STABILITY OF AFLATOXIN IN MALAWIAN TRADITIONAL NON – ALCOHOLIC MAIZE BASED-BEERS AND THE INHIBITORY ROLE OF GARLIC AND GINGER

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DECLARATION

This thesis is my own original work and it has not been submitted to any other institution for similar purposes. Acknowledgements have been duly made where other people's work has been used. I bear the responsibility for the contents of this paper.

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To my wife Prisca and daughter, Grace for the encouragement

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ABSTRACT

Maize-based sweet beer is popular beverages consumed among Malawians mainly in the Central Region. However, maize grains and malts, the main ingredients for the brewing process are prone to contamination of mycotoxins including aflatoxin. However, the fate of the aflatoxin during the brewing process is not clearly understood. Therefore, the current study was carried out to understand the effect of brewing processes on aflatoxin contamination in the beer. Six beers prepared with different aflatoxin contamination levels (45µg/kg to 183 µg/kg) were analyzed at different stages using Aflatest® immunoaffinity fluorometric assay to determine the effect of heat, fermentation and interaction of thereof. Furthermore, filtrate at different stage were analysed for aflatoxin. Subsequent experiments evaluated the inhibitory effect of ginger and garlic on aflatoxin biosynthesis and their effect on consumer acceptability of beer prepared from herbal treated malt. Fermenting and boiling the mixture caused about 20% and 33% aflatoxin reduction respectively with no interactive effect between the two factors. Fermenting the beer for 24 hours led to further reduction of aflatoxin to about 37%, a content that remained constant for up to 8 days. Aflatoxin content detected in beer filtrate remained between 10 to 12% throughout the brewing and storage period. Both ginger and garlic inhibited 90% of aflatoxin in the malt and had no significant effect on sensory properties on beer produced. The use of aflatoxin free raw materials during preparation of beer is important if safety of the consumers is to be guaranteed.

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ACRONYMS AND ABBREVIATIONS

AFB1	Aflatoxins B1
AFB2	Aflatoxins B2
AFG1	Aflatoxins G1
AFG2	Aflatoxins G2
AFs	Aflatoxins
a_w	Water activity
CAST	Council for Agricultural Science and Technology
DON	Deoxynivalenol
FAO	Food and Agriculture Organisation
FB_1	Fumonisms B1
FB_2	Fumonisms B2
FDA	Food and Drug Administration
FUM	Fumonisms
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFPRI	International Food Policy Research Institute
LOD	Limit of Detection
LUANAR	Lilongwe University of Agriculture and Natural Resources,

MAPAC	Malawi program for aflatoxin control
NRC	Natural Resources College
NSO	National Statistics Office
OTA	Ochratoxin A
pH	Power of hydrogen
ppb	Parts per billion
ppm	Parts per million
USDA	United States Department of Agriculture
WHO	World Health Organisation
ZEN	Zearalenone

CHAPTER 1

INTRODUCTION

1.1 Background Information

Cereal-based beverages are widely consumed in the world, cherished by both rural and urban population and they are associated with social, religious, nutritional and therapeutic values and beliefs (Aka et al., 2008; Mager, 2010). Most studies on beverages carried out in Africa have shown that the most consumed cereal based beverages are traditionally processed compared to those produced industrially (Gadaga et al., 2013; Aka et al., 2014; Tafere, 2015).

In Malawi, maize (*Zea mays L.*), also known as corn, is one of the common cereal ingredients for beer brewing, particularly in the central region. However, maize, just like most cereals is prone to pre- and post-harvest toxigenic fungal colonization and mycotoxin contamination (Milani 2013). This is also common even with other naked cereal grains such as wheat (*Triticum aestivum*), sorghum (*Sorghum bicolour L.*), finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum L.*). Cereal grains such as maize become contaminated with mycotoxins when molds such as *Aspergillus flavus, Penicillium parasiticus, Fusarium graminearum, Fusarium*

culmorum, Fusarium roseum and *Fosarium moniliforme* grow on the grain. Cereals under drought stress conditions and those damaged by insect pests are vulnerable to aflatoxin contamination (Desai et al., 2008; Guo et al., 2008).

Mycotoxins, which include aflatoxin, are the secondary metabolites of fungi which are poisonous and have undesirable effects to animals or humans when exposed. (Bennett & Klich, 2003). Several outbreaks of mycotoxicoses diseases in humans and animals such as nephropathy, various types of cancer, alimentary toxic aleukia, hepatic diseases, various hemorrhagic syndromes among others caused by various mycotoxins have been reported after the consumption of mycotoxin-contaminated food and feed respectively (Reddy & Raghavender, 2007; Carvajal & Castillo, 2009; Reddy et al., 2010).

Considering their negative impacts, mycotoxin-contaminated cereals must be discarded. However, this is not normally the case in most developing countries like Malawi due to persistent food shortage which results in the utilization of these cereals for human consumption (e.g. diverted to beer production) and animal feed thereby transferring of mycotoxins along the food chain. (Bennett & Klich, 2003; MacLachlan, 2011). Studies have also shown that local grains used for beverage contamination (mostly in low – income rural setting with much reliance on home – grown crops) are highly contaminated either from poor pre-harvest or post-harvest handling as better quality grains are sold for family income (Chibudu et al., 2015; Ayalew et al., 2016; Misihairabgwi et al., 2017; Ogara et al., 2017).

The risk of some mycotoxin's contamination, for example aflatoxin, increases in beer brewing process during the malting step which involves raising moisture content of the grains and the humidity of the environment (Milani, 2013). The risk is much greater in an African setting since malting process is mostly done under typical home environments where fungal colonization is not controlled (Adekoya et al., 2018) with possibility of aflatoxin been carried over to the final product (beer). Most studies have reported high incidences and levels of mycotoxin contamination in cereals, cereal based foods and feeds as well as in the traditional opaque beers (Kedera et al., 1999). Opaque beer is a weak alcoholic beverage produced by the process of alcoholic fermentation from a starch source, water and yeast as defined by Mawonike (2017). Some studies have reported almost 100% carry-over of mycotoxins from malted grains into beer and/or fermentation residues (Hanschmann & Krieg, 2006; Schaafsma et al., 2009). In contrast, other researchers have reported significant reduction of mycotoxins following alcoholic fermentation (Garda et al., 2005; Meca et al., 2010). Despite numerous reports of mycotoxin incidences in beers worldwide (Rubert et al., 2012; Burdaspal & Legarda, 2013; Varga et al., 2013), the effect of fermentation and other brewing processes involved in the production of Malawian non-alcoholic or sweet beer, is yet to be documented. Additionally, documentation of these effects on the mycotoxin quality of Malawian sweet beer would inform the introduction of safe and effective strategies for the management of mycotoxin in the Malawian sweet beer that would not compromise its sensory quality.

There are a number of approaches carried out by most researchers to manage or to detoxify the toxin in food (Hell & Mutegi, 2011; Monda & Alakonya, 2016; Ojiambo et al., 2018). The use of hot water treatment of cereals, plant extracts, chemical methods such as

ozonation, and use of lactic acid bacteria starter culture during malting and fermentation strategies have been reported to detoxify and control mycotoxin development during beer brewing process (Suzuki 2015; Pascari et al., 2018).

1.2 Problem statement

The production and consumption of maize based non-alcoholic beverage is a common practice in Malawi, particularly in the central region. The beverage is consumed by adults for nutritional and social purposes and to some extent used as a weaning beverage for under five children. Unfortunately, the production of the beverage often involves the use of poor grade maize grains and malts that are commonly contaminated with various mycotoxins including aflatoxins, which can sometimes be carried over and detected in the final product (Kenji et al., 2000; Matumba et al., 2011). However, the effects of different processes on the carryover of aflatoxins into the final product are not well understood. Moreover, there are limited studies that focused on inhibiting aflatoxin development in Malawian maize based non-alcoholic beverage. Therefore, the present study was an attempt to assess how different processes would affect the mycotoxin quality of sweet beer and exploring ways of controlling mycotoxins in the final product.

1.3 Research objectives

1.3.1 General Objective:

- To understand the effect of various production processes on the level of aflatoxin contamination in Malawian maize-based non-alcohol opaque beer.

1.3.2 Specific Objectives:

1. Establish the trend of aflatoxin reduction during fermentation and boiling of the Malawian maize-based non-alcohol opaque beer.
2. Determine the distribution of aflatoxin between the liquid and solid phases of maize-based non-alcoholic beer
3. Assess the effect of garlic and ginger application to maize on aflatoxin level in malt
4. Determine the effect of the herbal treatment of maize during malting on the sensory properties of maize-based non-alcoholic beer.

1.4 Thesis organization

Chapter one introduces this research study. In chapter two, the literature review is provided while materials and method are given in chapter three. The fourth chapter deals with results and discussion. In chapter five, conclusions and future perspectives are provided

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of maize production and consumption in Malawi

Maize is a most dominant and important staple cereal food crop in many Sub-Saharan countries (Ekpa et al., 2018; Ekpa et al., 2019). In Malawi, maize is the main stable food and is grown by 97% of farming households in all districts and it counts for over 60% of the total food consumption, 60% of energy, 48% protein consumption, and more than two-thirds of caloric availability. Besides, maize is also a source of micronutrients and vitamins: It is a source for 67% of iron, 65% of zinc, and 56–72% of the less ramified B vitamins (Ecker & Qaim, 2011). USDA (2018) reported that, to about 3000 thousand metric tons of maize is produced from an area about 1.7million hectare in the country, which represents more than 70% of the total arable land (NSO, 2005). This contributes significantly to diet of more than 80% of the population, with a consumption of 182kg per capita per year (Matumba et al., 2009; Mwalwayo & Thole, 2016). Maize is consumed in different forms which include nsima (a stiff or thickening maize porridge), boiled green corn, roasted corn and can also be processed into beverages such as distilled alcoholic (kachasu), alcoholic fermented drink (chibuku) and non-alcoholic fermented drink (thobwa) among others (JAICAF, 2008).

With the special interest, Malawian maize-based non-alcoholic or sweet opaque beer, locally called thobwa is prepared by boiling the fermented the mixture of maize porridge and cereal malt. This maize-based non-alcoholic beer traditionally is consumed mostly during cerebrations.

2.2 Mycotoxins

Mycotoxins, the toxic secondary metabolites produced by filamentous fungi, are found in a wide range of agricultural commodities worldwide (Bhat et al., 2003; Fredlund et al., 2013; Mnggawa, 2015; Tralamazza, 2016). It is estimated that mycotoxins contaminate about 25% of the world agricultural crops (FAO, 2011)

There are over 800 different kinds of mycotoxins produced by more 150 species of fungi (Whitlow et al., 2003; Stein et al., 2017). However, most researchers have discovered and recorded the common types of mycotoxins which include Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), Patulin, Ochratoxin A (OTA), Fumonisim B_1 (FB_1), Trichothecenes and Zearalenone (ZEN) (Scudamore and Patel, 2000; CAST, 2003; Hooker and Schaafsma,, 2005; Abbas et al., 2006; Ayalew, 2010; Warth et al., 2012; Probst et al., 2014; Okeke et al., 2015; Chilaka et al., 2017; Ogara et al., 2017).

Mycotoxins are produced by different moulds which are able to colonise a wide range of agro produce right from the field to the time of consumption. They are naturally produced in response to stress caused by environmental extremes, food shortage or competition from other micro-organisms (Losada et al., 2009). Hence the mycotoxins play a defensive role for the fungi. Wherever growth factors are permissible, fungi may grow and produce these

mycotoxins. When mycotoxins are ingested, inhaled, or absorbed through skin, they may reduce appetite and general performance, and cause sickness or death in humans and animals (Reddy et al., 2010). These mycotoxicoses (diseases caused by mycotoxins) often remain unrecognized by medical professionals, except when large numbers of people are involved (Fung & Clark, 2004). Mycotoxins have been reported to cause a variety of toxic effects including hepatotoxicity, teratogenicity, and mutagenicity, resulting in diseases such as toxic hepatitis, hemorrhage, edema, immunosuppression, hepatic carcinoma, equine leukoencephalomalacia (LEM), esophageal cancer, and kidney failure (Carvajal & Castillo, 2009; Dönmez et al., 2003). Not only do the mycotoxins affect humans and animal health, studies have also reported their negative effects on food crop quality, food safety as well as food security when crops are contaminated. Mycotoxins have been reported to reduce seed quality, protein and carbohydrate content, reduce the germination capacity as well as seedling damage hence lead to reduction in crop yield (Sharfun-Nahar & Hashmi, 2005; Mendoza et al., 2017). Mycotoxins contamination have contributed negatively to the food security in most African countries including Malawi where food supply is limited and often of poor quality (Misihairabgwi et al., 2017; Gbashi, 2018).

2.2.1 Occurrence of Aflatoxins

Aflatoxins are produced by several species of *Aspergillus*, particularly, *Aspergillus flavus* and *Aspergillus parasiticus* (Richard & Payne, 2003; Varga et al., 2011). The former is predominant in maize while the latter being more common in peanuts than maize (Whitlow & Hagler, 2003). These two species are the most studied of known approximately 250 species of *Aspergillus* and they are very dangerous to human health through contamination

of food (Bennett et al., 2003; CAST, 2003; Klich, 2007; Yin et al., 2008). The chemical structures of the four major aflatoxins produced in nature are shown in Figure 1 (a to d)

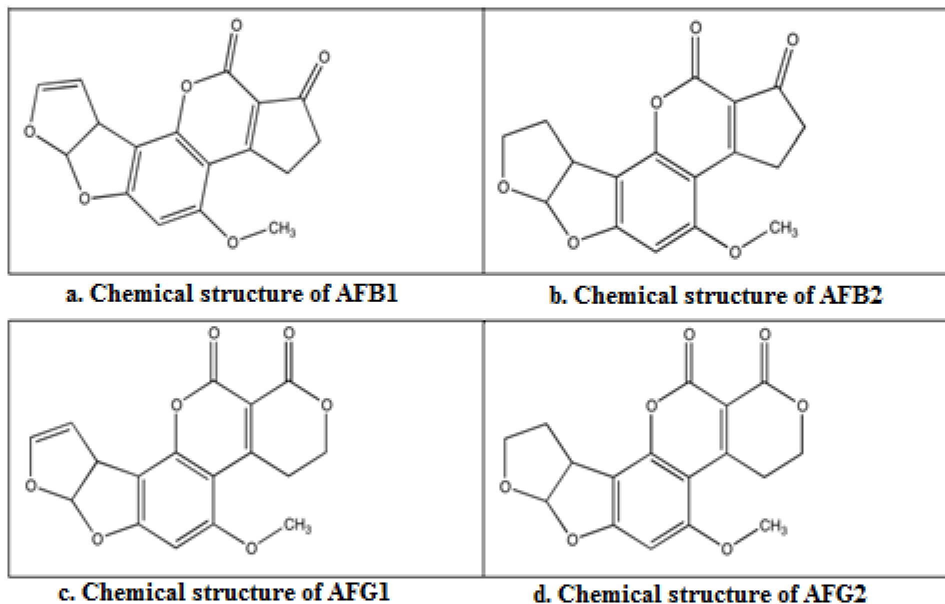

Figure 1: Chemical structures of four major aflatoxin molecules

Aflatoxin B1 (AFB_1) is the most potent toxin and carcinogenic mycotoxin, and it is classified as group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) (Klich, 2007).

Human foods that are particularly susceptible to aflatoxin contamination are those cultivated in the tropics and subtropics including maize and peanuts (Yin et al., 2008). Researchers have recorded that *Aspergillus flavus (A. flavus)* is more common in tropical and sub-tropical climates between 35 degrees north and south of equator and it is found in soils across the globe (Klich, 2007). Aflatoxins have also been reported in temperate countries of Europe and North America (Patel et al., 2015)

2.2.2 Conditions necessary for Aflatoxins growth.

The general perception of the presence of aflatoxin in food crops were thought to be only after harvest but further studies indicates that the toxins are available even during growth in the fields prior to harvest (Diao et al., 2015; Lavkor & Var, 2017). Aflatoxin are produced in crop plants when they are in the field, at harvest, during post-harvest operations as well as when the crop products are in storage. However, the researchers have shown the rate and level of production are dependent on temperature, humidity, time of planting, crop variety, post-harvest handling and storage conditions (Bruns, 2003; Diao et al., 2015).

Several researchers have reported a different behavior of *A. flavus* isolate regarding temperature with optimum value varying from 25 – 38ºC as well as humidity over 80% regardless the media (Garcia et al., 2011; Marin et al., 2012). For instance, Lahouar and others (2016) in their recent study showed that *A. flavus* grow in a wide narrower range of water activities (0.94 – 099a_w) and temperature range 25 – 37ºC. This finding is similar to what Medina et al. (2017) reported. On production and growth, Milan (2013) specified that the production of *Aspergillus species* responsible for aflatoxin favours 33°C and water stress condition of 0.99 $a_{w,}$ and its growth favours 35ºC and 0.95 a_w. Similar work by Pitt and Miscamble (1995) showed that the impact of ecological factors on the growth of these species were similar with minima of 0.82 $_{aw}$ at 25 °C and 0.81 $_{aw}$ at 30 and 37 °C.

2.3 Aflatoxin and their occurrence in maize

Maize has become one of the major sources of human exposure to aflatoxin due to its susceptibility to aflatoxin contamination (Scarpari et al., 2014). It is a common practice in most African developing countries to store or sell high quality maize and use the moldy and highly contaminated for consumption and as well as for production of maize based beverages (Mwalwayo et al., 2016). These practices put the population at health risk. Several studies have reported a number of cases in maize and their products to contain unacceptable levels of aflatoxins (Bankole & Mabekoje, 2004; James et al., 2007; Hell et al., 2008; Kpodo & Bankole, 2008; Akowuah et al., 2015; Sowley, 2016; Kachapulula et al., 2017; Okoth et al., 2017).

In West African countries different researchers have reported significant aflatoxin contamination levels in maize. In Ghana and Nigeria in a study carried out by Perrone et al. (2014), a mean of 330µg/kg with a maximum concentration of 1900µg/kg of aflatoxin concentration were detected in the maize samples sourced from household stores. In the same study, maize samples sourced from market stores a mean of 84µg/kg with a maximum of 480µg/kg of aflatoxin concentration were detected. Warth et al. (2012) in quantifying the mycotoxins in food and feed in Burkina Faso, a mean of 24µg/kg of aflatoxin contamination was detected in more than 10% of the samples studied. In Cote d'voire a mean of 107.9µg/kg was detected in 51 maize flour samples studied (Kouadio et al., 2014)

In Cameroon, the Central Africa, researchers also reported different aflatoxins levels in maize. Ediage et al. (2014) in their study to explore differences in mycotoxin patterns from agro-ecological region in maize reported a range of 2 - 645µg/kg aflatoxins contamination. In another study of mycotoxin in different food carried out by Njobeh et al. (2010) detected

a mean aflatoxin level of 51µg/kg. On contrary, Abia et al. (2013) detected a range of 0.1 to 3µg/kg of aflatoxin contamination in maize based food in Cameroon. High levels were also reported in East African countries. Several studies on maize carried out in the region reported the mean of over 100 µg/kg (Lewis et al., 2005; Probst et al., 2014; Kamala et al., 2016).

In Southern Africa, the notable high levels of AFs in maize were reported in Zambia, South Africa as well as Malawi (Rava, 1996; Mwalwayo et al., 2016). In a study carried out in Zambia by Kachapulula et al., (2017) aflatoxins were detected in maize samples studied with an average of 16 µg/kg. In the review study on maize in South Africa conducted by Rava (1996) the highest aflatoxin contamination level in maize recorded was 50µg/kg. In Malawi, a number of researchers have reported aflatoxin contamination in maize samples ranging from 0.7 to 400 µg/kg (Matumba et al., 2013; Matumba et al., 2015a; Mwalwayo et al., 2016). A study carried out by Matumba, et al. (2013) a range of 1 up to 382 µg/kg of aflatoxin contamination were detected in all maize samples studied. Some of the researchers reported the aflatoxin contamination level on average maximum of 26.2 µg/kg (Kachapulula et al., 2017). Other previous studies, however, have reported low levels of contaminations in other countries. In Uganda, for example, maize samples studied had no detectable aflatoxins levels (Kaaya et al., 2006).

2.4 Aflatoxin levels in traditional beers

The safety of African traditional beverages, as far as aflatoxin contamination is concerned, is influenced by mainly the quality of the raw materials used to produce the beverages (Salgueiro et al., 2010). Some of these raw materials are highly contaminated with aflatoxin which might be carried over to the final product (beer) thus making the beverages not safe

for consumption. Traditional beverages (alcoholic and non-alcoholic) are usually made from cereal grains, which include maize, millet, sorghum and barley (Aka et al., 2014)

Several studies have reported significant levels of aflatoxins in maize based beers in most African countries as well as outside Africa (Matumba et al., 2011; Darwish et al., 2014; Matumba et al., 2014; Ayalew et al., 2016; Mwalwayo et al., 2016; Chilaka et al., 2017; Peters et al., 2017; Ogara et al., 2017). In India, for example, Peters et al., (2017) reported that about 6% of the 420 traditional beer samples studied detected contamination of AFB_1 ranging from 0.05 to 230µg/Kg and about 2% were contaminated with AFB_2 ranging from 1.2 to 32µg/Kg. In another separate study, in France, 65% of traditional beer sample analysed had aflatoxin concentration ranges from 0.07 to 45.18µg/Kg (Peters et al., 2017).

In case of Africa, for example in Ghana, Peters, et al., (2017) detected aflatoxin contamination ranging from 0.07 to 38µg/Kg in about 64% of 422 traditional beer samples studied. In South Africa high levels were also detected of which about 9% of 35 traditional beer samples studied, had aflatoxins contamination whose concentration ranges from 12 to 400 µg/Kg (Odhav & Naicker, 2002). In Malawi, Matumba, et al. (2014) detected aflatoxins with average of 90 µg/Kg in 8 of the 9 maize-based opaque beers sampled from Chewa rituals.

In other brewing processes, mainly in Europe and other developed countries, the spent grains are filtered and studies have demonstrated that such practices reduce aflatoxin contaminations (Fandohan et al., 2005). Studies carried out in Canada reported very low levels ranges 0.007 to 0.0112 µg/Kg in beers just like in beers studied in other developed countries as well as beers processed from filtrates (Mably et al., 2005). No study however,

has compared the toxin found in the Malawian maize-based whole beer (with spent grains) and its filtered product.

2.5 Effect of processing on aflatoxin

Processing operations have the potential to either remove (reduce) or produce (increase) the aflatoxin contamination and make the food product safe to eat/drink or not. Most studies have reported on the effect of processing operations such as brewing process, on aflatoxin levels (Nkwe et al., 2005; Bationo et al., 2015; Kaushik, 2015; Karlovsky, 2016). In most studies the effect of brewing process on aflatoxin has been demonstrated by comparing aflatoxin content levels in brewery raw materials (malt and cereals) and in the product (beer). Negligible levels in the beer are reported in most studies regardless the significant aflatoxin levels detected in the raw materials. In Burkina Faso for example, Batioano et al. (2015) detected an average range of 97.6±88.2 μg/kg of aflatoxin in 25% of the malt samples but did not detect any aflatoxin beers samples.

The reduction of aflatoxin in cereal based beers could be the effect of processes such as boiling, dilution, fermentation process as well as pretreatment of the raw materials such as sorting, washing, winnowing, steeping, dehulling, milling as well as roasting as reported by most researchers (Fandohan et al., 2005; Chibudu et al., 2015; Matumba et al., 2015b; Okeke et al., 2015; Karlvosky et al., 2016). However, there is limited information available on the extent to which processing activities in Malawian maize-based beer brewing affect the aflatoxin content.

2.5.1 *Effect of pre-treatment of raw materials for brewing on aflatoxin*

Malting is one of the important pre-steps (pre-treatment on grains) which plays a vital role in fungal development in malted grains. It is a process of readying cereals to be used in brewing, and it involves steeping (soaking), germinating and drying sub-steps. Studies has shown that the process provides the conditions that favor the production and growth of aflatoxin. Chibudu, et al. (2015) reported that due to the sub-steps involved in malting process, moisture content in grains increases hence creates favourable conditions for aflatoxin development. In a study by Schabo et al. (2020) in Brazil on aflatoxins development during malting process, a range of 229.35 to 455.66µg/kg of the aflatoxin were reported to develop on malted grains after steeping step. In Africa, in a study carried out by Kenji et al. (2000) in Kenya and Malawi, aflatoxin concentrations of up to 1020µg/kg were detected in malted grains. Studies have reported that poor storage conditions of malting grains also promote aflatoxin development (Nawar, 2008; Chibudu, et al. (2015). This is common mainly in Sub - Saharan countries where the malting process is done using local setting without control measures. It is recommended therefore to use controlled environment to avoid aflatoxin contamination of malted grains (Ezekiel et al., 2018; Mastanjevic et al., 2019). However, there is limited information on how aflatoxin contamination can be avoided during malting at household level.

2.5.2 *Thermal effect on aflatoxin*

During the brewing process, heat is applied at different stages depending on the type of beer to be produced and the production methodology. Heat can be applied before brewing process during pre-treatment (grains) and/or when brewing during boiling. During sample pre-treatment, grains can be roasted, for example in Ethiopia, in preparation of Borde (sweet beer) as well as Keribo and Tella (alcoholic) grains are roasted (Tafere et al., 2015;).

Similarly, in Uganda the production of Malwa (sweet beer) and Kwete (alcoholic) involves roasting of the cereal grains and studies have shown that this reduces aflatoxin contamination. (Muyanja et al., 2010; Aka et al., 2014). In Malawi and in other African countries such as South Africa, Ethiopia, Kenya, Zambia, Uganda, Botswana boiling of the mixture (maize and malt) is involved in tradition brewing process (Lyumugabe et al., 2012).

Many studies carried out, outside Africa, have shown the positive effect of heating (boiling) process in beer brewing to reduce aflatoxin contamination levels. For instance, a study carried out by Oluwafemi et al. (2004) reported that 99.5% of the aflatoxins were destroyed in contaminated corn in 30 min at 250°C, compared to only 20% at 100°C. This study indicated that thermal degradation is the function of the degree of temperature. Higher temperatures may completely degrade the toxins to negligible levels.

Most researchers have demonstrated that aflatoxins are thermally unstable at different temperatures (Hussain et al., 2002; Bullern et al., 2007; Kabak, 2009). For example, Oluwafemi et al. (2004) recorded that aflatoxin thermal instability increases when temperature is around 250ºC. Raters and Mastissek (2008) also reaffirmed that aflatoxin are not 100% thermally stable and they pointed out that the aflatoxin do not survive any temperature higher than 160ºC. All these temperatures are far much higher than the temperature at which maize porridge boils (around 96ºC) as determined by Beswa et al. (2020). Malawi preparation of maize based beers firstly involves the production of maize porridge using wood as a source of energy. Firewood is categorized as the least fuel energy source (Burnette & Director, 2010) hence its effect on aflatoxin stability would be questionable. There is little or no information on the effect of cooking temperatures on

aflatoxin stability in maize based beverage when using firewood. Therefore, it is important to assess the effect of temperatures used for brewing traditional beer in Malawi using firewood on aflatoxin stability.

2.5.3 Effect of fermentation process on aflatoxin

Fermentation is a metabolic process in which sugar is consumed in the absence of oxygen. As a key process, several studies have shown that the contamination levels of aflatoxin in fermented products decrease during fermentation (Matumba et al., 2015a; Matumba et al., 2015b; Johnstone et al., 2012; Nagatomi et al., 2012; Mokoena et al., 2005; Alvurez et al., 2000). In the study on fate of aflatoxin B_1 during the fermentation of alcoholic beverages by Inoue, et al. (2013), about 70% of the original aflatoxin was detected after 7 days. The study also showed that the degradation was rapid only in the first 24 hours in which 80% of the toxic was detected in the beer. They pointed out that the concentration of AFB_1 decrease during fermentation was due to increased production of the hydrated derivative AFB_{2a} which is less toxic compared to AFB_1. In another separate study, Inoue, et al. (2013b), found out that aflatoxin decreased to 20% of their initial concentration due to fermentation which is in agreement with study findings reported by Pietri, et al. (2010). Despite of the reports on effectiveness of bacteria starter culture in aflatoxin degradation during fermentation (Wacoo et al., 2019; Shu et al., 2018), information is lacking on whether natural fermentation would degrade of aflatoxins to zero

2.6 Management of Aflatoxins using plant products

A number of approaches (strategies) have been taken for mycotoxin decontamination in food products by several researchers (Ismail et al., 2016; Herzallah et al., 2008; Hassan & Zhou, 2018). However, no single approach has proven to be completely successful in

removing the toxins and retaining the nutritional and functional properties of the food commodity. Some researchers have developed interest in inhibition properties of the plant extracts against growth of aflatoxigenic fungi (Yingprasert et al., 2013; Njoki et al., 2017). The organic extracts from the plant contain bioactive compounds which are responsible for antifungal activities (Tian and Chun, 2017).

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are reported to have antifungal properties and inhibit form of aflatoxin (Ismaiel et al., 2012; Abd EL – Aziz et al., 2012; Moon et al., 2018). Ismaiel, et al. (2012) in their study on treating *A. flavus* keratitis demonstrated the effect of garlic on aflatoxin growth. They found that, exposure of 2.88g/ml of garlic cause a reduction range 36.4 – 75.5% of possible aflatoxin growth from 30 minutes to 1 hour. In another recent study carried by Negara and Washe (2019) showed that 50mg/L of aqueous garlic had highest effect with 35.5% in 30 minutes' time of exposure and 68.3% in 1 hour in maize samples. The same study also showed about 16% degradation was caused by 50mg/L of ginger in 30 minutes and 22% in 1 hour. In another study by Abd EL – Aziz et al., (2012) showed garlic caused 93.20% aflatoxin reduction while ginger caused 57.75%. Latif et al. (2006) as they were studying the efficacy of plant extracts in controlling fungal infection, garlic caused 72% reduction in toxigenic fungi which was more effective than ginger which caused only 32% reduction. All these studies used aqueous forms of herbs. Nevertheless, there is of assess the effect of these antifungal medicinal plants (garlic and ginger) on aflatoxins management in maize based beer brewery.

Since malting process creates suitable conditions for mycotoxin development, the use of garlic and ginger to control the development of such mycotoxins would be ideal

considering their antifungal properties. However garlic and ginger powders, based on their sensory characteristic, have the potential to compromise the sensory properties of the final product depending of the amounts used (Cho et al., 2007; Lee et al. 2009; Kishk and Elsheshetawv, 2013). With limited information on the effect of ginger and garlic on sensory properties of maize based food products, it is imperative to study such effect on the Malawian maize based sweet beer.

CHAPTER 3

MATERIALS AND METHOD

3.1 Maize and malt samples

Eleven visually mouldy dried maize malts (6-10 kg each) were bought from different sellers at Msundwe market in Lilongwe district, central region of Malawi. Visually mouldy maize spared for beer brewing was sourced from households in villages around Msundwe market. Both the maize and the malt samples were separately ground to pass sieve # 20 (opening size = 0.85 mm) using Romer Labs Series II ® mill (Romer Labs, Inc., Union, MO, USA), thoroughly mixed and subsamples analysed for total aflatoxin concentration as described in later section (Section 3.3). Aflatoxin content of the eleven malt samples and six maize samples are presented in Appendix 1, with the total aflatoxin concentrations in malt samples ranging from 6 to 312 µg/kg and the maize samples having total aflatoxin concentration ranging from 225 to 985 µg/kg. Only the six highest aflatoxin contaminated malt and maize samples were subsequently used in the brewing experiment. Examples of the visually mouldy samples of maize sourced in the study are shown in Figure 2.

Figure 2: Visually mouldy maize samples

Clean maize samples from a farmer around Msundwe area (Lilongwe district) were analysed for total aflatoxin concentration as described section 3.3 and those with no detectable aflatoxins (LOD=1 μg/kg) were used in a malting experiment and preparation of non-alcoholic beer for sensory evaluation study.

3.2 Study design

Five randomised experimental designs were performed to achieve the broad study objective. The first two experiments were performed specifically to achieve the first specific objective. Of the two, the first experiment was to establish the trend of aflatoxin reduction during the brewing maize-based non-alcoholic beer and the second was to determine the effect of boiling, fermentation and the combination thereof on aflatoxin in maize-based non-alcoholic beer. The third experiment was performed to determine the

distribution of aflatoxin between the liquid and solid phases of maize-based non-alcoholic beer. The forth experiment was performed to assess the impact of ginger and garlic application to maize on aflatoxin level in malt. The fifth (last) experiment was carried out to determine the effect of herbal treatment of maize during malting on the sensory properties of maize-based non-alcoholic beer. The first three experiments were conducted within April and May in 2017 while the last two experiments were conducted in October, 2017.

3.2.1 Determining the trend of aflatoxin reduction during brewing (Experiment # 1)

Two sets of experienced brewers (women) were engaged to prepare the sweet beers at two different sites (home of one of the women). During preliminary trials, the brewers were provided with unlimited supplies of raw materials (water, ground maize and ground malt) and left to freely (without interference) perform the brewing based on their expertise. However, a researcher and the two research assistants involved in the study recorded the time taken or intervals for various steps and the quantities of raw material used (initial – final). Informed by these preliminary trials, a standardized brewing protocol (time and quantities) was set and subsequently utilized by the two sets of brewers under researchers' supervision. The two sets (brewer set 1 and brewer set 2) were engaged to prepare six beer samples (each brewer prepared 3 beer samples) using contaminated raw materials with different aflatoxin concentration levels (Appendix 1) the brewers were cautioned not to drink the beer as it contained high aflatoxin levels.

The standardized procedure involved preparing a porridge from 15 litres of water and 4.5 kg of aflatoxin contaminated maize flour in a 20 litres metallic pot, covered with a metallic lid, and boiling the mixture on an open wood fire for 80 minutes. The porridge was then cooled to about 45°C (estimated) prior to addition of 0.5 kg ground malt with vigorous stirring. A sample was then drawn and immediately analysed for aflatoxin concentration as described in section 3.3. Subsequently, samples were drawn at all critical stages of beer preparation and during storage one after the other and aflatoxin concentration determined as outlined in Figure 3. The beer samples after preparation were stored in plastic buckets at room temperature in an iron sheet roofed house for 8 days.

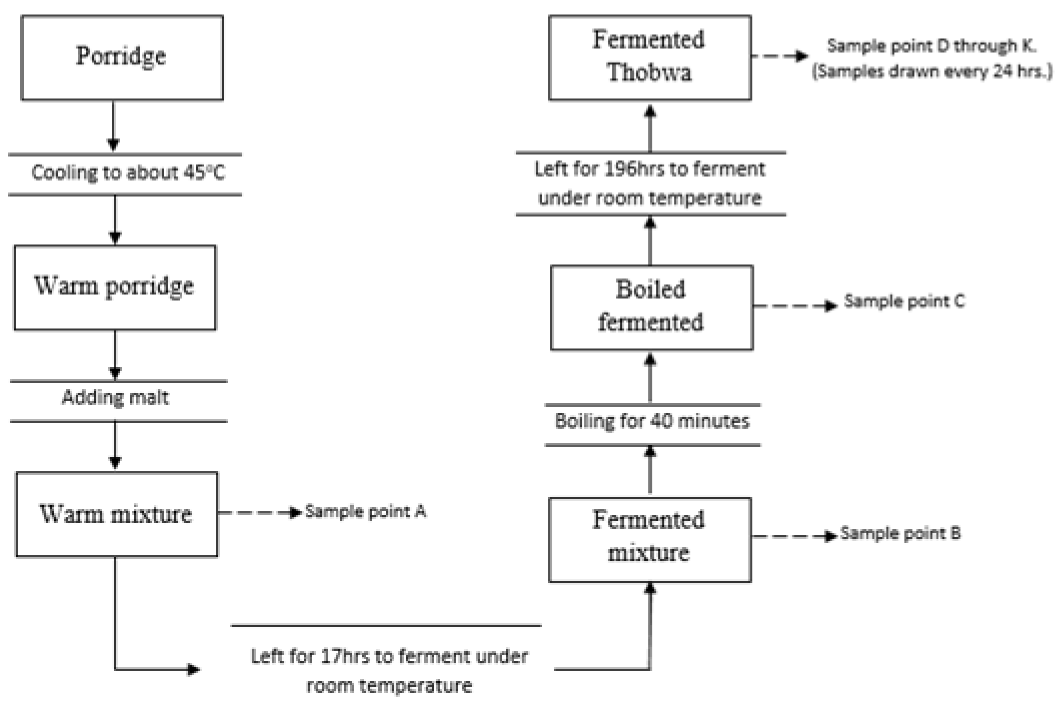

Figure 3: Scheme of sweet beer brewing (Experiment # 1)

Finally, aflatoxin levels at each stage were computed and expressed as percentage of the original mixture of the porridge and malt using the formula:

$$\frac{Aflatoxin\ content\ at\ a\ given\ stage}{Aflatoxin\ content\ soon\ after\ mixing\ porridge\ and\ malt\ (stage\ A)} \times 100\%$$

3.2.2 Determining the effect of boiling and fermentation on aflatoxin in sweet beer (Experiment # 2)

The second experiment (2^2 factorial design) which was aimed at determining the effect of boiling and fermentation processes in isolation as well as their interaction on aflatoxins in maize based sweet beer was superimposed onto the Experiment #1 at one brewer site (beer samples from brewer set 1). The experiment involved two variables (boiling and fermentation) at two levels coded 'no' (absent) or 'yes' (present). The level of aflatoxin detected in the three mixtures at sampling point A (first output from the design) was used to determine the original concentration where both processes were absent. The proportion of aflatoxin remaining in mixtures at sampling point B (second output) was used to determine the effect of fermentation only and the proportion of aflatoxin remaining in beers at sampling points C (third output) was used to determine the effect of combination thereof (all displayed in Figure 3). To realize a full factorial design, a boiling step, which is not practiced in normal brewing process, was introduced immediately after mixing porridge and ground malt (Sampling point A; Figure 3) to obtain sampling point herein referred to as point X (Table 1) for the elucidation of the singular effect of boiling beer. Thus, the proportion of aflatoxin remaining at sampling points A, B, C and X were statistically analysed and were computed and expressed as percentage of the original concentration of the mixture at point A using the formula:

$$\frac{Aflatoxin\ content\ at\ a\ given\ point}{Aflatoxin\ content\ soon\ after\ mixing\ porridge\ and\ malt\ (stage\ A)} \times 100\%$$

Table 1: Factorial design matrix for the boiling and fermentation of beer

Boiling		Fermentation: No	Fermentation: Yes
	No	No Fermentation No boiling (Sampling point A in Figure 3)	Fermentation No boiling (Sampling point B in Figure 3)
	Yes	No Fermentation Boiling (Sampling point X, a non-traditional step)	Fermentation and Boiling (Sampling point C in Figure 3)

3.2.3 Determining the distribution of aflatoxin in sweet beer (Experiment #3)

The Experiment #3 was superimposed onto Experiment 1 with the aim of determining the distribution of aflatoxin between the liquid and solid phases of maize-based non-alcoholic beer. In this regard, sub-samples of mixtures/sweet beers at sampling points A through to stage E (Figure 3) were taken into the laboratory and filtered using VICAM fluted filter paper # 1289 (Whatman 2V, Whatman Middlex) to obtain the liquid phase (clear liquid without spent grains). The filtrates were then analysed for aflatoxin content as described in section 3.3. The aflatoxin levels of the filtrate were computed and expressed as proportion of whole mixture/sweet beer at each sampling point to determine the proportion of aflatoxin in liquid phase at each point using the formula:

$$\frac{Aflatoxin\ content\ in\ the\ filtrate\ at\ a\ given\ sampling\ point}{Aflatoxin\ content\ in\ the\ whole\ of\ mixtures/beer\ \ at\ a\ given\ sampling\ point} \times 100\%$$

The aflatoxin levels of the filtrate were also computed and expressed as proportion of the whole mixture at point A (mixture of porridge and ground malt) to determine the effect of the processing on the proportion of aflatoxin in liquid phase using the formula:

$$\frac{Aflatoxin\ content\ in\ the\ filtrate\ at\ a\ given\ sampling\ point}{Aflatoxin\ content\ in\ the\ whole\ mixture\ at\ point\ A} \times 100\%$$

3.2.4 Assessing the impact of garlic and ginger application to maize on aflatoxin level in malt (Experiment #4)

The forth experiment was carried out based on assumption that garlic and ginger have ability to inhibit development of aflatoxin. The experiment was carried out at a household around Lilongwe University of Agriculture and Natural Resources, Natural Resources College (LUANAR-NRC) in Lilongwe district. In this experiment, clean white maize (20

kg) sourced from a household in Msundwe area within Lilongwe district was winnowed and carefully hand sorted to remove broken and mouldy grains. The clean maize lot was then apportioned into 12 equal portions (1.5 kg each) using a riffle-divider (Humboldt Testing Equipment, Norridge, IL). The portions were laid in an experiment involving three treatments with 4 replications namely: (1) garlic treated maize, (2) ginger treated maize and (3) control (with no herbal addition). Each portion was steeped in ground water (3*l*) for 48 hours. For herbal treated grains, 2 g of already prepared powdered garlic or ginger purchased from Lizulu market in Lilongwe district, were added to water in which maize grains were steeped and thoroughly stirred. After draining out the steeping water, each sample was separately tightly tied in hessian sack and left to germinate for five days in a warm storeroom with a window fitted with a transparent plastic sheet to maintain warm temperature. Water was sprinkled over the bags every morning and afternoon to maintain optimal condition for mould development. Malted samples were then separately sun dried for three days and ground into flour using Romer Labs Series II ® mill and subsamples analysed for aflatoxin content as described in section 3.3. The level of the aflatoxin contamination developed on herbal treated samples and the control were computed and expressed as percentages of the aflatoxins developed on untreated samples using the formula:

$$\frac{\textbf{Aflatoxin content on herbal treated malt samples}}{\textbf{Aflatoxin content on untreated malt sample}} \times \mathbf{100\%}$$

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3.2.5 Determining the effect of herbal treatment of maize during malting on the sensory properties of maize-based non-alcoholic beer.

The fifth experiment involved sensory evaluation of three sweet beer samples prepared from the three malt types as described under Experiment #4 to determine whether participants could detect a difference between herbal treated beers and non-treated ones. Conversely, the evaluation aimed at ascertaining whether the herbs altered the sensory properties of the beers. A total of 45 volunteer villagers (21-49 years) performed a tetrad discrimination test in which four samples were presented in random order; two samples of one set and two samples of another set. i.e. the first evaluation involved 2 untreated non-alcoholic beer samples and 2 garlic treated non-alcoholic beer samples followed by the second evaluation which involved tasting of the other 2 untreated non-alcoholic beer samples and 2 ginger non-alcoholic beer treated samples. The samples were presented in approx. 20 g aliquots in approx. 60 mL clear plastic cups and each sample was coded with a randomized four-digit number. Each of the panelists was asked to taste in order to perceive similarities based on taste and flavor and then group the four samples in two groups of two samples, based on similarity. Each panelist carried out both evaluations one after other. The panelists were asked to fill in their observations on the score card as given in appendix 4. The number of panelists that were able to correctly distinguish one set from another in each of evaluation was determined. It is noteworthy that none knew the predictions being tested and the participants had fasted, except for water, for at least one hour prior to the first evaluation and in-between evaluations.

3.3 Total aflatoxins determination

Extraction and clean-up of aflatoxin from sample test portions was performed using a modified version of the manufacturer's instruction for the Aflatest® immuno-affinity procedures for popcorn (VICAM, 2014) at LUANAR - NRC in food analysis laboratory and Chitedze Research Station. Samples of maize-based non-alcoholic beer drawn at different stages at brewery site were immediately cooled to freeze and taken to the laboratory for analysis. Aflatoxin was extracted from the maize-based non-alcoholic beer samples and filtrates test portions (50 g test portion + 5 g NaCl) with 200 mL of HPLC grade methanol (100%) and blended at high speed for 2 minutes. Similarly, extraction of aflatoxin from 50g flour samples test portions involved 200 mL of HPLC grade methanol/water (160+40 v/v) blend at high speed for 2 minutes. The triturated mixture was filtered through Sartorius grade 1289 fluted filter paper to remove particulate matter. A 10 mL volume of the filtered extract was diluted with 40 mL distilled water, mixed and filtered through a glass-fibre filter papers of 1.5 µm diameter. A 20 mL (1 g sample equivalent) of the diluted extract was passed through Aflatest® immuno-affinity column at a flow rate of about 1-2 drops/second. The column was washed twice with (10 mL) distilled water at a rate of (2 drops/second) to remove maize intrinsic compounds and finally the aflatoxins were selectively eluted with 1 mL of 100% HPLC grade methanol into a glass cuvette. One mL of Aflatest® developer (developer/ water of 1+9, v/v) was added to elute in the cuvette and mixed using vortex before reading the sample in a calibrated VICAM Series-4EX Fluorometer

3.4 Statistical analysis

Microsoft EXEL (Microsoft Corperation, Redmond, WA) and SPSS version 21 (IBM Corp, Armonk, New York) were used for data analysis and data presentation. Summary results are reported as means± SD unless otherwise indicated. The differences among means were analyzed by one-way ANOVA (Experiments #1, #2, #3 and #4). Results showing significant differences were subjected to post-hoc Tukey's test. The GLM univariate analysis was used to study the effect of the boiling, fermentation and their interaction on aflatoxin degradation (Experiment #2). Discrimination testing (tetrad tests) were analyzed using binomial statistics (chance probability= 1/3) (Experiment #5). The level of confidence required for significance was set at $p \leq 0.05$ for all statistical analyses.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Reduction of aflatoxin levels during brewing

The six sweet beer samples had different original concentrations ranging from 45 to 183 µg/kg due to different concentrations of malt and maize samples as displayed in table 2**.**

Table 2: Aflatoxin concentrations of raw materials and original concentrations in beer samples

Beer Sample	Maize malt used (µg/Kg)	Maize flour used (µg/Kg)	Original concentration in the beer mixture (µg/Kg)	Brewer
1	156	735	127	1
2	312	985	183	1
3	63	365	45	2
4	97	225	85	1
5	140	620	123	2
6	265	780	135	2

The reduction of aflatoxins in all the six sweet beers samples is graphically presented in Figure 4. All beer samples showed the same trend in terms of reduction, only a mean of 77 ± 9% of the aflatoxins were detected in the beers after 17 hours of fermentation (Stage B). Boiling the 17-hour fermented mixtures for 40 minutes reduced the aflatoxins to a mean of 46 ± 6% of the original levels (Stage C). Testing the finished product (beers) after 24 hours of storage showed further reduction of aflatoxins and only 36 ± 5% of the very original aflatoxin concentrations (16 - 66µg/kg) were detected in the beers (Stage D). Subsequently, the aflatoxin concentration in all sweet beer samples remained constant at average of 36 ± 5% (16 - 66µg/kg) for eight days of evaluation (stage D-K).

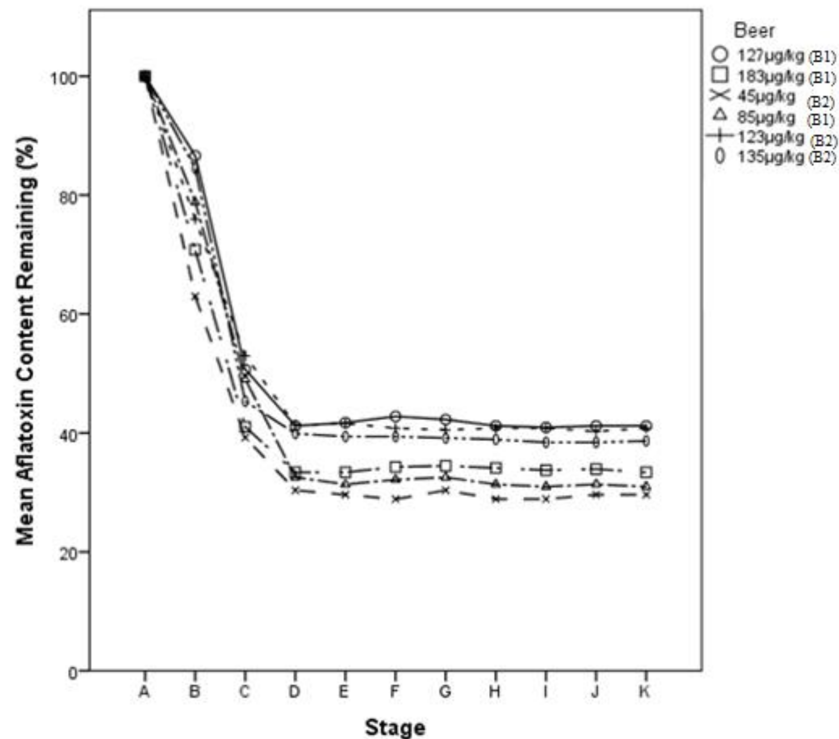

Figure 4: Fate of aflatoxin during brewing and storage of sweet beer

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'A' represents, warm mixture of porridge and malt on the onset of the brewing process; 'B" is 17-hour fermented mixture before boiling; 'C' is 17-hours fermented mixture (beer) after boiling for 40 minutes, 'D' through to 'K' are fermented beers one to eight days after boiling, respectively. 'B1' represents beer samples from brewers set 1 and 'B2' represents beer samples from brewers set 2.

4.1.2 Effect of boiling and fermentation on aflatoxin

The results on effect of boiling, fermentation and combination thereof on three beer samples from brewer 1 are displayed in the Figure 5. Boiling the beer mixtures prior to fermentation (not a normal procedure, only performed in this experiment to elucidate singular effect of boiling) caused a mean reduction of 33.4±0.5% in aflatoxin levels. The actual concentrations of the aflatoxins in beer samples 1, 2 and 3 after boiling process only were 84.7±1.15µg/kg, 121.3±2.08µg/kg and 57±1.73µg/kg respectively. Fermenting non-boiled mixtures (normal procedure) caused a mean reduction of 20.6±8.5% in aflatoxins (110.3±1.5µg/kg, 130.3±2.8µg/kg and 76±3µg/kg in beer samples 1, 2 and 3 respectively). The combination of the two processes in total caused a mean reduction of 53.1±5.2% in aflatoxins. The actual concentrations of the aflatoxins in beer samples as a result of the two processes are 64.3±2.3µg/kg, 75.3±2.3µg/kg and 41.7±1.15µg/kg for beer samples 1, 2 and 3 respectively. Statistically, the effects of these three (boiling, fermentation and combination thereof) are significantly different.

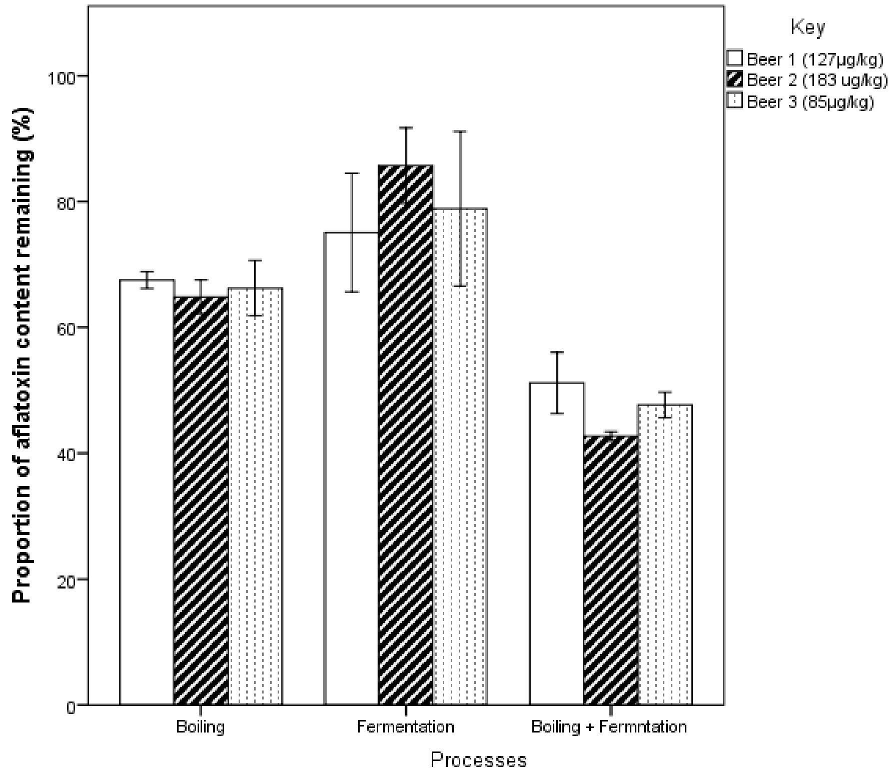

Figure 5: The effect of boiling, fermentation and combination thereof on aflatoxin content in three beer samples

The general linear model (GLM) univariate analysis of the results displayed in Figure 5 clearly demonstrated that boiling and fermenting the beer mixtures as processes in isolation have significant effect at $P < 0.05$ (Table 3). However, the combination of the two processes (interaction) had additive effects on the reduction of aflatoxins as there was no noticeable significant surplus effect.

Table 3: Effect of fermentation and boiling on percentage aflatoxin content during in the sweet beer during brewing process

Predictor	Unstandardized Coefficients		Standardized Coefficients	T	Significant
	B	Std. Error	Beta		
(Constant)	97.333	3.399		28.633	.000
Temperature	-30.667	2.776	-1.123	-11.049	.000
Fermentation	-17.667	2.776	-.647	-6.365	.001

4.1.3 The proportion of aflatoxins present in liquid phase of the beers

The proportions of aflatoxin content detected in liquid phase (filtrate) of the beers with respect to the whole beer mixtures during five stages of brewing are plotted in Figure 6. The proportion of aflatoxin in the filtrate remained statistically ($P < 0.05$) constant (10.4 - 12.2%) throughout the brewing process. However, if considered with respect to the original concentration of aflatoxins in the whole mixture (porridge plus malt) measured at the onset of the experiment (Stage A), the proportions of aflatoxin content in liquid phase had declined following 17-hour fermentation process (Stage B) and the boiling of the fermented mixtures (stage C).

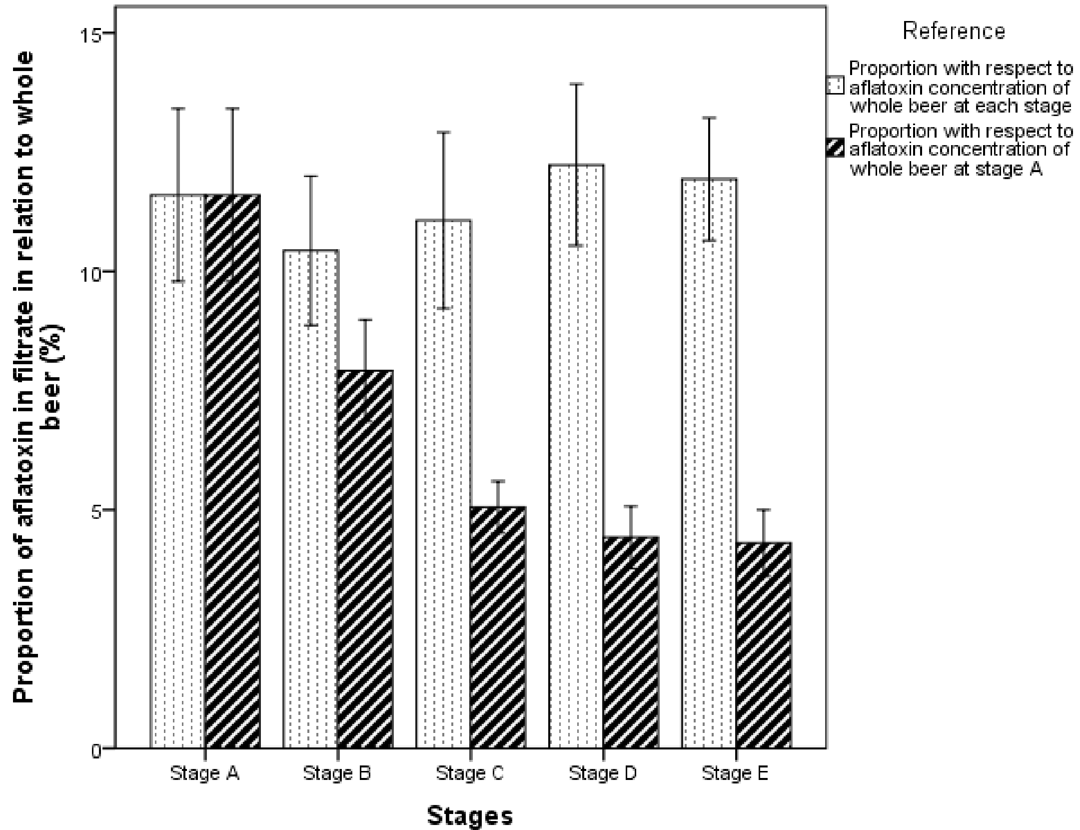

Figure 6: Fate of aflatoxin content in filtrate during brewing and storage of the sweet beer.

‘A’ represents, warm mixture of porridge and malt on the onset of the brewing process; ‘B” is 17-hour fermented mixture before boiling; ‘C’ is 17-hour fermented mixture (beer) after boiling for 40 minutes, ‘D’ and ‘E’ are fermented beers on first and second day after boiling respectively.

4.1.4 The efficacy of garlic and ginger in controlling aflatoxin contamination during malting

The effectiveness of controlling aflatoxin contamination in the malts by singular additions of garlic and ginger to maize is depicted in Table 4. Both garlic and ginger, significantly

($P < 0.05$) controlled aflatoxin contamination of the malts with a similar power (a factor of ~15).

Table 4: Effect of the garlic and ginger treatment on aflatoxin development and growth in malt

Sample Treatment	Total Aflatoxins detected	
	Concentration (µg/kg) Mean ± SD	Percentages (%)
Untreated	87.83 ± 19.45^{a}	100
Garlic	6.77 ± 2.93^{b}	7.7
Ginger	5.95 ± 2.79^{b}	6.8

4.1.5 Tetrad sensory evaluation test

The results of tetrad discrimination tests for beer samples that were prepared from malt treated with and without herbs are presented in Table 4. Only 36% and 40% of the panelists noticed a difference between the beer samples that were prepared using untreated malts and those from garlic-treated or ginger-treated malts, respectively. Considering that the probability of correctly discriminating between 2 samples by chance in a tetrad test is 33%, binomial proportion tests showed that both proportions (36% and 40%) were statistically insignificant ($p > 0.05$). Therefore, there were no noticeable differences between samples prepared using treated and untreated malts.

Table 5: Ability to perceive differences of beer made from herbal treated malts from those that were not

Herb	Total number of panelists	Number of panelists who correctly differentiated the beers	% panelists who correctly differentiated the beers	P-value
Garlic	45	18	40	0.199
Ginger	45	16	36	0.412

4.2 Discussion

Aflatoxins are potent hepatotoxic and carcinogenic mycotoxin, ubiquitous in maize grains and maize malts in Malawi (Matumba et al., 2013, Kenji et al., 2000). Therefore, strategies aimed at controlling its (aflatoxin) synthesis during malting; and the deep understanding of its (aflatoxin) degradation or stability during brewing of the maize-based non-alcohol opaque beer are absolutely necessary for the development of feasible approaches for minimizing consumer risks. The maize-based non-alcohol opaque beer is a popular beverage in Malawi which is consumed in large quantities by all gender categories including infants, therefore the aflatoxin presence may constitute a significant health risk factor.

The present study has for the first time broadly examined the effects of fermentation and boiling on aflatoxin levels during brewing of Malawian maize-based non-alcohol opaque beer. The results indicate that both fermentation and boiling of beer mixtures during brewing process result in significant aflatoxin reduction with the latter (boiling) having the greater effect. Although this study did not investigate the mechanism of the aflatoxin reduction, it can be postulated based on the findings from other studies that fermentation

led to irreversible microbial binding/conjugation (Hamad et al., 2017) and/or enzymatic transformations of the aflatoxins thereby making them escape analysis of its parent forms (Adebo et al., 2017; Wang et al., 2018). Likewise, it can be prostrated from existing literature that boiling may have also resulted to hydrolytic opening of the lactone ring in the aflatoxin followed by heat-induced decarboxylation, leading to the loss of the methoxy group from the aromatic ring (Nicolás-Vázquez et al., 2010; Saalia & Philips, 2011).

There are concerns over safety of thermal or chemical degradation products of mycotoxins. In some instances, thermal treatments have been reported to yield products that are as toxic as their parent mycotoxins or even worse (Dombrink-Kurtzman et al., 2000; Voss at al., 2010) or reversible under simulated gastrointestinal tract conditions e.g. the case of aflatoxin and nixtamalization of maize (Méndez-Albores et al., 2004). However, in case of aflatoxins, several toxicological studies using cytotoxicity assays on mammalian cell lines or live animal subjects have proven that aqueous boiling and fermentation produce aflatoxin degradation products with far reduced toxicity and carcinogenicity (Aiko et al., 2015; Liu et al 2017). For instance, Aflatoxin B2a degradation product of bacterial conversion of Aflatoxin B1 has shown to exhibit lower DNA-binding capacity than Aflatoxin B1. Therefore, the reduction of the aflatoxin levels observed in the present study suggests that the beers had comparatively lower toxicity than their original mixtures. However, it is noteworthy that in the present experiment the beers were not completely safe for human consumption since significant proportions (36-46%) of the parent aflatoxin compound were still detected in the ready to drink product and the concentrations were above both the median for total of aflatoxins limits used worldwide (10 µg/kg; FAO 2004)

and total aflatoxin limit for human consumption (20 µg/kg) enforced by the United States Food and Drug Administration. Thus, while brewing can significantly improve the mycotoxin safety of the beers, it is necessary to ensure that the raw materials (maize and malts) contain as low as reasonably achievable mycotoxins with proper handling during storage and processing to guarantee consumer safety particularly in infants and children.

The stability of the aflatoxins during brewing witnessed in the present study is of particularly importance considering the common practice by Malawian farmers who separate shriveled and mouldy grains (with high aflatoxin levels) from health proportion and spare them for brewing of non- and alcoholic beers consumed during tribal rituals commemorating of the dead. This practice is likely to result in high aflatoxin dietary exposure from beers in high aflatoxin contamination years as already demonstrated by an earlier study (Matumba et al., 2014).

Under the present investigation, fermentation proved to be slow and less efficient. Seventeen hours of natural fermentation of the beer only degraded 20% the toxins. Contrarily, in recent study, addition of bacteria starter to a culture medium resulted in about 70% aflatoxin degradation within 24 hours (Shu et al., 2018). Strikingly, more than 90% aflatoxin degradation was achieved following probiotic enrichment of kwete (a commonly consumed traditional fermented cereal beverage in Uganda) within 12 hours of fermentation and, a complete degradation of the aflatoxins was attained within 24 hours of fermentation (Wacoo et al., 2019). However, in Malawi, preparation of non-alcoholic maize based beer does not traditionally involve addition of fermentation starters although

it has increasingly become popular practice among alcoholic brewers in recent years (Mrs Nkumba, personal communication). Fermentation starters in this case compete or inhibit the toxigenic molds development (Wolf-Hall & Schwarz, 2002; Pascari et al., 2018). Given the many nutritional benefits associated with probiotics (Nagpal et al., 2012) and the aflatoxin degradation capability of some strains, promoting a culture of probiotic enrichment of the non-alcoholic maize based beer seems like a wiser strategy.

Aflatoxin degradation ceased after boiling at both brewer sites involved in the study which clearly indicates possibility of the phenomena. Unfortunately, this study did not investigate the reason for this observation and leaves the researchers speculating that boiling might have eliminated the aflatoxin decomposing microbes and that the acidic environment might have hindered subsequent re-colonization of the aflatoxin detoxifiers or hampered enzyme activity (Teoh et al., 2004).

The novelity of this study dwells in its ability to allow an investigation of the singular effect of boiling beer on aflatoxin through the use of full 2^2 factorial design and GLM univariate analysis. A full 2^2 factorial design has four outputs and in this study, outputs were given by the two processes (boiling and fermentation) at two levels (absent and present) and GLM univariate analysis was used to elucidate the effect of each process on aflatoxin reduction. Under normal practice, after mixing porridge and ground malt, the mixture is left overnight to ferment. However, in the present study, the introduction of a non-traditional boiling step after mixing porridge and ground malt allowed the elucidation of the singular effect of boiling the beer. The appreciable (33%) degradation of aflatoxin

arising from the single effect of boiling witnessed in the present study which is higher than degradation arising from fermentation alone (20%) is of scholarly significance. Since its discovery in the 1960, aflatoxins have been regarded to be thermally stable (World Health Organization, 1998), consequently not much work has been performed to investigate conditions that may enhance its efficacy. While aflatoxins are indeed quite stable to dry heating at temperatures below its thermal decomposition temperature of 267°C (Kabak, 2009), aflatoxins seem to be unstable under moist heat and the present findings reaffirms such phenomenon (Asghar, 2011). Owing to the potential loss of toxicity accompanying aqueous thermal degradation of the aflatoxin, it is worth understanding and optimizing the thermal degradation conditions.

The study has demonstrated the unequal distribution of aflatoxin between liquid and solid phases with liquid phase containing less aflatoxin. The comparatively lower levels detected in filtrate of the beers partly explains the disparity between aflatoxin levels recorded in opaque beers of Africa and clear beers brewed in Europe (Lulamba et al., 2019). While the absence of aflatoxins in beers made in Europe could be primarily due the low aflatoxin levels in the raw materials used, the processing effect cannot be ruled out. In clear European beer brewing process, lautering, is employed to separate the clear liquid wort from residual grains which is similar to filtration process employed in this study to obtain the liquid phase. In the current study, about 89% of the toxic remains in the solid phase and only small proportion (on average 11%) migrates into the liquid phase. Aflatoxins are poorly soluble in water (IARC, 2002) consequently during the lautering or filtration aflatoxins predominately remains in the spent grains (Rodrigues & Chin, 2012), thus making the clear

beers comparatively safe from aflatoxin contamination than opaque beers. Therefore, optimizing the production of maize based clear beverages could offer the best option though it is not 100% safer.

The aflatoxin degradation trend obtained in filtrates showed the similar effect as demonstrated by the whole beer mixture in this study. Both boiling and fermentation processes resulted into significant reduction in aflatoxin. This observation is similar to what Chu et al. (1975) observed while studying the stability of aflatoxin in the brewing process of clear beers. The pattern of degradation of aflatoxin they found is similar to the pattern observed in the current study.

The inhibition power of ginger and garlic powders on aflatoxin development on maize during malting process observed in the current study differs from most study findings where aqueous garlic has been reported to be more effective in preventing aflatoxin development than aqueous ginger (Latif et al., 2006; Abd EL – Aziz et al., 2012). The form of the herb (powder) used in this study might be responsible for such observation. The current study did not investigate the aflatoxin inhibition mechanism by the herbs but it can be hypothesized based on the findings from other studies on efficacy of the plant extracts on aflatoxins. Previous studies have proven that the antifungal properties of garlic is due to its extracts such as allicin which reacts with thiol groups of various enzymes there by affecting enzymatic biosynthesis of aflatoxin, and ajoene which varies the morphological properties of the fungal cells by disrupting the plasma membrane and mitochondrial functions (Harris et al., 2001; Prabahar et al., 2011). Three extracts from ginger (c-

terpinene, isoborneol, and citral) have been reported to inhibit aflatoxin production and growth by either modifying or interfering with the expression of aflatoxin biosynthesis genes (Liang et al., 2015; Moon et al., 2018)

The efficacy of herbs in deterring aflatoxin synthesis exhibited in present study offer a lot of promise as means of ensuring safety of opaque beers. The failure of the panelists to detect the presence/application of garlic and ginger in the beer (though garlic and ginger flavors were detectable before being added to the steeping water) indicate that flavor compounds in the herbs had disintegrated during malting and brewing. This is an important finding not widely documented in the literature which offers a practical remedy for aflatoxin contamination during malting (Gorjanovic et al., 2010; De Roos et al., 2019). Both ginger and garlic are grown across Malawi and many African countries and therefore could be easily accessed by craft brewers. However, there is need for further research to optimize the concentration of the herbs in the steeped maize/malt before the technology is rolled out. Studies have demonstrated that the sensory properties are affected by the concentration or the amount of the herbal used (Simon et al., 2018). In the current study only one dose (2g) of garlic and ginger were used during malting and there is a need to investigate the effect of varying the herbal dose on both inhibition efficiency of aflatoxins and sensory characteristics of the beer.

The study had several limitations. The study was modeled based on a recipe mainly used in the central region of Malawi which differs from other regions with the country. Even within the central region, there are likely to be some variations particularly in terms of

cooking time, cooking temperatures, water-matrix ratio, fermentation periods and temperatures and storage facilities. In the present study, all these parameters were held constant except for the cooking temperatures which were out of control of the researchers. Moreover, it is likely that the quality and a quantity of microbiota differs from one environment to the other and yet the present study was limited to one location (Lilongwe) and one season. The study also used small sample size and few replications. All these are likely to affect aflatoxin degradations outcomes. The study randomly recruited panelists in a sensory discrimination test without considering the ability to differentiate small differences in taste. Nonetheless, being the first study of its kind, the current results provide useful insights that would inform the design of future comprehensive studies on fate of aflatoxin during brewing Malawian maize-based opaque beer and management strategies aflatoxin during malting.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The present study has shown that boiling and fermentation reduces aflatoxin with boiling superior to fermentation. It has also proven that filtration reduces aflatoxin. Therefore, if fermentation, boiling and filtration are done in that order, they may results into the best reduction rate of aflatoxins. The study has also shown that a substantial amount of aflatoxin would still remain in the beer after the brewing process and storage. This, therefore, calls for the need to use aflatoxin contamination free maize and malt for brewing. The study has also demonstrated the effectiveness of using ginger and garlic during malting to inhibit aflatoxin development in malt without altering the sensory characteristics of the final beer product. This offers the best alternative way to control development of aflatoxin during malting conditions.

5.2 Future perspectives

The study has provided many insights, however, it could be interesting to extend it in the following directions:

- Further studies on the fate of aflatoxin during brewing Malawian maize-based opaque beer in different parts of Malawi.
- Explore the effect of other forms of garlic and ginger, besides powder, on aflatoxin inhibition during malting and brewing Malawian maize-based opaque beer.

- Identify and quantify aflatoxin thermal degradation or matrix bound products that arise from the non-alcoholic beer preparation
- Assess the toxicological safety aflatoxin thermal degradation or matrix bound products that formed during the beer preparation.
- Explore and optimize other factors (beside temperature and time) that affect the aflatoxin degradation or matrix binding during the beer preparation

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APPENDICES

APPENDIX 1: Contamination levels of raw materials

Sample Code	Maize malt used (µg/Kg)	Maize flour used (µg/Kg)	Brewer
A	156	735	1
B	312	985	1
C	63	365	2
D	97	225	1
E	140	620	2
F	265	780	2
G	21		
H	6		
I	42		
J	11		
K	26		

APPENDIX 2:Quantities of ingredients for Thobwa preparation

Woman	Amount of maize flour (gm)	Amount of water	Cooking time (min)	Malt Sample	Amount of malt (gm)	Volume of porridge (approximately)	Actual Boiling time (min)
Brewer 1	4472.4	15L	66	1	491.8	5.5L	40
	4436.8	15L	76	2	500.6	5.5L	39
	4545.7	15L	82	3	512.3	5.5L	40
Average	**4484.9**	**15L**	**74.7**		**501.57**	**5.5L**	**39.7**
Brewer 2	4512.9	15L	72	4	525.8	5.8L	40
	4431	15L	75	5	481.1	5.8L	40
	4543.2	15L	83	6	513.4	5.8L	40
Average	**4495.7**	**15L**	**76.7**		**507.77**	**5.8L**	**40**
Overall Average	**4488.8**	**15L**	**75.7**		**504.67**	**5.65**	**39.8**

APPENDIX 3: Aflatoxin unit conversion

	Unit 1	Unit 2
1	1µg/Kg	1ng/g
2	1µg/Kg	1ppb
3	1µg/Kg	0.001ppm
4	1µg/L	0.001ppm
5	1µg/L	1µg/Kg
6	1ppm	1000µg/Kg

APPENDIX 4:Sensory evaluation data collection tools

UNIVERSITY OF MALAWI

Chancellor College

INTRODUCTION

This is sensorial activity on maize based beverage (Thobwa) as part of my project research activities. Your participation and assistance will be highly appreciated.

Number of a Panelist: ________________ Date: ______________

Instructions:

1. You are provided with two kinds thobwa each divided into two to give four samples labeled **9184, 8914, 4819** and **1498**
2. Taste the sample and group them into two groups based their similarities using the four digit labels. Record your answer by ticking the following table
3. After each taste rinse your mouth with clean water provided

	Group A 2 Similar	Group B 2 Similar
9184		
8914		
4819		
1498		

ANY COMMENT:

___

___

THANK YOU FOR YOUR PARTICIPATION

UNIVERSITY OF MALAWI

Chancellor College

INTRODUCTION

This is sensorial activity on maize based beverage (Thobwa) as part of my project research activities. Your participation and assistance will be highly appreciated.

Number of a Panelist: ________________ Date: _____________

Instructions:

1. You are provided with two kinds thobwa each divided into two to give four samples labeled **6521, 5612, 2561** and **1257**
2. Taste the sample and group them into two groups based their similarities using the four digit labels. Record your answer by ticking the following table
3. After each taste rinse your mouth with clean water provided

	Group A 2 Similar	Group B 2 Similar
6521		
5612		
2561		
1257		

ANY COMMENT:

____

____

THANK YOU FOR YOUR PARTICIPATION