establish

the reduction of aflatoxins in the foods as consumed, with a view to advise the millers and consumers on how much lime to add during milling or preparation.

The Government of Malawi to make it a policy for lime to be added to *Likuni phala* during manufacturing in amounts that will bring the levels of aflatoxin to below the tolerances during preparation of the porridge. The recommended addition will be between 0.1% - 0.4%.

Studies be carried out on other aflatoxin prone products consumed in Malawi with the view to establish the points in post-harvest handling or preparation for consumption where intervention can be instituted to bring down aflatoxin contamination.

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APPENDIX

APPENDIX I: SENSORY EVALUATION QUESTIONNAIRE

Name (Optional): _____

Date: _____

INSTRUCTIONS

i. In this exercise, there are six (6) samples of "*Likuni phala*" porridge.
ii. You will be presented with one (1) sample at a time to evaluate your likeness
iii. With the water provided, rinse your mouth before and after tasting each sample and spit into the empty cups provided.
iv. Do not swallow the sample during tasting, spit out into the cups provided
v. Please evaluate your likeness of the various attributes of the given sample using the following scale:

7. Like very much

6. Like moderately

5. Like slightly

4. Neither like nor dislike

3. Dislike slightly

2. Dislike moderately

1. Dislike very much

vi. Before you begin tasting, evaluate your likeness for colour, appearance and odour (smell) and assign the scores.

Please assign the corresponding number of the degree of likeness against each samples attributes after tasting in the table below:

ATTRIBUTES	SCORES					
	Sample 791	Sample 180	Sample 233	Sample 648	Sample 407	Sample 527
Colour						
Appearance						
Odour (smell)						
Mouthfeel						
Taste						
Overall acceptance						