REDUCTION OF AFLATOXIN CONTAMINATION OF MAIZE MEAL FOODS IN THE NORTH-KIVU PROVINCE OF DEMOCRATIC REPUBLIC OF CONGO BY NIXTAMALIZATION

By

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Reg. No: A56/8927/2017

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SAFETY AND QUALITY OF THE UNIVERSITY OF NAIROBI

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY,

2019

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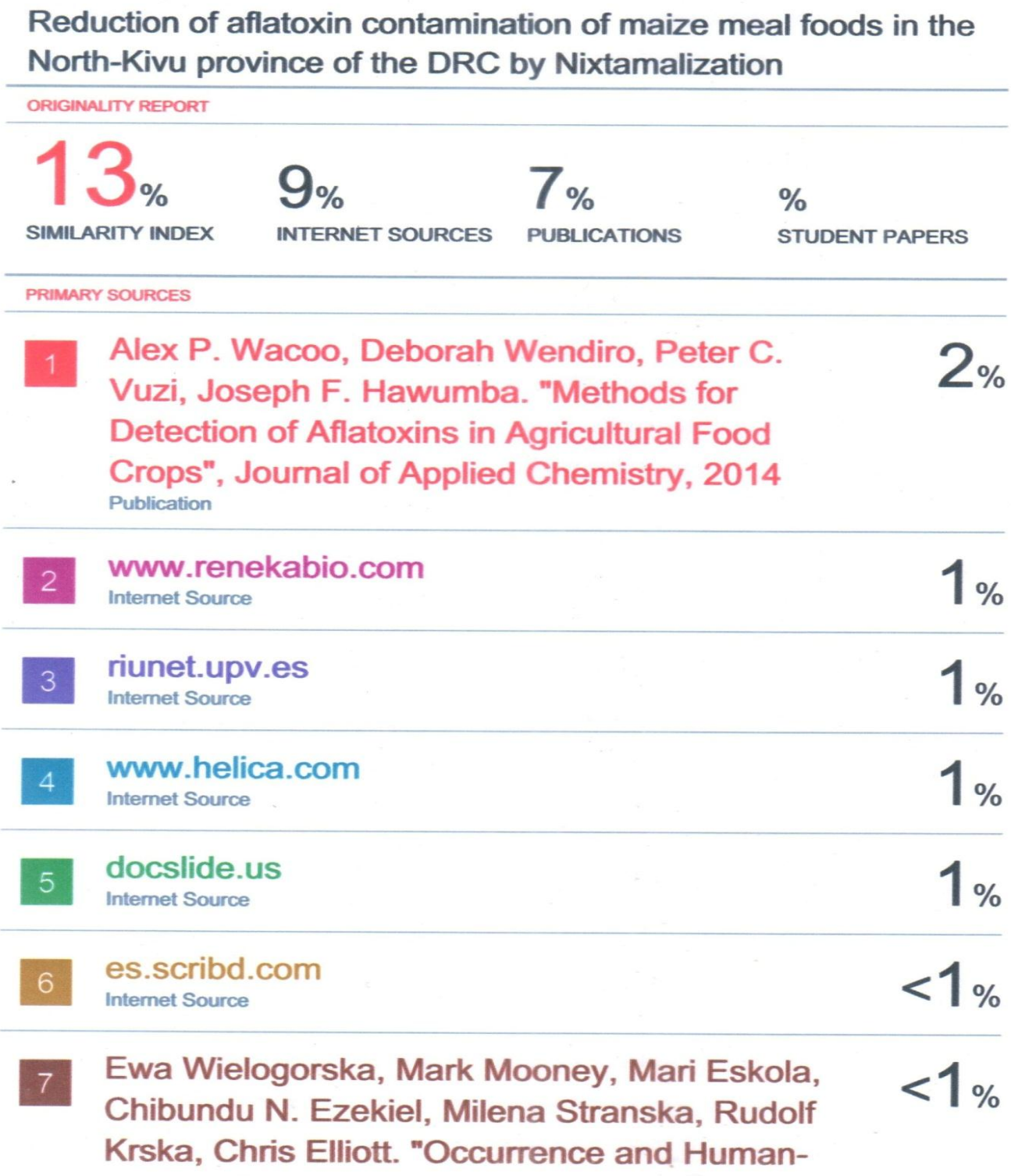

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ACKNOLWEDGEMENTS

Special thanks to my supervisors Prof. Jasper K. Imungi and Dr. Lucy Njue for their support and guidance in writing this dissertation. I thank you for your availability for consultation and for offering constructive guidance.

I would also like to thank my professors from the "Université Catholique du Graben" for their moral support and encouragements.

Special gratitude also goes to my parents for their moral, spiritual and financial supports.

I would also like to thank the lab-technician Peninah Ateku for her support during laboratory work.

Finally, I would like to thank my friends, especially Thomas Roc, Acsa Igizeneza, Mbusa Héritier, Deborah Waluvera, Kavira Vayitsura Alice, Mondo Mubalama and Patrick Bayeni for their motivation and encouragements.

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LIST OF ACRONYMS

DRC**:** Democratic Republic of Congo

$Ca(OH)_2$: Calcium hydroxide

MG: Maize Grain

MM: Maize Meal

M: Market

R: Range

S: Sample

GR: grilled maize meal

NG: Non-grilled maize meal

HPLC: High Performance Liquid Chromatography

LOQ: Limit of Quantification

LOD: Limit of Detection

Af: Aflatoxin

A: Aspergillus

ELISA: Enzyme-Linked Immunosorbent Assay

SSA: Sub-Saharan Africa

RH: Relative Humidity

OPERATIONAL DEFINITIONS

Mycotoxins: toxic secondary metabolites produced by several fungi;

Aflatoxins: secondary metabolites of the fungi Aspergillus flavus and Aspergillus parasiticus;

Nixtamalization: Alkali cooking of especially maize and cereals and their products.

Ugali: a product obtained by mixing maize meal in boiled water in the ratio of 0.5:1 for 7 to 10 minutes.

Acute toxicity: immediate absorption of high doses of toxin

Chronic toxicity: Daily consumption of low doses toxin

Grilled maize: grilled maize kernel at below 100°C for 30 minutes

Lime-cooking: cooking under alkaline condition

Maize meal is a meal ground from dried maize. It is a common staple food, and is ground to fine, medium, and coarse consistencies, but not as fine as maize flour which is very finely ground

ABSTRACT

The Democratic Republic of Congo relies on maize as one of the staples food for human, for animal feed and for industrial uses. However, it has been shown that maize and maize products are good substrates for growth of molds and production of aflatoxin. Levels higher than the tolerance of 10ppb of aflatoxin have been reported in maize consumed in DRC. This study was therefore intended to asses the level of aflatoxin-contamination of maize and maize products consumed in the three main cities (Butembo, Beni and Goma) of the North-Kivu Province of DRC, in which maize is increasingly becoming the main staple food consumed as *ugali.* The study also evaluated the effect of lime-cooking of ugali on its levels of aflatoxin as eaten with the aim to reduce to the tolerance of 10ppb for total aflatoxin. The acceptability of ugali cooked with lime was evaluated using sensory testing procedures. The ugali analyzed consisted of that from the grilled and from the non-grilled maize meal. The study started with a survey of households to establish the type of ugali consumed (from maize meal, cassava flour or mixtures with cassava flour) and the method for preparation of ugali for consumption. For the survey, a sample of 384 households was used. The sample was partitioned between the three Cities proportionate to their populations. The sample size was calculated using Cochran formula.

For aflatoxin assessment, a total of 30 samples of 2kg each of maize kernels were collected, 10 samples from each of the three Cities, in each city, from five (5) principal open air markets. The samples of maize kernels from Butembo consisted of five grilled and five non-grilled. Each of the 2Kg of maize kernels was separately milled in a local mill and then, from each, 1kg of the meal was packaged in Kraft Paper bags. The maize meal samples were then packaged in Kraft paper bags and taken to the Laboratories of the Department of Food Science, Nutrition and Technology, University of Nairobi and stored in a cool dry place, away from direct sunlight to

await further processing and analyses. The ugali cooking simulated the method practiced in households and the same method was applied for both grilled and non-grilled Ugali. The data from the survey was collected using the structured questionnaire. Aflatoxin was analyzed as total aflatoxin using ELISA Kit method according to the manufacturer instructions. Moisture was determined by drying about 5g samples accurately weighed in a porcelain crucible in a thermostatically controlled air oven at 105^{o}C to constant weight according to the AOCC method. Sensory evaluation was done using a seven-point hedonic rating scale (1 = Dislike very much and 7 = Like very much). Data showing the aflatoxin content for maize meal and nixtamalized ugali, moisture content, Calcium content and sensory evaluation were subjected to one way ANOVA using Genstat® Discovery 13thEdition at 95% confidence interval ($P \leq 0.05$). Results from the survey showed that the methods of cooking ugali and the types of ugali consumed in households differ from one city to another.

Results showed that the mean levels of aflatoxin in all the maize meals collected in the three cities are above 10ppb, the tolerance level of aflatoxin in the maize meals for human consumption. The means ranged between 18.34 and 20.98 ppb, the highest mean was for Goma city. The moisture contents of many of the meal samples, and obviously also the maize samples that were milled, were above the optimum for storage except for the grilled samples. That implies that the toxin producing molds would continue to grow and produce the toxin during storage. Cooking of ugali using the conventional method did not reduce the aflatoxin in the food to below the tolerance. However, cooking the ugali with addition of 1%-3% calcium hydroxide (lime) reduced the levels of aflatoxin to below the tolerance of 10ppb for total aflatoxin. This ugali was acceptable to the consumer as indicated by the results of acceptability testing by a

laboratory panel. Cooking with lime also increased the nutritional value of the ugali with respect to calcium.

The study concluded that the maize and maize meals consumed in the three major cities of North Kivu Province are contaminated with aflatoxin at levels way above the 10ppb for total aflatoxin, higher levels occurring in Goma than in the other two Cities. The moisture content of most maize samples that were milled was also higher that the optimum for storage to prevent growth of the toxin producing molds. Cooking of the ugali with addition of 1% calcium hydroxide on the basis of the maize meal reduced the aflatoxin levels of the ugali to below the tolerance, and the ugali still remained acceptable to the consumer.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Globally, cereals take an important place both in human food and in animal feed. Also, cereals may be used as source of industrial energy as they yield by fermentation, biogas or bioethanol (Valentine *et al*., 2012; Rosegrant *et al*., 2001). The most commonly consumed cereals globally include rice, wheat, maize, barley and to a lesser extent sorghum and millet, which constitute the crucial staples food required by billions of people (Sarwar *et al*., 2013). Cereals are the source of metabolic body energy. More than 50% of everyday caloric intake globally is from cereals (Peter *et al*, 2013).

The Sub-Saharan Africa (SSA) relies on maize as the basic food crop used for various foodstuff favorites and socio-economic upbringings. It grows in diverse agro-ecological zones and it is adapted to different farming systems (Olaniyan, 2015). Maize is beneficial for the good health as it provides vitamin B-complex which is beneficial for skin, hair, heart, brain and improves digestion. It was furthermore reported by Shah *et al.* (2016) that the essential fatty acids, especial linoleic acid in maize oil are of great importance for the body as they maintain blood pressure, regulate blood cholesterol and prevent cardiovascular diseases (Kevith, 2004). The importance of maize in SSA is identical to the one of rice or wheat in Asia, where the high consumption rates are in the Eastern and Southern Africa (ESA). It is estimated that about 208 million individuals in SSA use maize as their staple food and as a source of income; the estimated arable land of SSA is 200 million ha and maize occupies 33 million ha, according to FAOSTAT, (2015).

In the Democratic Republic of Congo (DRC), after cassava and banana, cereals take the third position with an overall average annual yield of about 1.55 million tons including 74% maize,

23% rice, 3% millet and 1% wheat. The great maize quantity comes from Kasai and Katanga regions with an estimated production of 70 % (FAOSTAT, (2015).

According to Bunambo (2011), maize gives acceptable grain yields in all the agro-ecological zones, it is well adapted to a broad range of environments and can be cultivated twice per year. At the same time, some varieties of maize are considered drought resistant. These characteristics make maize as a crop for food and nutritional security and cash crop by farmers. In 2000, the estimated maize production was 1,184,000 tones, but in 2014, the production decreased to 1,174,427 tones. This drop was attributed to climate change, insufficiency of inputs and insecurity, as well as diseases and pests such as Maize Lethal Necrosis Disease and caterpillars (FAOSTAT, 2017). However, maize still remains a main staple food for DRC. It is consumed in various forms as green boiled and roasted then as dry as cooked mixture with beans, as dry maize meal cooked to porridge, ugali or prepared into maize beer. The consumption of maize flour is increasing in the major cities of North-Kivu (Goma, Beni, Butembo) because of the expansion of the population, shortest cultural cycle and the variation of diet (Hoof, 2011).

Maize consumption in DR Congo like in many other SSA countries is however, delimited by aflatoxin contamination. This problem is aggravated by the fact that the consumers in the village have very little knowledge on aflatoxin. In DRC, the acceptable levels are 5ppb for AFB1 and 10ppb for total aflatoxins (FAO, 2004). A comparatively study of Kamika, Tekera and Ngboluo (2016) on the occurrence of aflatoxin in maize sampled in DRC along the food supply chain using analytical method gave data in the range of 70.65 and 98.20% with a precision of RSD<15% excepting AFG1 with an RSD of 18.05%. However, the area of interest on aflatoxin in DRC is still empty. Only few researches have been conducted.

Aflatoxin is a mycotoxin that negatively affects food security in Africa mostly in vulnerable groups of people. It is a fungal infection expressed by the production of secondary metabolites in agricultural products due to *Aspergillus flavus, Aspergillus parasiticus* and rarely, *Aspergillus nomius* (IARC, 2002). Aflatoxins are very detrimental to human health because they may cause illness and death, suppress immune systems, and cause liver dysfunction and ultimately cancer. In children, the liver dysfunction compromises digestion of protein and causes retarded growth and development, including retarded mental development (Egal *et al*.., 2005). In Africa, many people suffer from chronic exposure which leads to cancer but acute toxicity which leads to sickness and death has also been stated in some countries (Azziz-Baumgartner *et al*., 2005). Gong et al (2002; 2004) reported that 99 % of the children showed positive reactions for an aflatoxin biomarker in West Africa.

A part from health implications, aflatoxins also has economic implications for example it causes post-harvest loss by causing grain spoilage leading to reduced availability for consumption. Moreover, it lowers growth rate of the animals fed on aflatoxin-contaminated grains, so that the productivity is lowered. Products from African countries are always unable to comply with the stricter regulations of developed countries in which acceptable level of mycotoxins have been lowered, thus the exportation is reduced (Bandyopadhyay, 2016).

Food processing procedures involved in aflatoxin reduction include several methods among which sorting, cleaning, wet and dry milling, grain washing, dehulling, roasting, baking, frying, nixtamalization and extrusion cooking (Fandohan *et al.* 2008). Since long time ago, nixtamalization or cooking in alkali is a method that has been used in South America for tortillas preparation (Méndez-Albores *et al*., 2004). Traditionally, it has as purpose the enhancement of nutritional characteristics of maize such the production of resistant starch good for diabetics,

change in protein functionality to acquire gluten characteristics, intensification calcium in the product if lime or calcium hydroxide is used and availability of niacin. But late on, Méndez-Albores *et al*., (2004) and Afoakwa *et al*., (2007) reported that the nixtamalization process is one of the methods that reduce the aflatoxin-rate in maize and maize foodstuffs.

This cooking destroys the lactone moiety of aflatoxins by hydrolysis. At the high cooking temperatures, (100°C and above), the Alkali solution arouses the breakup of the lactone ring, followed by the carboxylation and then, the reaction may progress further, leading to the loss of methoxy group from aromatic ring (Nkhabu, 2011).

1.2. PROBLEM STATEMENT

Maize is a staple food in DRC and is consumed by both children and adults. The incidence of aflatoxin-contamination in maize is high (70.65 and 98.20%) as reported in the supply chain. With the lack of information and knowledge on aflatoxin in DRC, maize and maize products are still being processed in local mills regardless to the methods that reduce aflatoxin in the products to the acceptable levels. In households, maize and maize products, especially Ugali and porridge are prepared using methods that do not reduce the levels of toxin sufficiently enough in the foods as eaten, meaning that the people are exposed to high sub-lethal doses of the toxin from consumption of maize products.

The effect of the method of nixtamalization during ugali cooking proposed in this study has never been tried in DRC, thus it is still not known.

1.3 JUSTIFICATION

This study was designed to assess the reduction of aflatoxin contamination in maize meal and the possibility of lowering it by bringing it down to below using the process of Nixtamalization at the stage of cooking of *ugali* from maize meal so as to the tolerances in maize and maize products which are 5ppb for AFB1 and 10ppb for total aflatoxins (FAO, 2004). Maize was chosen because it is becoming the major component of ugali eaten in DRC. The establishment of the efficient nixtamalization method for reduction of aflatoxin in Ugali as it is eaten in households will reduce the risk of exposure of consumers to aflatoxin.

The efficiency of the reduction of aflatoxin levels by cooking ugali with Calcium hydroxide will also be a guidance to the DRC government in articulating a policy to add the chemical to the product during processing or cooking.

1.4 OBJECTIVES

1.4.1 Main Objective

The main objective of this study was to assess the levels of aflatoxin in the maize meal consumed in DRC and the effectiveness of lowering the toxin in the ugali by cooking with addition of alkali (nixtamalization).

1.4.2 Specific Objectives

1. To determine the Aflatoxin contents of maize meal from markets of three cities (Beni, Butembo and Goma) of North-Kivu of DR Congo
2. To establish the methods of cooking *ugali* from maize meal at the household level
3. To determine the aflatoxin contents of ugali cooked from the meals with addition of alkali (lime)

4. To determine the calcium contents of ugali cooked from the meals with addition of alkali (lime)
5. To establish the acceptability of ugali cooked with addition of lime by doing sensory evaluation

1.5 HYPOTHESIS

H1. Maize meal from the markets in the study area contain aflatoxin levels above the national tolerance

H2. The methods of cooking of *ugali* from maize meal will be different among households

H3. Cooking of ugali with addition of lime will have a positive effect on the reduction of the aflatoxin levels

H4. The addition of $Ca(OH)_2$ will increase the Ca content of the nixtamalized ugali

H5. Cooking of ugali with addition of lime does not cause significant change in the sensory attributes

CHAPTER TWO: LITERATURE REVIEW

2.1 Maize Production and Utilization in Democratic Republic of Congo

Maize is produced in different agro ecological zones of DRC and it is slowly becoming a basic food especially in the urban areas. The maize is prepared for consumption in various ways. Besides a food crop, maize is also utilized as an industrial crop for various products. Maize and maize products are also used as animal feeds.

2.1.1 Production

In DRC, agriculture is one the most important activities upon which the population relies. Staple crop growing varies according to area, although cassava and maize are major staples cultivated nationwide followed by other food crops, comprising bananas, groundnuts, rice, potatoes and other tubers (especially sweet potatoes and yams), palm, legumes (dry beans, cowpeas, and soybeans), sugarcane, horticultural products, and fruits. Additionally, households may produce cash crops such as coffee, tea, and palm products and in some zones, animal rising is practiced. (SNSA, 2012).

Among rice, millet, sorghum, wheat and maize, cereals cultivated in DRC, maize and millet are of important production. However, maize is highly consumed by the population and it is produced in all the provinces of the country, with high national production of 1,155,720 tons or 63% in Katanga, Bandundu and the 2 Kasai, all together in 2007 (FAO, 2009). The national production of maize as recorded by US Department of Agriculture's Foreign Agricultural Service (USDA FAS) was of 1,1 million tons 2014/ 2015 season and 1,2 thousand tons in the 2015/ 2016 season and it was predicted that the production of 2015/ 2016 season will remain the same in 2017/ 2018 season (USDA FAS, 2016, www.millermagazine.com/english.grain-and-flour-market-in-congo-and-central-africa/, 07 August, 2018). However, the national production is

unable to cover the needs of the population, thus the country depends on national, regional and international imports to meet the domestic requirements. The regional suppliers for imported maize grains and flour are Rwanda, Tanzania, Uganda and South Africa. Also, others important quantities are imported from Italy, United State of America and Brazil (DRC-2017-12-PB-EN-0.pdf, www.fews.net , 07 August, 2018).

2.1.2 Utilization

In DRC, as well as in other parts of America and Africa, maize and maize products have been introduced in the culinary habits of all the population and especially become a raw material for the breweries. In first time, maize is largely used for self-consumption and it is a source of variable cash income depending on the farm. Corn by-products are also an important source of animal feed. Finally, from a food-based crop, maize has progressively become a cash crop with greater interest to technical developments (DGPER, 2010).

As an important source of energy, maize is used in local diets as households substitute away from other starch- based foods. It is consumed in many different forms, fresh boiled maize, fresh roasted maize or prepared in a dough-like consistency known as *fufu* or *ugali* from maize flour. Uji or porridge is also made from maize flour as well as a traditional beer called *mandale* or *mandrakwa.*

Unlike in bordering countries Rwanda, Uganda, Zambia, among others, the processing infrastructure in many DRC's Provinces is not well established basically due to the lack of a consistent/reliable electricity source. Thus, in maize-consuming areas, people prefer imported maize flour over imported maize grain as it is easier to prepare. Imported maize grain is believed to be firmer than local maize grain, making it more challenging and expensive to process (FEWS NET DRC Staple Food Market Fundamentals, 2015, http: // www.fews.net , 8 August, 2018)

2.1.3 Methods of Preparing Maize Foods for Consumption in Democratic Republic of Congo

The main maize foods prepared in DRC include:

1. Ugali is a hard product obtained by mixing maize meal in boiled water in the ratio of 0.5:1 for 7 to 10 minutes. The product is related to be ready by it smell and its thickness. Ugali can be eaten with meat, fish or vegetables;
2. Porridge is a liquid product made from a mixture of a small quantity of maize flour (1/4kg) in hot water (1litter). The product is stirred until it boils and it is removed from the source of energy and then sugar may or not be added in it for taste. Porridge served in a cup and can be taken with bread or alone.

2.2 MYCOTOXINS

Mycotoxins are non- essential compounds produced by several fungi. About 200 types of fungi of the deuteromycetes collection are known to be responsible for the production of about 300 different mycotoxins (Cole RJ and Cox RH, 1981). However, among these them, only twenty are found in food and feed with concentrations that severely compromise both human and animals health. The commonly known mycotoxins of health relevant include fumonisins, ochratoxin A, trichotheceens (e.g. deoxynivalenol, zearaleonone), patulin, cyclopiazonic acid and sterigmatocystin. In this group, aflatoxins are world widely related to be the main threat due to toxicity and occurrence (da Rocha et al; 2014).

2.3. Fungi Producing Mycotoxins

Aspergillus flavus is a broadly spread fungus in the environment and it is often found in several cereals such as peanuts, corn and rice (Saini et al, 2012).

Aspergillus flavus is a morphologically complex species with phenotypes which vary widely. Based on its sclerotia size, the species is divided into two major morphotypes: L strains or Group I with sclerotia >400 mm in diameter and S strains or Group II with sclerotia <400 mm in diameter (Horn, 2005). Both Aspergillus flavus S and L strains are responsible for B1 and B2 aflatoxins-production but strains S can also produce aflatoxins G1 and G2 and they are geographically distributed worldwide (Tran-Dinh et al.; 1999).

Aspergillus flavus produces exclusively AfB1 and AfB2, while Aspergillus parasiticus is able to synthesize all four principal aflatoxins (AfB1, AfB2, AfG1 and AfG2) (Moss, 1989) with AfB1and AfG1 being the major metabolites but this fungus is rarely found in food and feed (AFSSA, 2009).

2.4. Aflatoxins (*Aspergillus flavus toxin)*

Aflatoxins are byproducts of Aspergillus flavus and Aspergillus parasiticus, dominant in tropical and subtropical food crops, primarily corn, peanuts, oilseeds and tree nuts worldwide (Wang *et al*, 2016). Corn and groundnuts are the more vulnerable crops to aflatoxin contamination and constitute the most important causes of aflatoxin exposure in humans with an amount beyond several billion regard to their highly consumption along the world (Strosnider *et al*., 2006). Aflatoxins were revealed in the 1960s during the identification of "Turkey-X-infection" which was the cause of more than 100,000 turkeys in England. Contamination was from the consumption of animals that were feed with the contaminated peanut meal (Bullerman, 1999).

2.4.1 Classification of Aflatoxins

Based on the structure, aflatoxins are classified in four major groups: aflatoxins B1 (Afb1), aflatoxins B2 (Afb2), aflatoxins G1 (Afg1) and aflatoxins G2 (Afg2) (Guo et al, 2009).

Aflatoxin B1 is known to be the highest poisonous form of the aflatoxins and the most effective to cause liver cancer in human (Richard and J. L; 2007).

2.4.2. Chemical structures of some aflatoxins

The chemical structures of some important aflatoxins are shown in Figure 1.

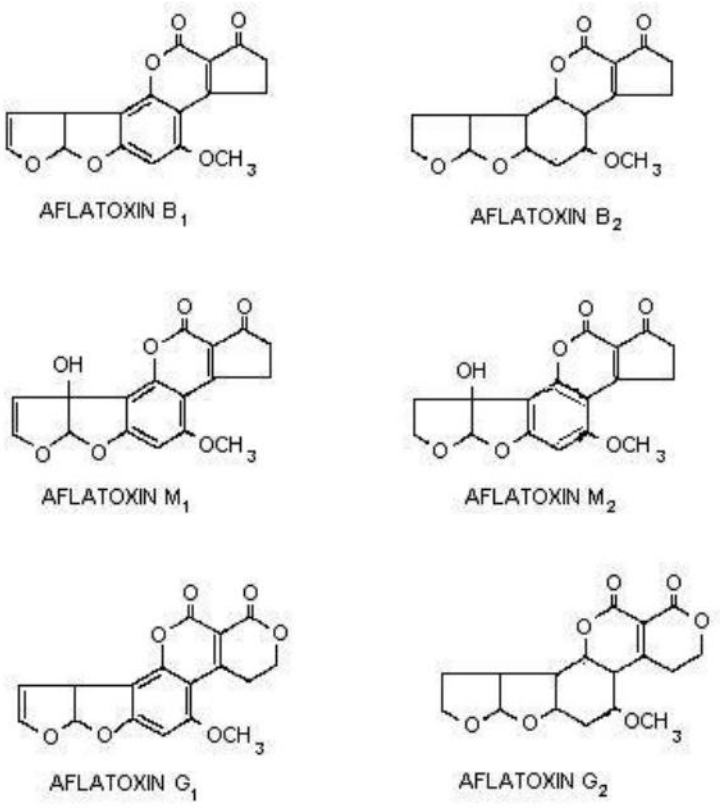

Figure1. Chemical structures of some aflatoxins (Cole and Cox, 1981).

2.4.3 Aflatoxins and Human Health

African countries are on high risk of Aflatoxin contamination where the highest level exposures were observed in West African countries (Gong et al., 2002; Gong et al., 2004).

Aflatoxin poisoning induces health threat to persons or animals known as aflatoxicoses. The exposure is widespread (99%) and children are more susceptible (Hell et al.; 2000). Daily consumption of low doses of aflatoxins in contaminated food may cause chronic aflatoxicosis

with low food consumption, retarded growth, destruction of immune-system and liver cancer (Farombi, 2006; Cardwell and Henry S, 2004) while immediate absorption of high doses of aflatoxins leads to acute aflatoxicosis.

In 2010, Liu and Wu showed that in Africa and Asian countries, the primary liver cancer Hepatocellular carcinoma (HCC) was associated with Afb1with outbreaks of 4.6%–28.2% in the world, mainly in developing countries with outbreaks of chronic hepatitis virus B (HBV) infection.

In Europe, the epidemiological data in 1999 showed that the incorporation of 1 ng of aflatoxins per kg of bodyweight daily over an entire lifetime increases the prevalence of liver cancer of 0.013 cases per year per 100,000 individuals (JECFA, 1999). The dose-response association for aflatoxins has been set for humans but the Daily Tolerable Intake (DTI) has not been set. Thus, the only realistic approach to prevent aflatoxin contamination is to reduce the exposure to the lowest level possible as set by ALARA (As Low As Reasonably Achievable).

2.4.4. Aflatoxin and Economy

Products contaminated with aflatoxin are of low economic importance since they are hazards to both human and livestock. Economic threats include price reduction, market restrictions or obligatory crop demolition impacting the income of farmers and numerous other feed and food industry participants (Isakeit *et al*.; 2011).

In the United States, mycotoxin induces an approximate average of economic loss of one billion dollars for which aflatoxins represents a large proportion (CAST, 2003).

2.4.5. Factors Influencing Aflatoxin Production in Maize and Products

The spread of Aspergillus flavus in nature results from several environmental conditions among which temperature and relative humidity (RH) are the most critical (Giorni et al., 2012). A RH

greater than 85% accelerates A.flavus growth (Al- Shikli et al., 2010).The growth of Aspergillus flavus and the production of aflatoxins may occur during pre-harvest as well as during post-harvest period mostly in the tropical and sub-tropical regions where conditions are generally optimum. Aspergillus flavus grow well at temperature 35 °C and 0.95 aw and aflatoxins are produced at 33 °C and 0.99 aw (Sanchis and Magan, 2004). A. flavus can still grow at farming crops in the pre-harvest period or during storing (Saini and Kaur, 2012).

Stress, especially drought stress is also an important pre-harvest factor for Aspergillus flavus growth while insect activity may also cause production of aflatoxin during storage (Al- Shikli et al., 2010).

2.4.6 Maize and Aflatoxins in Democratic Republic of Congo

The Democratic Republic of Congo population is exposed to high risk of aflatoxicosis since it relies on maize as an important food. In developing countries such as the DRC, there is still a lack of information concerning the natural occurrence of aflatoxin, its exposure to both humans and animals and strategies for controlling the propagation or infection of food crops and their products. Among few studies conducted in the area, a comparatively study of Kamika, Tekera and Ngboluo (2016) on the occurrence of aflatoxin in maize collected in the DRC along the food supply chain using analytical method gave data in the range of 70.65 and 98.20% with a precision of RSD<15% excepting AFG1 with an RSD of 18.05%. The limits of quantification (LOQ) and limits of detection (LOD) fluctuated from 1 to 2 μg/kg and 0.31 to 0.69 μg/kg respectively. Data from pre-harvest period samples also showed that 32% out of a total of 50 samples presented 1.5–51.23 μg/kg for AFB1 and 3.1–103.89 μg/kg for total aflatoxin. They also showed that the rate of aflatoxin increases as the chain of supply progresses. 100% of all samples exceeded the supreme limit of 10 μg/kg for total aflatoxin as established by the WHO at 300

times. Between the storage city and market, the rate of aflatoxin increased extremely at up to 500 times. The significant difference between store and market was less than 0.01.

2.5. METHODS OF REDUCING AFLATOXIN CONTAMINATION IN MAIZE

Numerous technologies have been used to reduce mycotoxin risk in 'high risk' foods, especially maize and groundnut at pre- harvest stage as well as at post-harvest stage (Bankole et al., 2003). Reduction of aflatoxins content is often attained physically (sorting, physical segregation, flotation, etc.), chemically (with calcium hydroxide, ammonia) and microbiologically by including probiotics or lactic acid bacteria into the diet.

Reduction of aflatoxin accumulation in products may be reduced using Good Agricultural Practices and Good Manufacturing Practices during pre and post-harvest periods. Among them, the most important include biological control of Aspegillus ssp, cultural practices, monitoring and crop destruction, grain drying, sorting, storage, however, the total control is not possible (Hell *et al.*, , 2008; Waliyar *et al*., 2015).

Physical methods may help in detoxification of aflatoxin in products by heat, gamma radiation, ultraviolet (UV), or visible light treatments taking in account conditions of each products. AfB1 is resistant to dry heat up to its melting point of 260°C; it is disintegrated at temperature 269°C (Ciegler *et al,* 1983).

Chemical methods have been proved to be more effective for destruction of aflatoxins and include chlorinating agents such as sodium hypochlorite, chlorine dioxide and gaseous chlorine; oxidizing agents such as hydrogen peroxide, ozone and sodium bisulphite and the hydrolytic agents which are acids and alkalis (Stoloff and Trager; 1965).

The alkaline cooking or lime treatment of foods is one of the techniques that have been applied for aflatoxin reduction in food products. It has been applied for longue in Mexico, especially on

maize for the preparation of tortilla in a process known as nixtamalization (Méndez-Albores et al., 2004). In this operation, the corn is soaked in an alkali solution and it improves the quality of the product like increased flavor, aroma, and hemicellulose dissolution in addition to the reduction of mycotoxins (Méndez-Albores et al., 2004).

In alkaline conditions, aflatoxins lose the stability and become unstable (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) and calcium carbonate $Ca(OH)_2$ (Karlovsky et al., 2016).

The use of ammonia has also been tested and its effectiveness has been reported high in aflatoxins degradation (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). In maize, ammoniation can decrease the aflatoxin concentration by more than 75% and according to Chelkowski et al., (1981) it can completely decompose OTA and lower aflatoxin concentration by more than 99%.in maize, wheat and barley.

In a study by Méndez-Albores et al., (2004) the reduction of aflatoxins through nixtamalization of corn flour used for tortilla making in Mexico was studied where 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 content of AFB1 by 94% from 495 to 28.5 mg/kg.

The physical methods of reduction of aflatoxin are shown on Figure 2.

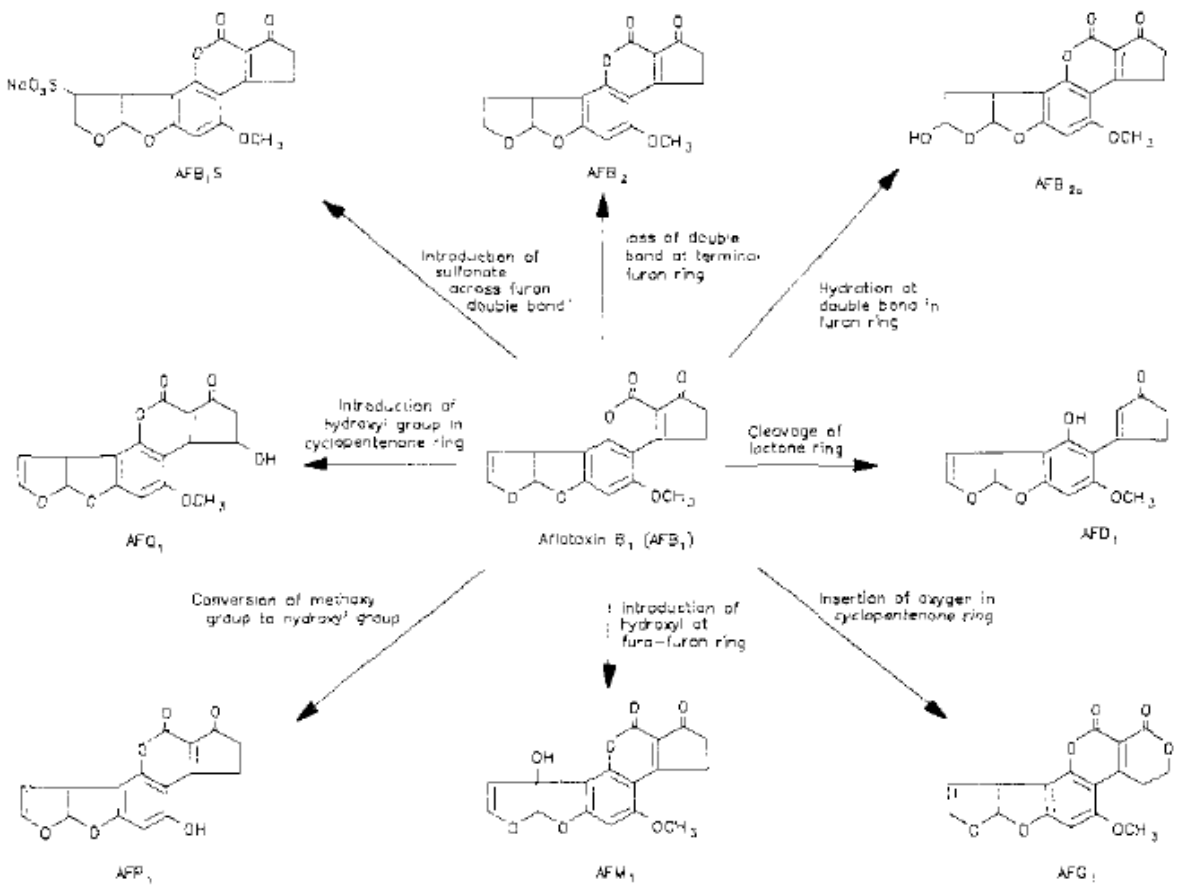

Figure 2: Physical methods of reduction of aflatoxin (Source: Samarejeewa et.al, 1989)

2.6. METHODS OF MEASURING AFLATOXIN.

Numerous aflatoxin detection and quantification methodologies have been established and many of them are based on electrochemical and optical principles such as fluorescence, UV-absorption, spectrometry, chromatography and immunochemical assay tests.

2.6.1 Chromatographic Methods

For this analysis, the mobile and the stationery phases interact physically and then the components to be separated are spread between the two phases (Wacoo et al., 2014). During the analysis, the sample 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).

2.6.1.1 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 utilization of pre-column derivatization with trifluoretic acid and post-column derivatization with iodine or bromine (Elizalde-González, 2011).

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

2.6.1.2 High performance liquid chromatography (HPLC)

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 static phase confined to a plastic or a glass tube and a mobile phase consisting of organic or aqueous solvents, which flow through the solid adsorbent (Wacoo et al., 2014).

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

The methods give 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. (2011) after analyzing 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 suggested 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).

2.6.2 Spectroscopic Methods

2.6.2.1 Fluorescence spectrophotometry

This technique uses the absorption in the Ultra Violet-visible section which is essential for unraveling the molecular structure of compounds (Wacoo et al., 2014). However, after absorption, some molecules emit 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 process can detect the 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 moreover a bit higher compared to 4 µg/Kg which was set for European standards (Wacoo et al., 2014).

2.6.2.2 Frontier infrared spectroscopy

The method is based on the fluctuation in molecular vibrations upon irradiation with infrared radiations and the vibrations by the bonds within the molecule are quantified (Wacoo et al., 2014). The different bonds of organic molecules vibrate at diverse frequencies considering that the atomic size, length and strength bond vary greatly in molecules which affects the capacity of the molecules to absorb infrared radiation (Wacoo et al., 2014)

2.6.3 Immunochemical Methods

These procedures work on the principle of specificity of binding between antibodies and antigens including receptors and ligands (Sargent & Sadik, 1999). The formation of the complexes between antibody-antigen or receptor-ligand is quantified through following the spectrophotometric variation in absorbance of photons of light energy (Wacoo et al., 2014).

2.6.3.1 Radioimmunoassay (RIA)

This was the first immunochemical technique developed and it relies on the competitive binding between a radioactive-labeled antigen and a nonradioactive antigen principle (Wacoo et al., 2014). The radioactive-labeled antigen competes with unlabeled 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 benefit of RIA is the ability to accomplish multiple analyses concurrently with high levels of sensitivity and specificity (Wacoo et al., 2014).

However, RIA has disadvantages which limit its use in aflatoxin analysis; it necessitates a pure antigen, the potential health hazards regarding the use of a radioactive isotope and the problems

linked with the storage and management of the radioactive waste (Tseng et al., 1989). This led to the development of safer non-radioactive alternatives like ELISA.

2.6.3.2 Enzyme-linked immunosorbent assay (ELISA)

This technique labels the antigens or the antibodies with enzymes and it's the common technique 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 method is also based on the specificity of antibodies for antigens. The labeling of either antibodies or antigens with an enzyme that can be identified easily by the use of specific substrates amplifies the sensitivity of the assay (Babu, 2010).

The major advantage of ELISA kit is that they are cheap and easeful to use, and do not require severe sample cleanup, they are not associated with healthiness 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 design was cross sectional with analytical components. Using a structured questionnaire, a sample of households was interviewed on the method of preparation of ugali from the maize meal. The results were used to simulate the cooking in the laboratory. Then samples of market maize meal were analyzed for aflatoxin contents. The maize meal samples were used for preparation of ugali with addition of lime. The ugali was analyzed for aflatoxin contents and sensory acceptability.

3.2 METHODOLOGY

3.2.1 Study Site

The study was carried out in DRC, the North-Kivu in the three major cities Beni, Goma and Butembo where maize is highly consumed.

The North Kivu is the province of the DRC with a high population estimated at 7.5 million and an area of 60 thousand km², while the North Kivu has 124 habitants/km^2. This province is located in the Equatorial in 0°58’ North latitude and 02°03’ South latitude; 27°14’ longitude West and 29°58’ East longitude. The East part is limited by the Republic of Rwanda and Uganda (South-East), The North- west by The Oriental province, the South-West by the Maniema province and the South by the South-Kivu. There are different climates in the North- Kivu with a high correlation between altitudes and temperatures: below 1000 meters: 23°C, at 1500 meters: 19°C and 2000 meters: 15°C.

The North- Kivu has six districts: Beni, Lubero, Rutsuru, Goma, Walikale and Masisi. Goma, Butembo and Beni are the major cities, with a total population of 2,201,304 distributed as

follows: 1 101 306; 744 838 and 355 160 respectively in 2017 (Democratic Republic of the Congo, https:// www.populationdata.net,2017, 16th July 2018). The positioning of North Kivu and the three cities are shown in the map of DRC Congo in Figure 3:

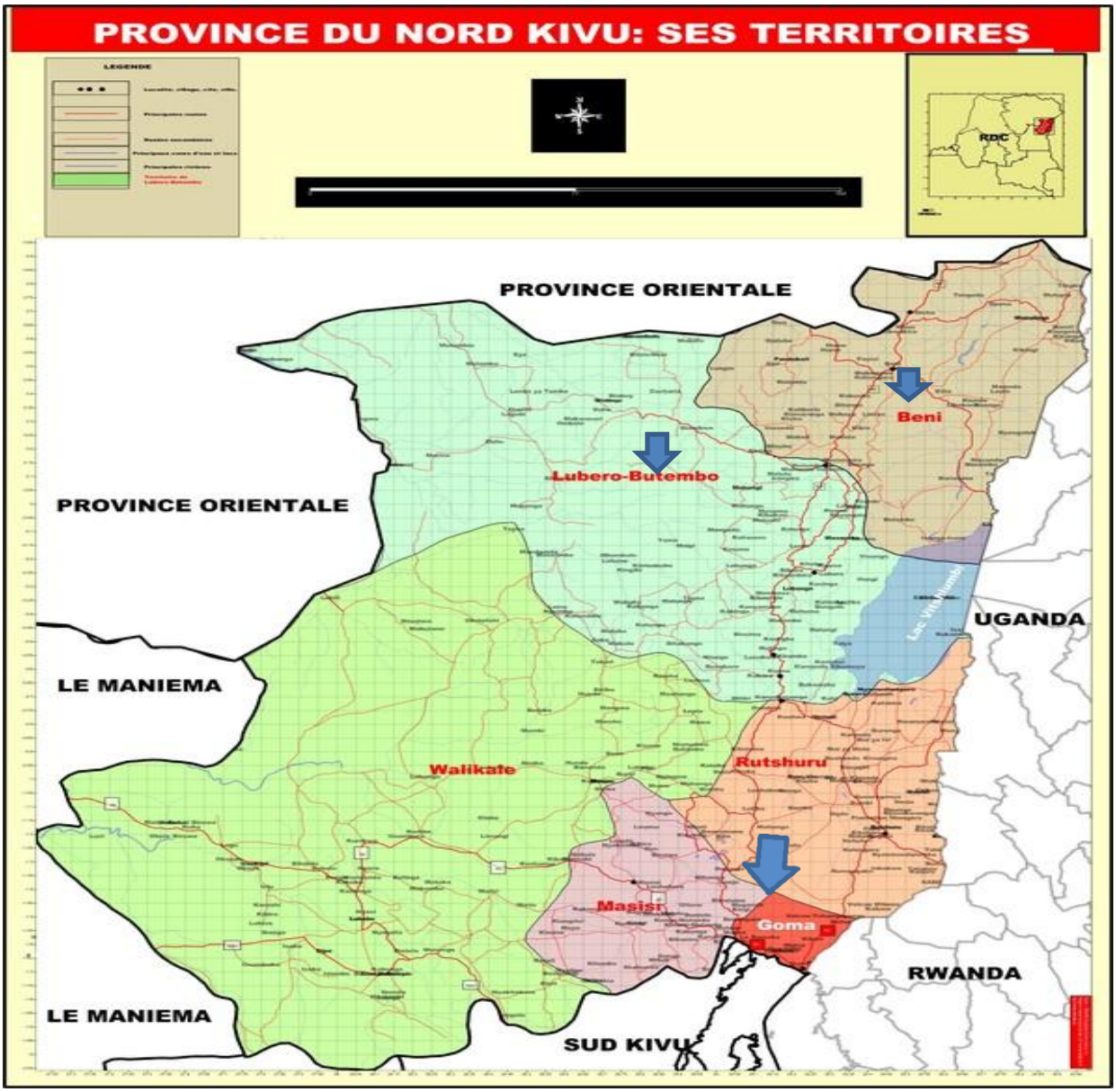

Figure 3. Map of North Kivu Territory/ DRC
Source: DRC maps (10/12/2010)

1. Butembo

Butembo city is in the North-Kivu in the Eastern of DRC, lying the West of the Virunga National park. Butembo has an area of 190.34 km^2 with an estimated size of the population 744 838. This city has 4 municipalities: Bulengera, Kimemi, Mususa and Vulamba.

Butembo city straddles the two territories of Lubero and Beni by the chiefdoms of Baswagha and Bashu.

The 'Rift Valley Occidental' on the MITUMBA Mountains is the close neighbor of Butembo city to the East and is part of the East African Rift. This city does not have the same relief in all its extent since it is built on several hills whose altitude varies in 1600 meters in the valleys and 2000 meters at the highest point located precisely in MATEMBE in Vulamba commune. The average is therefore more or less 1800 meters.

Butembo is characterized by a mild climate of mountains also called altitude climate distinguished by alternating a small dry season from December to February followed by a small rainy season from March to the end of June and this, constantly annual with very little significant disturbance.

The long dry season generally goes almost invariably throughout the years from July to mid-September and the great rainy season runs from late September to early December. The absolute lowest temperature was 13.4°C recorded in February 2015. The maximum absolute temperature was 25.3 ° C recorded in January 2015. The absolute amplitude was 8.4 ° C recorded in August with an average absolute temperature of 19.2 ° C. The average annual maximum temperature is 24.4 ° C. The hottest month of 2015 is February and the month of August is the coldest month. Butembo is located at 0°17’15’’ East, 0°17’40’’ North.

The city of Butembo is a center of commercial and industrial character, but the industrial character is the most dominant. On the ground, the trade is manifested by the presence of the various stores of articles, the shops, galleries and super markets all along the streets, the movement of import and export, the daily attendance of branches (Banks, cooperatives, micro finance...).

As for agriculture, it occupies a large part of the population of Butembo, it is traditional and it is a source of income for a certain category of people who live on the one hand from the production of their field (beans, maize, cassava, potato, sweet potato and all kinds of vegetables, ...) and other products of industry for export (coffee, tea, cinchona, ...).

In general, the inhabitants of the town of Butembo are Bantu of the Nande tribe, also called "Yira". These present themselves both as tribes and as an ethnic group. There are also other nearby tribes but very few. (https://fr.wikipedia.org/wiki/Butembo)

2. Beni

Beni city is in the north eastern of the Democratic Republic of Congo. It lies immediately on the west of the Virunga National Park and the Ruwenzori Mountains, on the shore of the Ituri Forest. Beni city has an area of 7,484Km^2 with an estimated size of the population of 355 160. Beni is subdivided into four municipalities: Beu, Bungulu, Ruwenzori and Muhekera. Beni is 25 km from the city of Oicha (capital of Beni territory). The city of Beni is situated on a plateau of small hills in the South-East and dominated by a plain to the Northeast as well as to the North-West. The geographical coordinates are as follows: between latitude 0 ° 29 '18' 'North and longitude 29 ° 27' 32 " East. Beni is characterized by a tropical climate (Af) with an annual temperature of 23.0°C and average annual rainfalls of 1582mm.

The principal activities include agriculture, livestock, Trade, Industry and crafts. Agriculture focuses on food crops: cassava, maize, rice, beans, soybeans, bananas, peanuts, potatoes, cabbages, etc and on perennial crops: cocoa, coffee, papaya, reforestation, oil palm. Breeding is based on poultry, goats, rabbits, cattle, Ovids...

The trade is based on: General trade (shops and boutiques); the importation of petroleum products (stations, deposits and Gaddafi), agricultural and forestry products, manufactured products, pharmaceutical products; food trade (government procurement); trade in products of services, etc.

Industry: it deals with soap, mineral water, wood, papain, brewery, palm oil, etc.

Crafts: it is based on carpentry, beauty salon, cutting and sewing...

(https://en.wikipedia.org/wiki/Beni, Democratic_Republic_of_the_Congo, 11th May, 2019).

3. Goma

Goma, the capital of North Kivu province in the eastern DRC is situated on the northern coast of Lake Kivu, next to Gisenyi, a Rwandan city . The Lake Kivu and the two cities are in the Albertine Rift, the western branch of the East African Rift system. Goma lies only 13–18 km south of the active Nyiragongo Volcano.

Goma city is located on the south of Equatorial between 141 ° South latitude and 29 ° 14 East longitude. It is bounded on the North by the territory of Nyiragongo, on the South by the province of South Kivu, on the West by the Masisi territory and on the East by the Rwandan Republic. Goma covers an area of 66,824 Km^2 or 11% of North Kivu province and has an estimated population of 1 101 306.

The city, built at the foot of volcanoes Nyiragongo and Karisimbi, is entirely covered with volcanic soils with a slight relief**.** Its altitude varies between 1401m at Lake Kivu and 2000m at

the point of addition with the Bukumu community. The city has only one highest point, Mount Goma.

With a generally temperate climate, softened by the winds blowing from Lake Kivu and volcanoes, the city generally knows two seasons:

- a rainy season from late August to mid-May, interspersed with a short dry season that runs from mid-December to mid-February and;

- a dry season from mid-May to the end of August.

The hydrography of the city of Goma includes only "Lake Kivu, the green lake and the black lake (https://en.climate-data.org/africa/congo-kinshasa/nord-kivu/goma-1074/**,** 11th May, 2019) Goma is subdivided into two municipalities, these into neighborhoods and these into avenues and cells. These municipalities are:

- The municipality of Goma covers an area of 33,372 square kilometers and has seven districts: Mikeno, Mapendo, Volcano, Katindo, Keshero, Himbi and Lac-Vert.

- The municipality of Karisimbi which covers an area of 33,452 Km^2 and includes the neighborhoods: Kahembe, Murara, Bujovu, Majengo, North Mabanga, South Mabanga, Kasika, Katoyi, Ndosho, Mugunga and Virunga.

Although located in the city, the city of Goma still includes neighborhoods being subdivided (Katoyi, Ndosho, Keshero) and who still lead a rural life (based on agriculture), others not yet divided and not densely populated (Green Lake, Ndosho, Mugunga) and other non-farmed but densely occupied, (Mabanga North and South, Mapendo, Mikeno, Kahembe).

As for the population of the city of Goma, it is diversified because it comes from different areas with different objectives. The various assets-geographical, commercial (trade), transport-predisposed the city to both internal and external migrations which explains the continuous

increase of the population (http://kikachris.over-blog.com/article-33121311.html, 11th May, 2019)

3.2.2 Sample Size Calculation for Type of Ugali and Cooking Methods Survey

The sample size was determined using Cochran's formula (1977) and the sample was proportionately allocated to the three cities on the basis of their population size.

N=$\frac{z^2 * pq}{d^2}$, where

n: sample size;

z: 1.96 at 95% CI;

p: proportion of the population with the desired characteristic (aflatoxin exposure);

q: p-1 and

d: acceptable degree of accuracy at 5% (0.05) level

n=$\frac{(1.96)^2 * 0.5 * 0.5}{0.05^2}$

n= 384

This sample was divided among the Cities proportionate to their populations as follows:

Goma: $\frac{1,101,306}{2,201,304} x384 = 192$

Butembo: $\frac{744,838}{2,201,304} x384 = 130$

Beni: $\frac{355,160}{2,201,304} x384 = 62$

3.2.3 Sampling Procedure

The schematic diagram representing the sampling procedure is shown in Figure 4

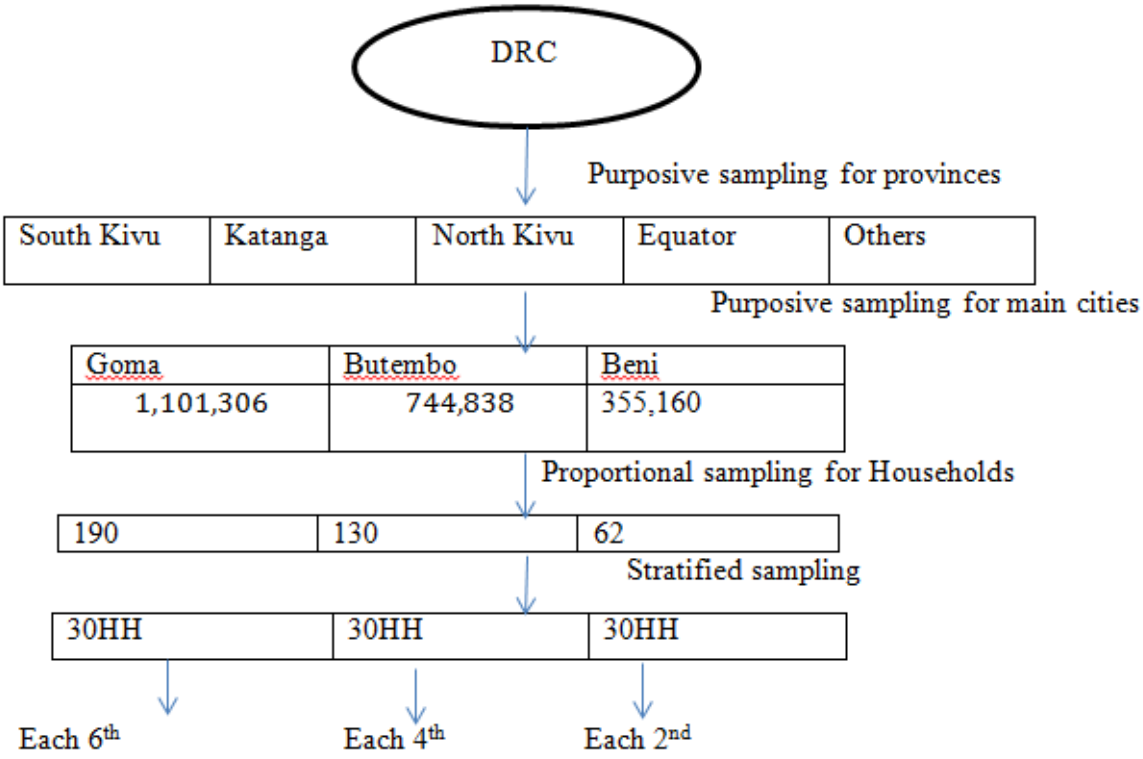

Figure 4: Sampling procedure

The North Kivu Province was purposively sampled from DRC because none study on aflatoxin in maize from this region has never been conducted and the three main Cities of the province (Goma, Beni, Butembo) were also purposively sampled because of the increment of the consumption of maize meal due to the expansion of the population, shortest cultural cycle and the variation of diet (Eco-Congo, 2011).

The sample of 384 households was proportionately distributed to the three cities according to their populations.

The sample of households in each City was used for the survey on the type of ugali and a sub-sample of 30 households from each was taken by stratified sampling and used for survey on method of ugali preparation.

3.2.4 Data Collection on Type of Ugali Consumed and the Method of Cooking

The data was collected using the structured questionnaire shown in the Appendix 1 as follows:

Type of ugali to determine whether it was maize or cassava only or maize-cassava ugali and the average proportions of maize meal and cassava flour in the latter ugali.

Method of ugali preparation to determine the ratio of flour/meal to water and the procedure of ugali preparation including time.

3.2.5 Collection of Maize Samples for Milling to Meals for Analysis

A total of 30 samples were collected from the three cities as shown in figure 5

MG: Maize Grain

MM: Maize Meal

M: Market

R: Range

S: Sample

V: Vendors

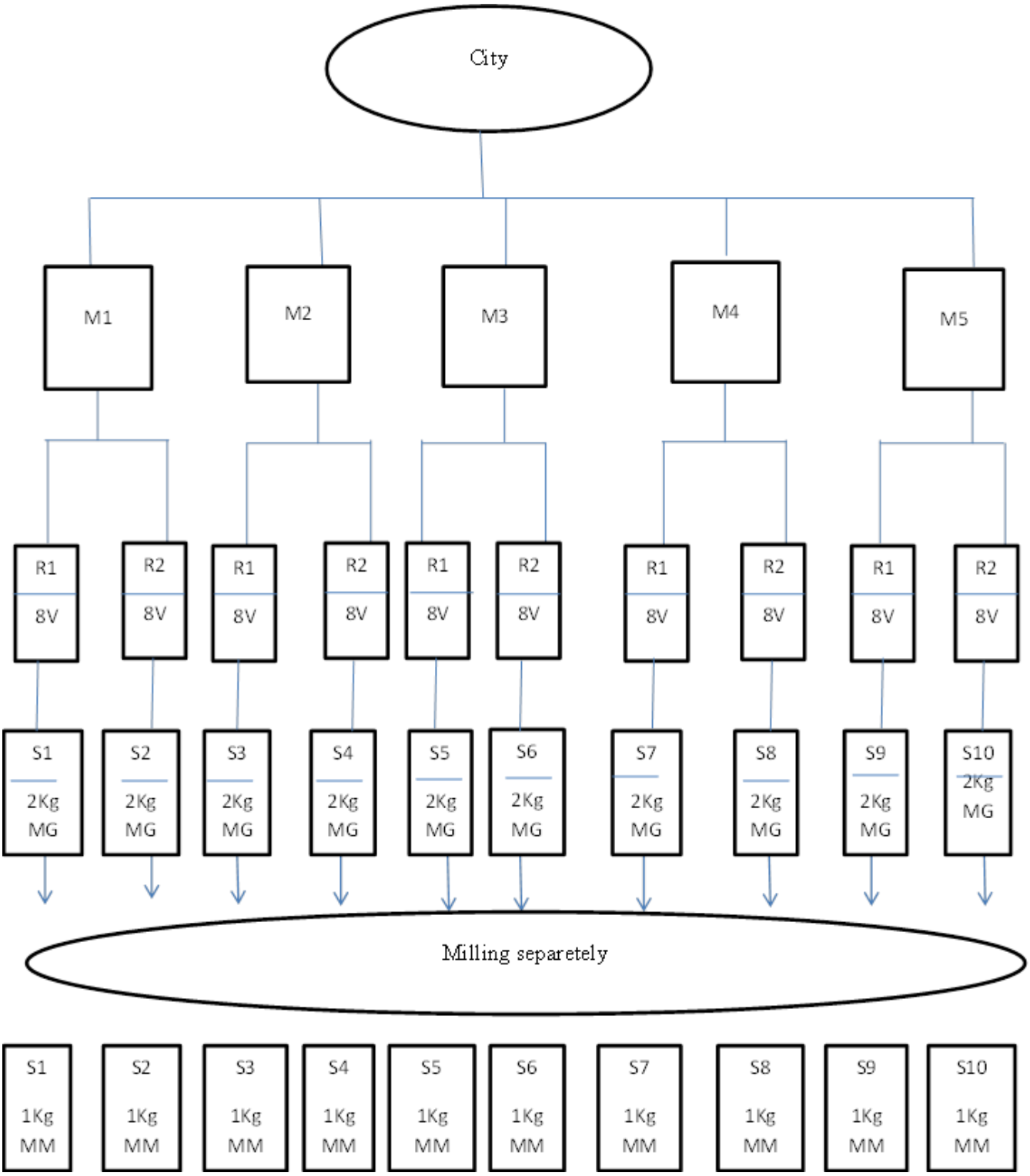

Figure 5: Collection of maize samples for milling and maize meals for analysis

This sampling procedure was applied in Goma and Beni cities in the same way but in Butembo it had a particularity before the milling stage. Here, the ten samples of maize grains were subdivided in two groups and then, five samples of raw maize grains were milled separately and the other five ones were grilled according to the information gotten from the survey and then were milled.

In each city, five (5) principal open air markets were selected and from each market, sixteen vendors were chosen randomly. The vendors were arranged in two rows; therefore eight vendors were randomly picked from each row, making a total of 16 vendors. From each of the eight vendors from one raw, a sample of maize grains of 0.25Kg was collected and all the eight samples were mixed together to form one composite sample of 2Kg. Thus, in each market two composite samples of 2Kg each were collected. Each of the 2Kg of maize grain was milled in the local meal and then, from each, 1kg of maize meal was packaged.

The maize meal samples were then packaged in Kraft paper bags and transported to the Laboratories of the Department of Food Science, Nutrition and Technology, University of Nairobi and stored in a cool dry place, away from direct sunlight to await further processing and analyses.

3.2.6 Production of Grilled Maize Meal

The maize for production of grilled ugali was grilled as shown on figure 6:

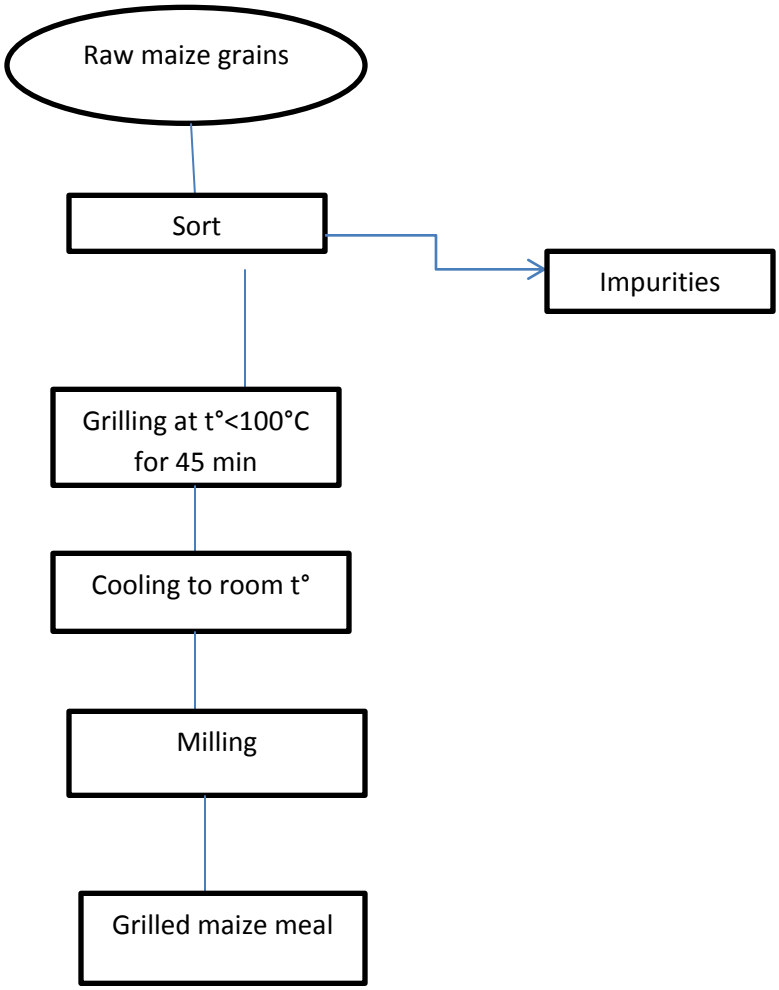

Figure 6: Production of grilled maize meal.

The traditional household grilling of maize kernels is as shown in picture below.

Source: Survey, 2018

For the production of grilled maize meal, the raw maize grains were sorted to remove impurities (stones, sand, damaged grains, ect) and then were spread on an open oven using fire wood as source of energy. The temperature used was below 75-89°C. The process was done by continually turning until the grains start turning from white color to brown for 45 minutes. Once ready, the grains were removed from the heat and were cooled at room temperature before the milling.

3.2.7 Milling of Maize

The maize (non-grilled and the grilled maize) was milled using a local hammer (posho) mill with a sieve of 0.3 mm of dimension as normally done for clients. All the millings were done in local hammer mills, as it is the practice in the three Cities.

3.2.8 Preparation of Ugali

The ugali cooking simulated the method practiced in households and the same method as applied for both grilled and non-grilled Ugali as follows:

1. **Ugali cooked with no alkali addition**

The ugali with no alkali addition was made by adding fifty grams (50g) of maize meal in 100 mL of boiling water and then the product was mixed for 7 minutes for complete cooking. The ugali were removed from the heat (hot plate) and were conditioned in sterile containers for analysis.

2. **Ugali cooked with alkali addition**

Different concentrations of 0; 2; 0.4; 0.6; 1; 2 and 3% of lime (Calcium Hydroxide) were added in ugali to determine the effect of Calcium Hydroxide on the aflatoxin content of ugali as eaten. The preparation of Ugali was done as follows:

For each concentration of Calcium Hydroxide, the calculated amount of Calcium Hydroxide was added in 100 mL of water as it is boiling and then 50g of maize meal was added after the dissolution of the lime and then the process continued as for ugali with no lime addition.

3.3 ANALYTICAL METHODS

3.3.1 Determination of Moisture Content

The weight of a previously dried and cooled empty dish was taken. Then about 2g of sample were weighed accurately in the dish. The dish and contents were placed air-oven at 105^0C and dried to constant weight. The dish and residue were transferred to a desiccator, cooled to room temperature, and then weighed.

The moisture content of the samples was determined in percentage as loss in weight of the sample using the AOCC method.

3.3.2 Analysis of Aflatoxins

Both samples, 25 samples of non-grilled maize meal, and 5 samples of grilled maize meal and 28 samples of ugali from 4 samples of maize meal with aflatoxin levels exceeding the limits (4 with no-lime addition and 24 nixtamalized ugali with 0; 2; 0.4; 0.6; 1; 2 and 3% of lime) were analyzed for total aflatoxins using ELISA Kit method, mark *helica* biosystems Inc with 96-wells, lot No. AF050818, as manufactured according to the GMP guidelines and quality control test as a unit (Catalogue No. 941AF01M-96).

Helica Total Aflatoxin Assay Principle

This Assay is a solid phase competitive inhibition enzyme immunoassay. Optimization of an aflatoxin specific antibody, which layered to a polystyrene microwell is done so that it cross reacts with all four subtypes of aflatoxin, B1, B2, G1 and G2. The extraction of the toxins from a milled sample is done with 70% methanol, and then a mixture of extracted sample and HRP-conjugated aflatoxin is prepared and added to the antibody-coated microwell. There is a competition between aflatoxin from the extracted sample and the HRP-conjugated aflatoxin to bind with the antibody-coated to the microwell. Microwell contents are poured and non-specific

reactants are removed by washing. An enzyme substrate (TMB) is added and a blue color develops. There is a direct proportional relation between the intensity of the blue color and the quantity of the conjugate, and an inverse proportional relation between the color and the aflatoxin concentration in the sample or standard.

Therefore, the power of the blue color will decrease as the concentration of aflatoxin in the sample or standard increases. The reaction is stopped by adding an acid stop solution which alters the blue color to a yellow color. The measurement of microwells is run optically by a microplate reader with an absorbance filter of 450 nm (OD_{450}). The Optical Densities of samples are compared to the ones of the kit standards and a result is interpreted.

Reagents

-1 pouch of Antibody coated microwell plate with 96 wells (12x8 well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody, ready-to-use

- 1 plate of mixing wells (Green) with 96 non-coated wells (12x8 wells strips) in a microwell holder, ready-to-use

- 6 vial Aflatoxin standards (Green cap) of 1.5 mL/vial of aflatoxin at the following concentrations: 0.0, 0.2, O.5, 1.0, 2.0 and 4.0 ng/mL in 70% methanol, ready-to-use

- 2 bottle of Aflatoxin HPR-conjugate (Blue cap) of 2x12mL of aflatoxin B_1 conjugated to peroxidase in buffer with preservative, ready-to-use

- 1 bottle of substrate reagent (Red cap) of 12mL stabilized tetramethylbenzine (TMB), ready-to-use

- 1 bottle of stop solution of 12mL acidic solution, ready-to-use

- 1 pouch of washing buffer of PBS with 0.05% Tween20, bring to 1 liter with distilled water and store refrigerated.

Extraction procedure

Twenty five milliliters (25mL) of methanol 70% were added to 5g of maize flour for extraction and then the mixtures were shaken in a sealed container for a minimum of 2 minutes. The mixtures were kept for 5 minutes to allow the particulate matter to settle, and then filtration of 5-

10 mL of the extract through a Whatman filter paper followed. The filtrate was collected to be tested.

Assay procedure

All samples were subjected to room temperature before analysis. The PBS-Tween packet was reconstituted by washing out the contents with a gentle stream of distilled water into a 1-liter container; one mixing well was placed in a microwell holder for each standard and sample to be analyzed, an equal number of Antibody Coated Microtiter Wells were put in another microwell holder. 200µL of the Aflatoxin-HPR Conjugate was dispensed into each mixing well; using a new pipette tip for each, 100µL of each standard and sample was added to suitable mixing well containing conjugate. Mix by priming pipettor at least 3 times. Using a new pipette tip for each, 100µL of content from each mixing well was transferred to a corresponding Antibody Coated Microtiter Well, and then incubation for 15 minutes at room temperature followed.

The contents from micro-wells were decanted into a discard basin and microwells were washed by filling each PBS-Tween wash buffer, then decanting the buffer into a discard basin. The operation was repeated for a total of 5 washes; the microwells were taped (face down) on a layer of absorbent towels to remove residual buffer. A required volume of 1ml/strip of Substrate reagent was measured and placed in a separate container and 100µL was added to each microwell. The microwells were covered to avoid light and then incubated for 5 minutes; a required volume of 1ml/strip of Stop solution was measured and placed in a separate container; 100µL was added in the same sequence and at the same place as the substrate reagent was added. The Optical Density for each microwell was read with a micro-titer plate reader using a 450nm filter and the OD of each microwell was recorded. The zero standard, the calculate % binding for

each standard and the sample as a percentage of the zero binging were set as 100% binding (B_0), (%B) and (%B/B_0) respectively.

3.3.3 Determination of Calcium Content of Ugali

Four grams of sample were precisely weighed into a porcelain crucible and put in a muffle furnace set at 500°C, then incinerated until a light gray or white ash of constant weight was obtained. To obtain the acid insoluble ash, the ash was covered with concentrated HCl and evaporated to near dryness on a boiling water bath. Then 25ml of a 10% HCl solution were added and were covered with a watch glass and boiled gently over a low flame for 10min. The liquid portion was filtered through an ashless filter paper, and then the deposit was washed with hot distilled water. The Calcium content was determined in the filtrate using an atomic absorption spectrophotometer according to the AOCC method and calculated using this formula:

$$\text{Ca (mg/kg)} = \frac{read\ value\ x\ extracted\ volume}{weigh\ of\ the\ sample}$$

3.4 SENSORY EVALUATION OF UGALI

Ten ugali samples were prepared with two (one grilled and one non-grilled) of the four maize meal which had above the tolerable levels of aflatoxins with varying concentrations of Calcium hydroxide (0%; 0.2%; 0.4%; 0.6%; and 1%) added.

The ugali were cooked simulated to the method practiced in households and were subjected to sensory evaluation by 10 randomly selected students who are familiar with ugali. The panelists were presented with coded samples and were requested to indicate their liking for each product on colour, odour, appearance, taste, mouthfeel and overall acceptance based on 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

3.5 STATISTICAL ANALYSIS OF DATA

Data showing the aflatoxin content for maize meal and nixtamalized ugali, moisture content, Calcium content and sensory evaluation were subjected to one way ANOVA using Genstat® Discovery 13thEdition at 95% confidence interval ($P \leq 0.05$). Variable means for measurements showing significant differences in the ANOVA were compared using the LSD. Values were judged to be significantly different by LSD if $P < 0.05$.

However, data from the survey on the type of ugali were analyzed using excel chart and the methods of ugali preparation analyzed only for means from the proportions of flour and water as done in the households.

CHAPTE FOUR: RESULTS AND DISCUSSION

The outputs and the discussions of the study are presented in this chapter four. The presentation format includes narratives, data Tables and Figures.

4.1 UGALI COOKING METHODS IN HOUSEHOLDS

The results of the survey showed that there are two methods of cooking ugali and for all the methods, the proportion of maize meal to water was of 0.5Kg of maize meal in 1L of water and the time of cooking varied between seven to ten minutes.

For the first method, porridge is made first with a small portion of maize meal and then when boiling, the remaining maize meal is added, the pot is covered for 2 to 3 minutes and then the mixed for seven to ten minutes until it becomes ready. Ugali is known to be ready by its smell and its thickness. This method is usually used for ugali from 100% non-grilled maize meal for it to be well cooked.

For the second method, maize meal is directly added to boiled water and then mixed for five to seven minutes for deep cooking. This method is manly done for ugali from grilled maize meal, or from cassava flour or for ugali from the mixed of cassava flour and grilled maize meal.

4.2 Types of Ugali Consumed by Households in the Three Cities

The results of the survey on the kind of ugali prepared and consumed in the households in Beni, Goma and Butembo cities are shown on Figure 7.

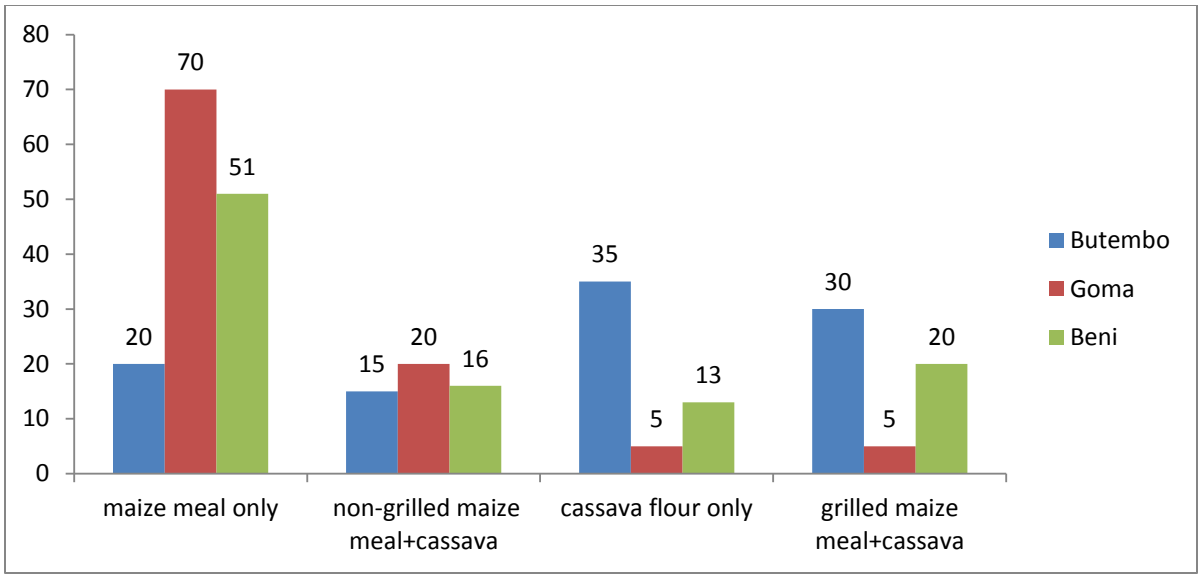

Figure 7: Type of ugali consumed in households in Beni, Goma and Butembo cities.

Figure7 shows that pure maize ugali is consumed most in Goma (70%). This is probably because Goma is populated by people from different tribes from all the parts of D.R.C who take maize meal ugali as their principal food in their diet. The other reason may due to the fact that Goma is close to Rwanda where maize is also mostly consumed, thus they are influenced by the diet of their neighbors. Figure7also shows that the other 30% of the other types of ugali (20% non-grilled maize meal+cassava flour; 5% cassava flour only and 5% grilled maize meal+cassava flour) are slightly consumed in Goma and this is mostly in families with low income (Survey, 2018).

However, consumption of Ugali from cassava flour only was highest in Butembo (35%) and lowest in Goma probably because of the monotribe of the population of Butembo (Nande) who originally cultivate cassava more than maize and since long times ago; cassava was the main food for this tribe. Finally, the consumption of ugali from maize meal only in Beni was intermediate between those of the other two cities (51%) but there was no significant difference

between the other types of ugali (16% non-grilled maize meal+cassava flour; 13% cassava flour only and 20% grilled maize meal+cassava flour) (P>0.05).

4.3 MOISTURE CONTENTS MAIZE MEALS

Results of moisture content of maize meal samples from milling of maize kernels collected in Beni and Goma (non-grilled) are shown in Table 1a and the ones from Butembo in Table 1b. In Butembo city, there were grilled and non-grilled samples, but in Beni and Goma, there were no grilled samples.

Table 1a: Percentage Moisture contents of Non-Grilled maize meal in Beni and Goma

Sample per city	**Mean moisture contents per city (%)**	
	BENI	GOMA
NG1	12.90 ± 0.01^{ab}	13.82 ± 0.07^{bcd}
NG2	12.46 ± 0.03^{bc}	13.49 ± 0.23^{cd}
NG3	12.45 ± 0.06^{abc}	13.20 ± 0.44^{d}
NG4	12.29 ± 0.11^{bc}	12.40 ± 0.01^{e}
NG5	11.61 ± 0.24^{c}	12.45 ± 0.04^{e}
NG6	13.53 ± 0.03^{a}	14.19 ± 0.47^{abc}
NG7	12.56 ± 1.46^{abc}	13.82 ± 0.51^{bcd}
NG8	11.72 ± 0.21^{bc}	13.62 ± 0.54^{cd}
NG9	12.12 ± 0.04^{bc}	14.51 ± 0.03^{ab}
NG10	12.42 ± 0.88^{abc}	14.62 ± 0.01^{a}
Mean*	**12.41± 0.67**	**13.61± 0.78**

*Mean ± SD (n=30). The mean values with the same superscripts are not significantly different at $p<0.05$

Table 1b: Percentage moisture contents of Grilled and Non-Grilled maize meal in Butembo

Sample per city	Mean moisture contents per city
NG1	13.16 ± 0.26^{a}
NG2	12.55 ± 0.12^{bc}
NG3	13.05 ± 0.32^{ab}
NG4	12.15 ± 0.21^{c}
NG5	12.38 ± 0.07^{c}
Mean*	**12.66±0.44**
GR1	8.63 ± 0.46^{b}
GR2	9.47 ± 0.41^{a}
GR3	7.35 ± 0.08^{c}
GR4	8.56 ± 0.27^{b}
GR5	8.36 ± 0.08^{b}
Mean*	**8.47 ± 0.70**

*Mean ± SD (n=10 for each city). Means values with the same superscripts are not significantly different at $p<0.05$.

Key: NG: Non-grilled maize; NG: Grilled maize

As shown in Table 1a, the mean moisture contents of samples from Beni city are significantly different from each other ($p<0.05$). It is assumed that the moisture contents of the meals represented the moisture contents of maize samples from which they were milled. The values ranged between 11.61 and 13.53% with the mean of 12.41%. Therefore, all the maize samples were within the general recommended optimum storage moisture set between 10 – 13% for cereals for aflatoxin prevention (Hell et al., 2008).

The means of non-grilled samples collected in Goma were also significantly different from each other ($p<0.05$). The values ranged between 12.40 and 14.62%, respectively. As shown in the Table 1a, only 20% of samples were below the recommended optimum storage moisture level. Majority of the maize from Goma was therefore insufficiently dried to prevent mold growth and production of aflatioxin during storage. The same risk of toxin production could be associated with the milled products during storage.

From Butembo city, two types of flours were collected. Five samples from the non-grilled maize and five from the grilled. The moisture contents of the flours from the non-grilled maize were in the same range as the moisture contents of the flours from Beni and were significantly different from each other ($p<0.05$). The moisture contents were also lower than the recommended levels for prevention of growth of Mycotoxigenic molds. Values ranged between 12.15 and 13.16%.

The moisture contents of the grilled samples were also significant different from one another with LSD=0.7844. The moisture contents ranged between 7.35 and 9.47%, much lower than the values for the no grilled samples probably due to the moisture removed during grilling. With these low moisture contents, the grilled maize meals may be kept for long without the risk of fungal growth and aflatoxin production as longer as the environmental conditions (relative humidity and temperature) are well controlled. The means of the Non-grilled samples from the 3 cities are between 12.41% and 13.61%, the recommended moisture content for cereals. However, these moisture contents are above the recommendation of 9 and 10% moisture content for flour storage for stability and longer shelf life as recommended by Nasir *et al* (2003) who found a great growth of Mold and insect infestation in flour stored at moisture greater than 10% compared to flour stored at lower moisture content 9%. Having these moisture contents greater

than recommended for storage, all the non-grilled maize meals are at high risk of Mold development and possible toxin production, and also insect infestation.

4.4 AFLATOXIN CONTENTS OF MAIZE MEAL SAMPLES

The results of the aflatoxin for non-grilled and grilled maize meal samples from the three Cities are shown in Table 2a for Beni and Goma and in Table 2b for Butembo.

The results of aflatoxin levels are calculated on as is basis. However, the deviation from the mean of all the moisture levels as shown in Tables 1a and 1b are less than 1. The difference between the values when expressed on moisture-free bases would not be expected to be significantly different from what they are. As shown in Table 2a, all the 10 samples of maize meal from Beni City were contaminated with aflatoxin. The levels ranged between 3.9 and 37.1ppb, with mean of 20.3ppb. Only 2 samples (representing 20%) had levels of aflatoxin lower than the national 10ppb tolerance for total aflatoxin. The aflatoxin contents are significantly different from one sample to another at $p<0.05$ and significant difference is well seen when the variable means are compared with the LSD of 1.8390.

Table 2a: Aflatoxin contents of non-grilled maize meal in Beni and Goma

Sample	Aflatoxin contents in ppb	
	BENI	GOMA
NG1	3.9± 0.2^{h}	37.8± 0.2^{h}
NG2	34.4± 0.0^{b}	4.9± 0.1^{e}
NG3	8.2± 0.0^{g}	47.1± 0.3^{a}
NG4	10.9± 0.1^{f}	6.0± 0.1^{d}
NG5	21.1± 0.4^{d}	19.3± 0.4^{b}
NG6	33.9 0.6^{b}	28.8± 0.3^{gh}
NG7	10.9± 0.5^{f}	8.1± 0.4^{c}
NG8	37.1± 2.4^{a}	28.9± 0.1^{f}
NG9	26.1± 0.4^{c}	4.0± 0.1^{c}
NG10	16.8± 0.2^{e}	4.5± 0.4^{g}
Mean*	**20.3± 11.7**	**21.0± 15.3**

*Mean ± SD (n=10 for each City). The mean values with the same superscripts are not significantly different at $p<0.05$.

Table 2b: Aflatoxin contents of maize meal in Butembo

	Aflatoxin contents in ppb
NG1	1.8± 0.2[e]
NG2	41.1± 0.1[a]
NG3	36.6 0.1[b]
NG4	6.9± 0.1[c]
NG5	5.3±0.0[d]
Mean*	**18.3±17.8**
GR1	18.6± 0.1[b]
GR2	5.6± 0.2[e]
GR3	14.1± 0.0[c]
GR4	9.8± 0.2[d]
GR5	39.2± 0.1[a]
Mean*	**17.4± 12.4**

*Mean ± SD (n=10 for each City). The mean values with the same superscripts are not significantly different at $p<0.05$.

All the samples in Goma were also contaminated with aflatoxin and shown significant difference at $p<0.05$ when means are compared with the LSD of 0.5959. Values ranged between 4.0 and 47.7 ppb with a mean of 20.3 ppb. Up to 50% of the samples had aflatoxin content exceeding the tolerance of 10ppb for total aflatoxin.

In Butembo City, all the grilled and non-grilled samples were positively aflatoxin contaminated with concentrations range between 6.9 and 41.1ppb for non-grilled with a mean of 18.3ppb for

non-grilled samples and between 5.6 and 39.2 ppb with a mean of 17.4ppb for grilled samples. Three out of five non-grilled samples (60%) were high than the tolerance among the non-grilled and for grilled samples, also 60% were above the tolerance. The mean aflatoxin content of the grilled maize meal sample was not significantly lower than the mean for the non-grilled samples, contrary to what was expected since grilling is done in the open and some oxidation would be expected. Probably the UV radiation from the sun did not break the maize sufficiently due to the high temperature of grilling, therefore reducing the extent of oxidative degradation.

The mean levels of aflatoxins in all the maize meals collected in the three Cities were above 10ppb, the acceptable level of aflatoxins in maize meals for human consumption. The means range between 17.4ppb in Butembo grilled and 21.0ppb in Goma non-grilled. The highest risk of exposure to aflatoxin is in Goma City where maize is highly consumed (70%) and the single sample with the highest level of aflatoxin was; however found in Goma City (47.1ppb). This is probably due to the high moisture content with which maize was stored. These outcomes are similar to the ones of Kamika *et al*., (2016) who conducted a comparatively study assessing the occurrence of aflatoxin in maize samples in DRC throughout the food supply chain and found the aflatoxin contaminated samples of maize ranging between 70.65 and 98.20% which expose consumers to both chronic and acute aflatoxicosis. With regard to moisture contents of the maize meals (table 1), the maize grains where stored under the recommended rate for cereals storage to reduce the aflatoxin production. However, the aflatoxin poison was produced in all the samples. This production of aflatoxin may had started in the maize since the field due to the lack of Good Agricultural Practices (GAP) and other facts like relative humidity, insect damages, temperature in the store, aeration in the store, package materials, etc might have been the sources of contamination as indicated by (Udoh *et aI*., 2000; Hell et aI., 2000b).

4.5 COOKING UGALI WITH ALKALI (LIME) ADDITION (NIXTAMALIZATION) TO REDUCE AFLATOXIN

Among samples with high level of aflatoxin, 4 (2 non-grilled: NG2 in Butembo city and NG3 in Goma city and 2 grilled: GR3 and GR5 in Butembo city) were cooked with alkali at different concentrations of Ca $(OH)_2$ (0; 0.2; 0.4; 0.6; 1; 2 and 3%) to test the effect of lime cooking on the reduction of aflatoxin in ugali as eaten.

4.5.1 Moisture Contents of Ugali Cooked with Nixtamalizationn

The moisture contents of the nixtamalized ugali are shown in Table 3.

Table 3: Moisture contents of nixtamalized ugali

Maize meal	Mean moisture contents (%) With percent lime						
	0%	0.2%	0.4%	0.6%	1%	2%	3%
NG2	65.1 ± 0.1^{a}	$63.7\pm1.8a^{b}$	62.4 ± 0.8^{ab}	61.2 ± 0.7^{b}	62.9 ± 0.8^{bc}	63.7 ± 0.4^{ab}	63 ± 0.2^{bc}
NG3	67.4 ± 0.3^{a}	63.2 ± 0.7^{c}	65.0 ± 0.2^{b}	64.6 ± 0.1^{b}	64.5 ± 1.5^{b}	63.0 ± 0.6^{b}	64.3 ± 1.7^{b}
GR3	67.5 ± 0.8^{a}	62.6 ± 0.31^{b}	63.84 ± 0.16^{abc}	60.2 ± 2.3^{c}	62.9 ± 0.2^{b}	62.0 ± 2.3^{b}	62.6 ± 1.3^{b}
GR5	65.3 ± 0.3^{a}	65.2 ± 2.5^{ab}	62.3 ± 0.9^{abc}	63.3 ± 0.1^{abc}	62.9 ± 0.1^{bc}	62.0 ± 1.8^{bc}	62.1 ± 1.6^{bc}
Mean*	66.6±1.3	63.7±1.1	63.4±1.3	62.3±1.9	63.3±0.8	62.7±0.8	63±0.9

*Mean ± SD (n=28). The mean values with the same superscripts are not significantly different at $p<0.05$

As Table 3 shows, the moisture contents are different from one another in all the ugali cooked with the addition of $Ca(OH)_2$ at different concentration. They are however inferior to the values of ugali cooked without addition of lime. This could be due to maize-starch gelatinization which facilitates hydration (easier and faster) and though, water is release and evaporates during cooking. These results agree with the findings of Chang and Hsu (1985) who studied the impact

of lime on moisture absorption and stated that the absorption of water in maize cooked in lime solution is greater than in maize cooked in normal water.

4.5.2 Changes of Aflatoxin Contents of the Nixtamalized Ugali

The aflatoxin contents of the nixtamalized ugali are shown in Table 4.

Table 4: Mean of aflatoxin contents in the nixtamalized Ugali with 0, 0.2, 0.4, 0.6, 1, 2 and 3% Ca$(OH)_2$

Ugali	Total aflatoxin (ppb) %Lime						
	0%	0.2%	0.4%	0.6%	1.0%	2.0%	3.0%
NG2	9.1 ± 0.3^{c}	16.9 ± 0.1^{b}	20.2 ± 0.4^{a}	17.39 ± 0.1^{b}	1	No detectable	No detectable
NG3	39.1 ± 0.3^{a}	27.8 ± 0.2^{c}	29.7 ± 0.2^{b}	30.1 ± 0.2^{b}	5.1	1.0	No detectable
GR3	4.3 ± 0.1^{a}	4.15 ± 0.02^{a}	3.5 ± 0.3^{b}	3.7 ± 0.1^{b}	No detectable	No detectable	No detectable
GR5	12.3 ± 0.1^{c}	14.9 ± 0.1^{b}	15.6 ± 0.5^{b}	17.5 ± 0.4^{a}	No detectable	No detectable	1.78

*Mean ± SD (n=28). The mean values with the same superscripts are not significantly different at $p<0.05$.

As shown in table 4, the addition of 0.2, 0.4 and 0.6% $Ca(OH)_2$ had a positive effect on the reduction of aflatoxin in the nixtamalized ugali. However, the reduction rates were not significant different from the ones in the non nixtamalized ugali. All the Ugali were cooked in the same conditions of temperature, cooking time and same proportion of water and flour according to the traditional methods. Normally, the reduction of aflatoxin would be increasing as the amount of added $Ca(OH)_2$ increases and would be greater in the nixtamalized ugali than in the non-nixtamalized Ugali (0% of lime). Yet, the results show an independent rate of reduction of aflatoxin in all the Ugali. This might be explained by supporting the research of Hwang *et al.*,

2006 who shown that the effectiveness and degree of reduction depends on numerous aspects, as well as aflatoxin concentration, the magnitude of binding between aflatoxin and food components, heat penetration, moisture content, pH, ionic strength, processing conditions and source of contamination (naturally or artificially). The aflatoxin-degradation might be the result of heat.

Aflatoxins are reported to be heat-resistant compounds at temperatures below 267 °C (Hwang *et al*., 2006). However, the results in table 4 show aflatoxin-degradation in the Ugali cooked at boiling water temperature and in an open pan. The effect of the heat on the non-nixtamalized ugali reduced the aflatoxin content at below the tolerable level in NG2 and GR3 ugali, slightly above the tolerable level in GR5 but in the GR3 ugali, the contamination level was still largely beyond the tolerance. These outcomes agree with the ones of Reddy and Rani, 2004 who reported that all heat treatment (boiling, roasting, baking and steaming) of maize products still have a significant effect on destruction of Aflatoxin in foodstuffs at percentages ranging between 50-70%. The results show a rate of decrement of aflatoxin with the hot lime treatment which is high than the one found by Price and Jorgensen (1985) who reported that the rate of reduction of the mycotoxin is 46%.

With the addition of 1% $Ca(OH)_2$, all the aflatoxin levels dropped to bellow the tolerance and by increasing the concentration of $Ca(OH)_2$, the aflatoxin levels steadily dropped to very low values as well as incalculable values. These are different that of Price and Jorgensen (1985) who reported that the effect of the addition of 2% of $Ca(OH)_2$ on the reduction of aflatoxin in tortilla was not significantly different with the one of 10% of $Ca(OH)_2$ during tortilla process. The difference in the results might be due to the fact of using the maize with natural aflatoxin contamination in which the aflatoxin was spread throughout the kernel where it is somewhat

secure from the action of alkali and heat or from oxidation of the open ring form as reported by Shotwell et al. (1974), Price and Sanchez, 1979; Ulloa-Sosa and Schroder (1969).

4.5.3 Calcium Contents of Ugali Cooked with Addition of Lime

The calcium contents of the ugali cooked with lime which reduced the aflatoxin contents to levels below the tolerance (1% - 3%) are shown in Table 5.

Table 5: Calcium contents of the nixtamalized Ugali

Maize meal	**Calcium (mg/100g) %Lime**			
	0%	1%	2%	3%
NG2	24.2 ± 0.8^{d}	171.5 ± 0.4^{c}	314.5 ± 3.2^{b}	409.3 ± 1.5^{a}
NG3	22.5 ± 0.8^{d}	395.6 ± 13.2^{c}	533.7 ± 8.5^{b}	652.3 ± 5.0^{a}
GR3	14.5 ± 0.6^{d}	255.3 ± 3.6^{c}	420.4 ± 0.9^{b}	503.7 ± 2.3^{a}
GR5	8.7 ± 0.5^{d}	303.9 ± 1.1^{c}	484.3 ± 6.4^{b}	850.55 ± 7.4^{a}

*Mean ± SD (n=16). The mean values with the same superscripts are not significantly different at $p<0.05$.

As shown in table 5, the value of Ca contents are significant different from one another and were increasing as the quantity of Ca $(OH)_2$ was raised. The addition of 3% had the highest increments that came from 24.2, 22.5, 14.5 and 8.7 to 409.3, 652.3, 503.7and 850.55mg/100g in NG2, NG3, GR3 and GR5 respectively.

These results are supported by Fernández-Muñoz *et al.*., (2004); Gutiérrez *et al*., (2007) who find that the increment in Calcium in the product is one major contributions of nixtamalization and help in solving the problem of osteoporosis caused in mainly post- and premenopausal women and elderly men due to the lack of Ca in the bones and Ca is beneficial for the growth of children. They found that the Ca increases at a rate of 750%.

4.5.4 Sensory Evaluation of the ugali cooked with lime

The sensory evaluation of the ugali cooked with lime at1% - 3%, which reduced the aflatoxin contents to levels below the tolerance are shown in Tables 6a and 6b

Table 6a: Sensory evaluation of the Grilled nixtamalized Ugali

	Attributes					
% Lime	Colour	Appearance	Odour	Taste	Mouthfeel	Overall acceptance
0%	5.1±0.1^{a}	4.5±0.2^{c}	5±0.1^{a}	5.3±0.1^{a}	5.1±0.1^{a}	5.4±0.1^{a}
1.0%	4.5±0.1b	4.8±0.1^{bc}	5.3±0.1^{a}	5.1±0.1^{a}	5.1±0.2^{a}	5.6±0.2^{a}
2%	5.2±0.1^{a}	5.2±0.1^{ab}	4.8±0.1^{a}	5.1±0.4^{a}	5±0.1^{a}	5.1±0.2^{a}
3%	5.3±0.1^{a}	5.5±0.1^{a}	5.2±0.1^{a}	4.7±0.1^{a}	5.1±0.1^{a}	5.3±0.1^{a}
Mean*	5.0±0.4	5±0.4	5.1±0.2	5±0.3	5.1±0.1	5.4±0.2

Mean ± SD (n=24). The mean values with the same superscripts are not significantly different at $p<0.05$.

The ugali cooked with 0%, 2% and 3% where slightly accepted for their color. On their appearance, the ugali with 2% and 3% were appreciated and on their odor, the ugali with 0%, 1% and 3% were well scored. The ugali with 0%, 1% and 2% scored well for their taste and all the ugali were slightly accepted for their mouth feel. For the overall acceptance, all the ugali with were accepted and the ugali with 1% was accepted with the highest score.

Table 6b: Sensory evaluation of the non-grilled nixtamalized Ugali

% Lime	Attributes					
	Colour	Appearance	Odour	Taste	Mouthfeel	Overall acceptance
0%	5.1±0.1[a]	5.5±0.1[a]	4.8±0.2[a]	5.1±0.1[ab]	4.9±0.1[a]	5.1±0.1[a]
1%	5±0.1[a]	5.2±0.1[ab]	5.1±0.1[a]	5.3±0.1[a]	5.3±0.1[a]	5.2±0.1[a]
2%	4.9±0.1[a]	5.1±0.1[ab]	4.9±0.1[a]	4.7±0.1[b]	4.2±0.1[b]	5.2±0.3[a]
3%	4.4±0.3[a]	4.9±0.2[a]	4.6±0.1[a]	3.9±0.1[c]	3.9±0.1[b]	4.8±0.2[a]
Mean*	4.9±0.2	5.2±0.2	4.9±0.2	4.8±0.6	4.6±0.6	5.1±0.2

Mean ± SD (n=24). The mean values with the same superscripts are not significantly different at $p<0.05$.

On colour, the ugali with 0% and 1% were preferred while the ugali with 0%, 1% and 2% were accepted for their appearance. Only the ugali with 1% was accepted for the odor and mouth feel. The ugali with 0% and 1% were preferred for the taste while for the overall acceptance, the ugali with 0%, 1% and 2% were slightly accepted. Like for the ugali with grilled maize meal, the ugali with 1% scored well among others.

Looking at the two tables, the ugali cooked with the addition of 1% lime were well scored both for both grilled and non-grilled maize meal.

Taking into consideration the reduction of aflatoxin and the amount of Calcium in the ugali, it was evident to cook the ugali with the addition of 1% of lime since this concentration reduces the aflatoxin to bellow the tolerance and increases the Calcium content of ugali without compromising the acceptability of the product.

Still the practice of nixtamalization is new in the area, the appreciation of the product will increase with time as consumers will be getting used to it.

CHAPTER FIVE: CONCLUSION AND RECOMMANDATIONS

5.1 CONCLUSIONS

The conclusion drawn from the research findings is that,

The results of the survey shown that there are two methods of cooking ugali and for all the methods, the proportion of maize meal to water was of 0.5Kg of maize meal in 1L of water and the time of cooking varied between seven to ten minutes

The types of ugali consumed in each of the main cities differ from one another according to the occupational tribe. The consumption of ugali from cassava only was high in Butembo while the consumption of ugali maize only was high in Goma and in Beni

Most of the maize meals from the three cities had levels of moisture above the recommendation of 9 and 10% moisture content for flour storage for stability and longer shelf life. Samples with the highest moisture content were found in Goma city

The means of aflatoxins in all the maize meals collected in the three Cities were above 10ppb, the acceptable level of aflatoxins in maize meals for human consumption

The moisture contents of the nixtamalized ugali samples with the addition of $Ca(OH)_2$ at different concentration were different from one another. They were however inferior to the values of ugali cooked without addition of lime.

The aflatoxin levels in the maize meal from all the three Cities were generally higher than the tolerance for total aflatoxin in the Country. However on cooking with addition of 1 – 3 percent lime, the aflatoxin contents of the ugali fell below the tolerable levels. The ugalis were acceptable to the panelists and their Calcium content increased.

Cooking ugali with addition of $Ca(OH)_2$ increased the contents of Ca

5.2: RECOMMENDATIONS

Further studies on the occurrence of aflatoxin in maize consumed in other parts of DRC should be done to cover the case on aflatoxin in the region and further studies on the other methods of reduction of aflatoxins should be carried out with other ready to eat products like porridge, boiled fresh maize; etc to reduce the exposure of consumers to aflatoxin-contamination

Farmers should be trained on the methods of harvesting and post-harvest handling of the maize including storage to reduce the aflatoxin contents of the kernels and the milled products to the tolerable levels

Farmers to be trained also on how to identify aflatoxin contaminated maize kernels and infection with the relevant molds

Studies to be carried out on other aflatoxin prone products consumed in DRC and on animal feeds and dairy products to aflatoxin-contamination.

The government of DRC should make a policy for 1% lime to be added in the maize meal at milling or during cooking of the ugali

Studies to be carried out on the aflatoxin contents of the milk of mothers and the exposure of the infants to the aflatoxin therefrom

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APPENDIX

Questionnaire

I am KAVGHO MUVUNGA Gloire, a Masters student at University of Nairobi in Food Safety and Quality.

I am doing a research on '*reduction of aflatoxin contamination of maize flour foods in three cities of north-kivu province of DRC by nixtamalization preparation for consumption'* and I am therefore to request for your help on providing me information on the following questions.

Determination of the cooking methods of Ugali from maize flour in households

Q.1 Do you take Ugali? Yes ☐ No ☐

Q.2 If yes, which type of ugali do you take?

A……..

Q.3 how do you prepare it? (Proportion of water and flour, cooking time according to the type of ugali consumed)

A……..