FACULTY OF PHARMACEUTICAL SCIENCES

MULTI-MYCOTOXIN ASSESSMENT IN TANZANIAN MAIZE FLOUR USING LC-MS/MS ANALYSIS

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Promotor: Prof. dr. Marthe De Boevre

A Master dissertation for the study program of Master in Drug Development

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SUMMARY

Introduction: Maize is the most widely cultivated crop in Tanzania and consequently serves as a staple food for a large portion of the population. It plays a crucial role in the country's food security and economic stability. However, maize is highly susceptible to fungal infections such as *Aspergillus* and *Fusarium*, which are responsible for the production of various mycotoxins. Contamination with these mycotoxins can cause severe health problems, ranging from acute to chronic effects. Additionally, it can lead to crop failures, resulting in economic losses.

Objectives: The objectives of this study were threefold. First, to simultaneously determine the levels of multiple mycotoxins in maize flour samples from three agro-ecological zones in Eastern Tanzania. Second, to identify variations in the prevalence of these mycotoxins between regions and to understand the correlation with climatic conditions. Third, to investigate the difference in mycotoxin concentrations between undehulled and dehulled maize flour to assess the impact of dehulling.

Methods: For this study, 90 maize flour samples were collected from three cities in Eastern Tanzania, with 45 samples being undehulled and 45 samples dehulled. From each city, 30 samples were collected from local mills, shops, and markets. Subsequently, in the laboratory of Ghent, the quantitative analysis ANAL-18 was conducted, where the maize flour samples were analysed for the presence of 23 different mycotoxins using Liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The highest concentrations of mycotoxins were found for fumonisins, followed by aflatoxins and diacetoxyscirpenol (DAS). Significant geographical variations were observed for fumonisin B1 (FB1), zearalenone (ZEN) and DAS. Overall, Morogoro exhibited the highest prevalence and contamination level of mycotoxins, possibly due to the city's more humid climate. Additionally, significant differences were observed between undehulled and dehulled maize flour for fumonisins and deoxynivalenol (DON), with considerably more contamination found in the undehulled maize flour samples.

Conclusion: The results of this thesis indicate widespread contamination of maize flour with multiple mycotoxins in Eastern Tanzania, with significant variations depending on the region and maize processing level. This underscores the need for stricter control measures and more effective prevention strategies to improve food safety and protect public health.

SAMENVATTING

Inleiding: Maïs is het meest geteelde gewas in Tanzania en vormt daarom een basisvoedsel voor een groot deel van de bevolking. Het speelt een cruciale rol in de voedselzekerheid en economische stabiliteit van het land. Echter, maïs is zeer gevoelig voor aantasting door schimmels zoals *Aspergillus* en *Fusarium*, die verantwoordelijk zijn voor de productie van verschillende mycotoxines. Besmetting met deze mycotoxines kan ernstige gezondheidsproblemen veroorzaken, variërend van acute tot chronische effecten. Verder kan het leiden tot misoogsten, met economische verliezen tot gevolg.

Objectieven: De doelstellingen van deze studie waren drievoudig. Ten eerste, het gelijktijdig bepalen van de niveaus van meerdere mycotoxines in maïsbloemmonsters uit drie agro-ecologische zones in Oost-Tanzania. Ten tweede, het identificeren van geografische variaties in de prevalentie van deze mycotoxines tussen de regio's en het begrijpen van de samenhang met klimatologische omstandigheden. Ten derde, het onderzoeken van het verschil in mycotoxineconcentraties tussen ongepelde en gepelde maïsbloem om de impact van het pellen te beoordelen.

Methoden: Voor deze studie werden 90 maïsbloemmonsters verzameld uit drie steden in Oost-Tanzania, waarbij 45 stalen ongepeld en 45 stalen gepeld waren. Uit elke stad werden 30 monsters verzameld afkomstig van lokale molens, winkels en markten. Vervolgens werd in het laboratorium van Gent de kwantitatieve analyse ANAL-18 uitgevoerd, waarbij de maïsbloemmonsters werden geanalyseerd op de aanwezigheid van 23 verschillende mycotoxines met behulp van LC-MS/MS.

Resultaten: De hoogste concentraties werden gevonden voor de fumonisinen, gevolgd door de aflatoxinen en DAS. Er werden significante geografische variaties waargenomen voor FB1, DAS en ZEN. Over het algemeen vertoonde Morogoro de hoogste prevalentie en contaminatiegraad van mycotoxines, wat mogelijks te wijten is aan het meer vochtige klimaat van de stad. Daarnaast werden er significante verschillen waargenomen tussen ongepelde en gepelde maïsbloem voor fumonisinen en DON, waarbij aanzienlijk meer besmetting werd aangetroffen in de ongepelde maïsbloemmonsters.

Conclusie: De resultaten van deze thesis wijzen op een wijdverspreide besmetting van maïsbloem met meerdere mycotoxinen in Oost-Tanzania, met aanzienlijke variaties afhankelijk van de regio en het verwerkingsniveau van de maïs. Dit onderstreept de noodzaak van strengere controlemaatregelen en effectievere preventiestrategieën om de voedselveiligheid te verbeteren en de volksgezondheid te beschermen.

ACKNOWLEDGMENTS

The realisation of this master's thesis has been a process of trial and error. The past four months have been a unique experience, during which I have acquired both theoretical and practical knowledge that will stay with me throughout my further academic career. I would like to take a moment to express my sincere gratitude to the people who have supported me throughout this journey.

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Without all of you, this would never have been possible.
My sincere thanks go to each of you!

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LIST OF ABBREVIATIONS

15-ADON:	15-acetyl deoxynivalenol
3-ADON:	3-acetyl deoxynivalenol
AFB1:	Aflatoxin B1
AFB2:	Aflatoxin B2
AFG1:	Aflatoxin G1
AFG2:	Aflatoxin G2
AFM1:	Aflatoxin M1
AFM2:	Aflatoxin M2
AFs:	Aflatoxins
AME:	Alternariol methyl ether
AOH:	Alternariol
Ar:	Argon
CID:	Collision Induced Dissociation
D:	Dona
DAS:	Diacetoxyscirpenol
DOM:	Deepoxy-deoxynivalenol
DON:	Deoxynivalenol
EFSA:	European Food Safety Authority
ENN B:	Enniatin B
ESI:	Elektrospray ionisation
EU:	European Union
FAO:	Food and Agriculture Organization of the United Nations
FB1:	Fumonisin B1
FB2:	Fumonisin B2
FB3:	Fumonisin B3
FUMs:	Fumonisins
F-X:	Fusarenone-X
GAP:	Good agricultural practices
GC:	Gas chromatography
GMP:	Good manufacturing practices
HPLC:	High-performance liquid chromatography
HT-2:	HT-2 toxin
IARC:	International Agency for Research on Cancer
LC:	Liquid chromatography
LC-MS/MS:	Liquid chromatography tandem mass spectrometry
LOD:	Limit of Detection
m/z:	Mass-to-charge ratio
MRM:	Multiple Reaction Monitoring
MS/MS:	Tandem mass spectrometry
MS:	Mass spectrometry
N_2:	Nitrogen

NEO:	Neosolaniol
NIV:	Nivalenol
OTA:	Ochratoxin A
PAT:	Patulin
Q1:	Quadrupole 1
Q2:	Quadrupole 2 (collision cell)
Q3:	Quadrupole 3
r:	Correlation coefficient
ROQ-C:	Roquefortin-C
S:	Sembe
SCF:	Scientific Committee on Food
SD:	Standard deviation
SOP:	Standard Operating Procedure
SPE:	Solid-phase extraction
STERIG:	Sterigmatocystin
T-2:	T-2 toxin
TBS	Tanzania Bureau of Standards
TFDA:	Tanzania Food and Drugs Authority
UPLC:	Ultra-high performance liquid chromatography
UV:	Ultraviolet
WHO:	World Health Organisation
ZAN:	Zearalanone
ZEN:	Zearalenone

LIST OF SYMBOLS

%	Percent
°C	Degrees Celsius
µg	Microgram
µg/kg	Microgram per kilogram
µg/mL	Microliter per mililiter
µL	Microliter
µL/s	Microliter per second
µm	Micrometer
eV	Electron Volt
F	Fahrenheit
g	Gram
g	Relative Centrifuge Force
kg	Kilogram
kV	Kilo Volt
L/hr	Liter per hour
mg	Miligram
mg/mL	Miligram per mililiter
min	Minutes
mL	Mililiter
mL/min	Mililiter per minute
mm	Millimeter
ng/µL	Nanogram per microliter
ppb	Parts per billion
rpm	Revolutions per minute
s	Second

PREFACE

According to the World Health Organisation (WHO), approximately 600 million people fall ill every year after eating food contaminated with pathogens, chemicals, toxins and 420,000 of these people die each year (1). Mycotoxin exposure stands out as a significant contributor to this issue, ranging from low to potentially dangerous levels. The presence of mycotoxins in food and feed can lead to significant health issues for both humans and animals, while also carrying economic consequences (2). Aflatoxicosis, in particular, is considered one of the most severe forms of mycotoxin poisoning due to its potentially fatal consequences. In 2016 and 2017, serious outbreaks of aflatoxicosis were reported in the Manyara, Dodoma and Kiteto regions of Tanzania. Both outbreaks were linked to the consumption of mouldy maize (3). In response to such repeated outbreaks, there has been a growing global awareness of mycotoxin contamination in both food and feed, leading to numerous ongoing studies in this field.

The term 'mycotoxin' was first used in 1962 during a veterinary crisis near London. Around 100,000 turkey poults died as a consequence of peanut meal contaminated with aflatoxins produced by *Aspergillus flavus*. As a result of the emergence of this Turkey X disease, scientists became aware of the dangers associated with mycotoxin-producing moulds. Subsequently, investigations extended to other components that could potentially also end up in the mycotoxin section (4).

This thesis investigates the contamination of maize flour samples with mycotoxins, collected from three agro-ecological zones in Tanzania. Together with Alisa Kovaleva, another thesis student, I travelled to Tanzania for four weeks to collect these samples. During our stay, we visited local mills, shops, and markets to obtain the required samples, giving us an in-depth insight into the maize supply chain in Tanzania. The direct contact with local residents gave us a better understanding of the food safety and security challenges in the region. Integrating these insights from Tanzania with the analysis of the samples at Ghent University provides a broader perspective on scientific research worldwide, with a specific focus on mycotoxin research in low- and middle-income countries.

1. INTRODUCTION

1.1. MYCOTOXINS

1.1.1. Characteristics of mycotoxins

Mycotoxins are low-molecular-weight compounds produced naturally as toxic secondary metabolites by species of filamentous fungi (5). Their function is believed to serve fungal defence or competition. Fungi that produce mycotoxins are called mycotoxigenic fungi. A large number of fungal species are mycotoxigenic fungi, but the most important producing genera are *Aspergillus, Penicillium, Fusarium* and *Alternaria* (4,6). These fungi are found throughout the world in a wide variety of environments, with warm and humid climates being particularly preferred (7). In general terms, mycotoxigenic fungi are not aggressive pathogens. However, specific species have the capability to infiltrate and thrive within plant tissues, producing mycotoxins throughout cultivation, harvesting, drying, transportation, processing, and storage stages. Remarkably, most mycotoxins withstand food processing and remain detectable because they are chemically stable (8). To date, more than 400 different mycotoxins have been identified of which approximately 30 receive attention in the contamination of food and feed (9). Mycotoxin-producing moulds can be found in a wide range of food and non-food products of both plant and animal origin including rice, wheat, soybeans, nuts, dried fruits, spices, cereals, meat and eggs (10,11).

1.1.2. Most prevalent mycotoxins

While numerous species of toxigenic moulds exist, only a select few mycotoxins are considered to be important for human and animal health. Literally hundreds of different mycotoxins have been identified, but the most commonly observed mycotoxins for food and feed safety include aflatoxins, fumonisins, ochratoxin A (OTA), patulin (PAT), trichothecenes (DON, T-2 toxin (T-2), HT-2 toxin (HT-2)) and ZEN). These can be considered as the most prevalent mycotoxin (12). In the following paragraphs, aflatoxins and fumonisins will be discussed in more detail.

1.1.2.1. Aflatoxins

<u>Occurrence</u>

Aflatoxins (AFs) are the most widely recognized and extensively studied mycotoxins. They are widely distributed secondary fungal metabolites that present significant health

hazards to both humans and animals (13,14). As mentioned above, aflatoxins were first isolated and characterised after the outbreak of Turkey X disease in the 1960s (4). Aflatoxins are produced by fungi belonging to the genus *Aspergillus*, with *Aspergillus flavus* and *Aspergillus parasiticus* being the main aflatoxin producers. *Aspergillus flavus* mainly colonises the above-ground parts of plants (leaves, flowers), while *Aspergillus parasiticus* prefers soil environments. Several other *Aspergillus* species also produce aflatoxins, including *Aspergillus bombycis, Aspergillus ochraceoroseus, Aspergillus nomius* and *Aspergillus pseudotamari*, but these are less common (15). These *Aspergillus* fungi have the ability to contaminate a wide range of foods before and/or after harvest including nuts, cereals, spices, dried fruits, cocoa beans, and figs. Moreover, they are commonly present in derived and processed products such as peanut butter, bread, beer, and baby food. Given that cereals serve as the foundation of animal feed, aflatoxins or their metabolites have also been detected in meat, milk, and dairy products. They grow mainly in warm and humid environments (14).

Physicochemical characteristics

There are a number of different types of aflatoxins, but the main aflatoxins found in food are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2). Figure 1.1 illustrates the chemical structures of the most widespread aflatoxins, which are typically characterised by a cyclopentane E-ring (B-type and M-type) and a xanthone ring (G-type). AFB1 and AFB2 derive their names from their blue fluorescence under ultraviolet (UV) light, while AFG1 and AFG2 are named for their green fluorescence (16). AFM1 and AFM2 are formed as metabolites of AFB1 and AFB2 and are excreted in the milk of animals exposed to AFB1 and AFB2 (14). Aflatoxins are considered to be polar compounds as they are soluble in polar solvents for instance chloroform and methanol. In addition, they are not stable at extreme pH values (less than 3 or greater than 10) and in the presence of oxidising compounds and UV light (17).

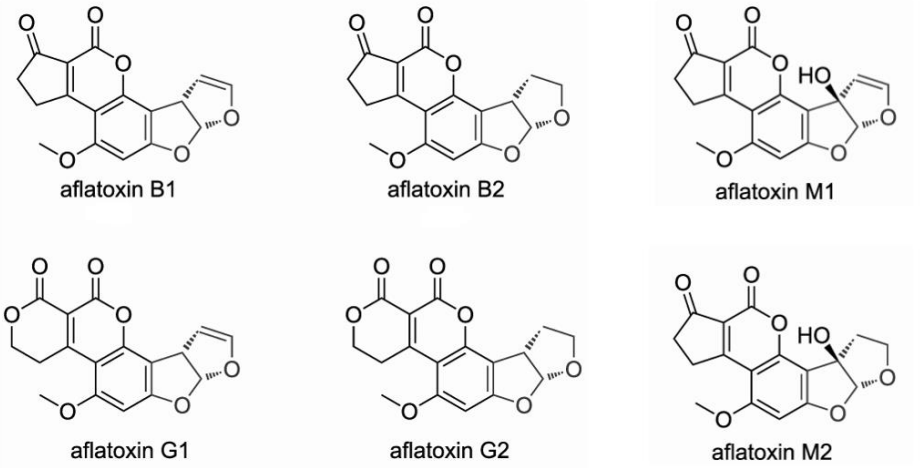

Figure 1.1. Chemical structures of different types of aflatoxins (18)**.**

Toxicology

Aflatoxins can be teratogenic, mutagenic and immunogenic to human and animal health. They pose a serious threat, particularly in Africa, where acute aflatoxicosis has resulted in numerous deaths. More specifically, AFB1 is the most important compound in terms of its occurrence and toxicity to humans and animals. In 2002, the International Agency for Research on Cancer (IARC) classified AFB1 in group 1: substances that are carcinogenic to humans. Several studies have shown that AFB1 is a major risk factor for the development of hepatocellular carcinoma (19). Moreover, aflatoxins can cause other toxic effects: immunosuppression, reduced milk and egg production, reduced reproductive performance, reduced feed utilisation and efficiency, anaemia and retarded growth in children (20–23). Additionally, several diseases such as Reye's syndrome, kwashiorkor and hepatitis are suspected to be linked to the consumption of aflatoxins (24).

1.1.2.2. Fumonisins

Occurrence

Fumonisins (FUMs) are mycotoxins produced by fungi of the genus *Fusarium*. The five most important toxigenic *Fusarium* species are *F. sporotrichioides, F. graminearum, F. verticillioides, F. poae* and *F. equiseti*. Among these, *F. verticillioides* is one of the most pathogenic *Fusarium* species infecting food and is often associated with maize contamination. While maize is a primary source of fumonisins, contamination can also occur in other grains such as wheat, rice, and barley. These fungi are found in temperate and warm climates (25).

Physicochemical characteristics

Multiple fumonisins have been identified, categorized into four groups (A, B, C, and P). However, the predominant fumonisins are fumonisins B1 (FB1), B2 (FB2), and B3 (FB3), which are shown in Figure 1.2. Among these three fumonisin analogues, FB1 is the most toxic and is most commonly found in agricultural crops (26). FUMs are considered to be polar as they are soluble in polar solvents such as methanol and water. The structure of these fumonisins is very similar, with the main differences being the number and position of the hydroxyl groups. The presence of a long chain hydrocarbon unit in the structure correlates with their toxicity (27).

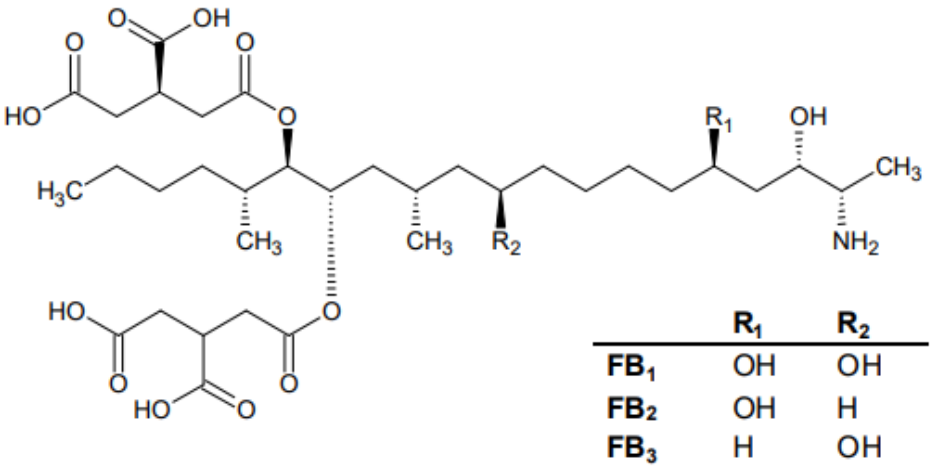

Figure 1.2. The general structural formula of fumonisins B (28)

Toxicology

Fusarium fungi and their associated fumonisins are a global food safety problem. Under favourable environmental conditions, particularly when maize is damaged by insects, the *Fusarium* fungus can cause a range of pathological conditions, including ear rot, stem rot and seedling blight. These conditions provide a suitable environment for the growth and production of fumonisins (29). The IARC has classified FB1 as Group 2B: substances that are possibly carcinogenic to humans. Several studies have shown an association between exposure to FB1 and the development of oesophageal cancer and leukoencephalopathymalacia in Africa, Asia and America (26,30,31). FB1 is also thought to have an adverse effect on embryo development as it could cause an increased risk of neural tube defects (32).

1.1.3. Impact of mycotoxin exposure on human/animal health

Exposure to mycotoxins can occur either directly through consumption of infected food or indirectly through animals fed with contaminated feed, particularly milk. In exceptional cases, exposure can also happen through inhalation (4). The intake of mycotoxins can cause a variety of detrimental health effects, posing a significant threat to both human and animal health. The health effects of mycotoxins exposure vary depending on frequency and quantity of mycotoxins ingested. Acute exposure occurs when individuals consume one or more doses of a particular toxin within a brief timeframe. These doses are at levels adequate to induce immediate health harm, and in some cases, fatality. An example of an acute exposure that was reported sporadically, is acute poisoning with AFB1 (19).

In cases of chronic exposure, consumers ingest amounts of toxins frequently and over long periods of time that are not high enough to cause acute health effects. Chronic exposure may result in long-term consequences including autoimmune illnesses, metabolic and biochemical deficiencies and cancer. It is often difficult to distinguish between acute and chronic effects, but in general the main risks of mycotoxin exposure to humans and animals are related to chronic exposure. However, most of the published problems associated with mycotoxins are cases of acute effects (33).

1.1.4. Economic impact of mycotoxin contamination

Besides the health effects on humans and animals discussed earlier, the occurrence of mycotoxins in food and feed causes annual economic losses (34). In Tanzania alone, mycotoxin contamination exacts a staggering economic toll, with estimated losses reaching millions of dollars annually. The economic impact of aflatoxins caused losses of up to 264 US dollars in 2014 (35).

Mycotoxin contamination can lead to reduced crop yields due to reduced growth, development and overall productivity. In addition, deterioration in the quality of infected crops can lead to rejection or downgrading by markets, resulting in financial losses for producers. These losses are compounded by the need to invest in costly food safety measures. This places a significant burden on crop producers (36). Such economic consequences spill over to all sectors further down the chain, such as food processing, livestock production and export markets, adding to the socio-economic toll of mycotoxin contamination. In fact, mycotoxin contamination results in loss of human and animal life

and associated medical and veterinary costs, as well as reduced livestock production (37). Despite these challenges, strategies to reduce mycotoxin risks are urgently needed, including pre-harvest interventions, post-harvest management techniques, biotechnological innovations and regulatory measures (38).

1.2. FACTORS INFLUENCING THE PREVALENCE OF MYCOTOXINS

The production of mycotoxins relies on factors from both fungi and crops. Optimal conditions are essential not only for fungal proliferation but also for crops to maintain continuous resistance against microbial infections and pests. Although mycotoxin contamination is a worldwide issue, multiple datasets indicate regional variations. Geographical factors and climatic disparities between locations are potential reasons for diverse mycotoxin production. Farming practices also affect the presence of mycotoxins in food and feed (38). In Norway, a study has shown that climatic and agronomic factors contribute 10-30% to mould and mycotoxin formation (39).

1.2.1. Climate

Tropical regions experience a higher occurrence of mycotoxins compared to temperate climates due to their elevated average temperatures and humidity levels. The ideal temperature and moisture levels for fungal growth and mycotoxin production differ depending on the fungal species and the specific type of mycotoxin (40). Ochratoxins and aflatoxins, for example, will be produced at higher temperatures than fumonisins and trichothecenes (41). When soil moisture falls below a specific level, most crops are less resistant to certain fungal infections. Drought and semi-arid to arid conditions also cause regular contamination with mycotoxins. As a consequence, changes in weather conditions have already caused outbreaks of mycotoxicosis (42). Understanding the interaction of environmental conditions, for instance temperature and humidity, is essential for developing effective strategies to control mycotoxin contamination in agricultural products.

1.2.2. Agricultural practices

In addition to climate, there are several factors that may explain the greater susceptibility of humans and animals in more tropical developing countries to exposure to mycotoxins. First, they have few means of agricultural production and the storage of agricultural produce is of poor quality, resulting in higher mycotoxin production.

Harvesting and storage of insufficiently dried crops causes the multiplication of fungi and the production of mycotoxins. Poor detection and quantification also make it difficult to monitor and control mycotoxins. Malnutrition further exacerbates the problem (43,44). Finally, the population has a much lower level of knowledge about mycotoxins, resulting in fewer or no good policies and actions to counter the problem. This lack of awareness perpetuates the cycle of mycotoxin contamination, posing significant risks to both public health and agricultural productivity (45,46).

1.2.3. Prevention and control of mycotoxins

Exposure to mycotoxins must be reduced to ensure global health. Prevention of mycotoxin contamination is seen as the primary measure to achieve this. It is important to make food producers and processors aware of practices that reduce fungal growth and mycotoxin contamination during the various processes leading to the final product. This can be achieved through proper education, high awareness and effective campaigns on good and improved agricultural practices (9,47).

The first step are good agricultural practices (GAP) both before and after harvest. Several articles indicate that the use of organic or mineral fertilisers, pesticides and herbicides reduces mycotoxin production. Other agronomic factors such as crop rotation, thorough drying (at least 60-90 days), adequate storage conditions, as well as sorting out damaged crops can have a positive impact. The aim is to minimise all stress factors on the crop (43,48). In addition, there are studies showing that organic farming systems would be better at reducing mycotoxins compared to conventional farming (49). Along with GAP, good manufacturing practices (GMP) is also important to ensure food safety during the production and processing of food. GMP involves maintaining strict hygiene measures. This includes regular cleaning and disinfection of equipment and storage facilities, preventing cross-contamination and implementing appropriate controls during the production process (48) .

Implementing these preventive measures can ensure the safety and quality of food. This includes taking preventive measures at all stages of the food production chain, from farm to consumer.

1.3. MAIZE AS STAPLE FOOD IN AFRICA

1.3.1. Global importance of maize

Mould contaminated foods such as maize are consumed daily in different parts of Tanzania. Therefore, it is essential to examine the occurrence of mycotoxins in maize flour in different agro-ecological zones of Tanzania. Maize (*Zea mays*) is one of the most widely grown cereals in the world. While it serves as livestock feed in higher-income countries, it holds vital importance as a staple food in regions such as Southern and Eastern Africa and Central America (50). With production reaching nearly 1.2 billion tonnes in 2022, maize has demonstrated the fastest growth since 2000 among major cereals including wheat, rice, barley, and sorghum. A visual representation is found in Figure 1.3. The versatility of maize extends across various sectors, including food, animal feed, biofuels, and industrial applications (51).

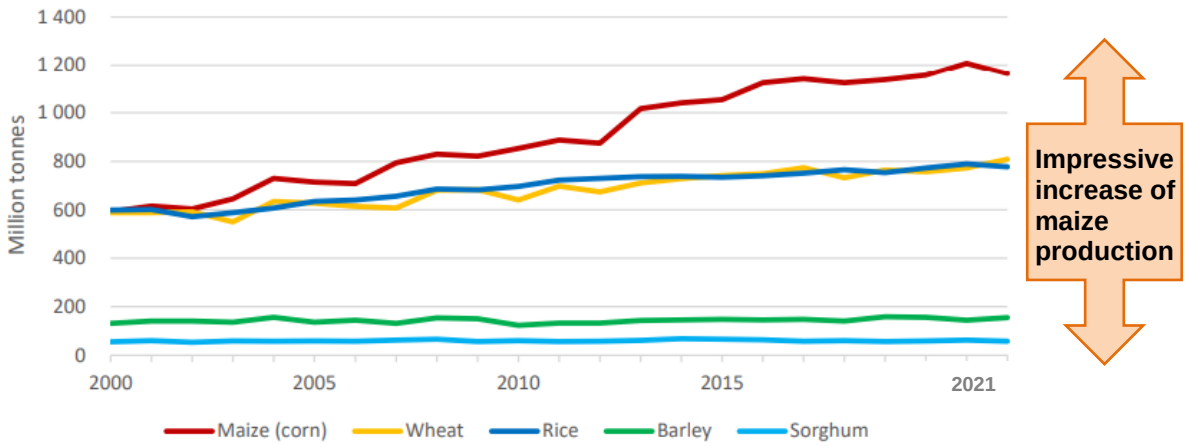

Figure 1.3. Graph of world production of top cereals sourced from FAO with maize having the fastest growth since 2000 among other cereals including wheat, rice, barley and sorghum (51)**.**

Maize as a versatile crop, plays a crucial role across continents. According to data from the Food and Agriculture Organization of the United Nations (FAO), Americans are the largest producers and exporters of maize. Together with Brazil, the United States accounted for 39% of global production in 2022. The vast majority of this production is used for animal feed and biofuels. In Africa and Asia, however, maize is a significant food crop for human consumption (51).

In East Africa alone, over 200 million people consume maize daily, with Tanzania accounting for approximately 48 million consumers (52). Maize is an important food crop in the region and is therefore widely used in various dishes, making it a mainstay of food security and nutrition in Tanzania. A traditional maize-based dish that is very popular in

Tanzania is ugali. It is a compact, doughy substance made by mixing maize flour with boiling water and then boiling it until it has a thick, firm consistency. Ugali is often eaten at all meals, from breakfast to dinner, and can be served with meat, vegetables or beans. Overall, ugali is an appropriate food choice, especially for those who are food insecure. It provides an affordable, nutritious and filling meal that contributes to the food security and well-being of people in Africa (53).

Maize crops are highly susceptible to infestation by several toxic fungi and are vulnerable to contamination by multiple mycotoxins. Studies have shown that aflatoxins and fumonisins are the most prevalent in the maize food chain. Together with groundnut, maize is the crop most exposed to mycotoxins due to its widespread consumption. This is known as carry-over of mycotoxins from food to humans (54).

Research on mycotoxins in Tanzania holds great significance, given that millions of people in Africa consume contaminated food on a daily basis. In recent years, maize has supplanted crops such as cassava, sorghum, and millet in certain African regions, driven by its ability to yield higher returns (55). As a result, there has been increased interest in research into the mycotoxin contamination of maize.

1.3.2. Difference between undehulled and dehulled maize flour

It is significant to note that there is a distinction between undehulled (dona) and dehulled (sembe) maize flour. Dehulled maize flour is obtained by removing the testa and splitting the cotyledons (seed leaves) of the maize kernel using a dehulling machine. Both undehulled and dehulled maize flour are then processed in a milling machine to produce refined maize flour. This process is illustrated in Figure 1.4. Undehulled maize flour is more yellow in colour and has a coarser texture than dehulled maize flour because it retains the outer husk. It also contains more fibre and nutrients, making it more nutritious for people. However, undehulled maize tends to contain more mycotoxins because they accumulate in the outer parts of the maize kernel. The dehulling process therefore influences the presence of mycotoxins in maize (56,57).

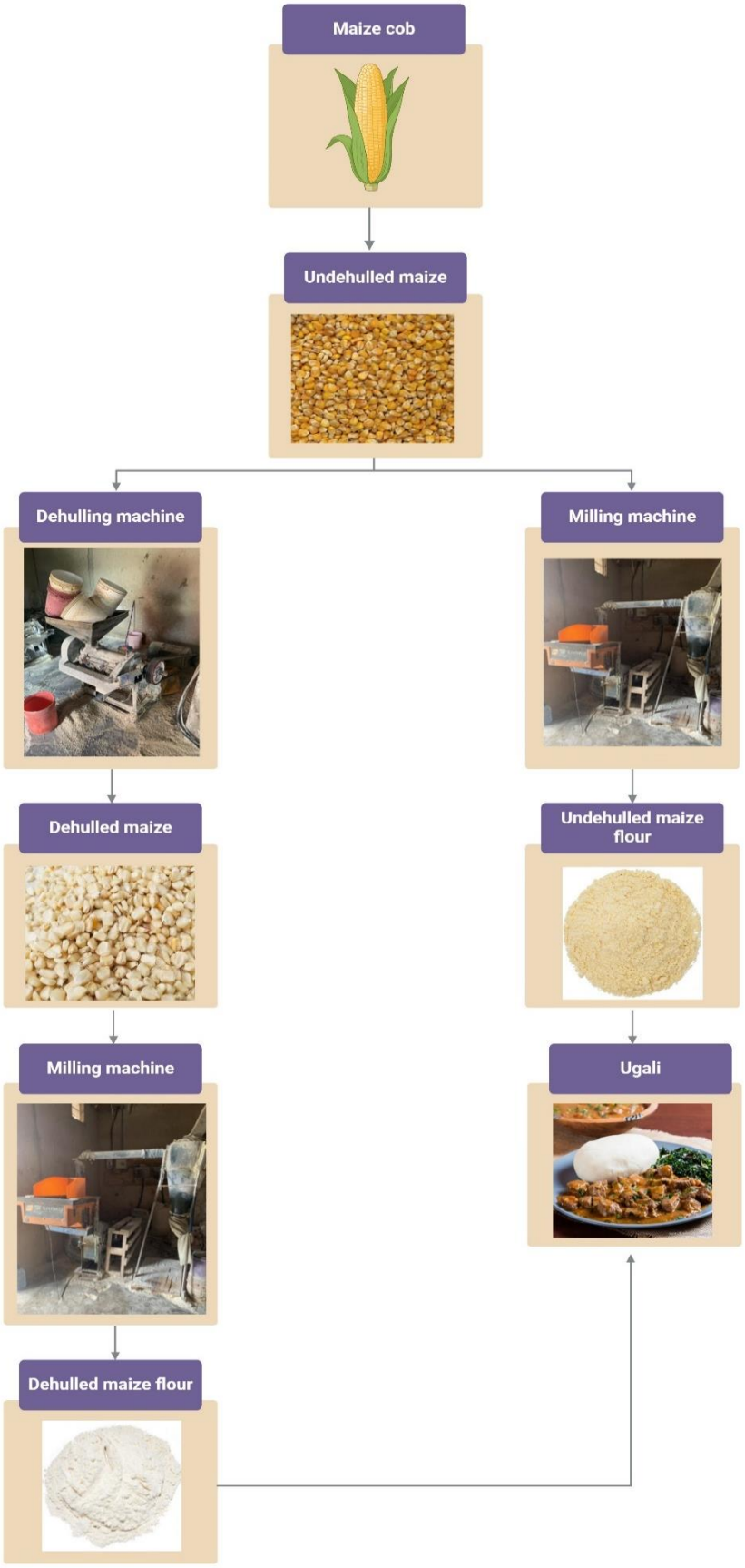

Figure 1.4. The process of going from maize to maize flour and to Ugali, from both undehulled and dehulled maize flour. This includes dehulling of the maize and milling of the maize flour.

1.4. MYCOTOXIN LEGISLATION

The ability of both humans and animals to detect mycotoxins and other chemical contaminants such as antibiotics and pesticides in food and feed is limited. As mycotoxin contamination poses significant health risks, strict measures are needed to control their occurrence and ensure food safety (38). The legislation for both Europe and Africa is discussed below.

1.4.1. Legislation in Europe

The European Union (EU) has established comprehensive legislation to regulate and control the presence of mycotoxins in food to ensure food safety in all Member States. This legislation, described in Commission Regulation (EU) No 2023/915 of 25/04/2023 (replaces the former Regulation (EC) No 1881/2006), sets maximum levels for mycotoxins such as aflatoxins, fumonisins, OTA, ZEN and PAT in different categories of food (58). These maximum levels may vary depending on the type of mycotoxin, the food product and other factors. To protect public health, products exceeding the maximum levels should not be placed on the market. The Scientific Committee on Food (SCF) concludes that aflatoxins are genotoxic carcinogens (59). Therefore, the European Commission has established that food for direct human consumption should not contain more than 5 µg/kg of AFB1, which is the most toxic form. In addition, the total concentration of all aflatoxins should not exceed 10 µg/kg. The maximum levels concerning maize-based food laid down in this Regulation are set out in Appendix I. This also includes the maximum levels for DON, T-2 and HT-2, which were recently supplemented as amendments to Commission Regulation (EU) No 2023/915 (60,61).

All Member States are required to carry out regular monitoring and control measures to enforce these specific limits, including sampling, laboratory analysis and reporting to the European Commission. The European Food Safety Authority (EFSA) carries out scientific risk assessments of mycotoxins to provide opinions to the European Commission. These opinions are used when drafting, revising and updating the European legislation on maximum levels of mycotoxins in food. In addition, food companies are required to maintain traceability and labelling standards to demonstrate compliance with mycotoxin regulations. Strict controls, scientific advice and cooperation between EU Member States aim to minimise the risk of mycotoxin contamination in food (62,63).

1.4.2. Legislation in Africa

In contrast, most African countries do not have regulatory limits for mycotoxins, and if they do, they typically apply only to international trade. Regulations on mycotoxins are based on knowledge of mycotoxin concentrations in food. This information can be obtained through the availability of research funding, which is often inadequate or unavailable in African countries. Limited resources, inadequate infrastructure, food insecurity and lack of capacity at the regulatory and enforcement levels also hamper the implementation and enforcement of mycotoxin regulations. As a result, mycotoxicosis outbreaks continue to occur in African countries in the 21st century (3).

The Tanzanian government has implemented several laws and regulations to control mycotoxin contamination. These are reflected in the Food, Drugs, and Cosmetics Act of 2003 (64). According to this, the Tanzania Food and Drugs Authority (TFDA) and the Tanzania Bureau of Standards (TBS) are responsible for ensuring food safety in Tanzania (65). Most of the proposed African mycotoxin legislation relates to aflatoxins. The East African Community has set the following limits in maize: 5 ppb for AFB1, 10 ppb for pre-total aflatoxins and 2,000 ppb for fumonisins. Despite these efforts, it remains difficult to effectively control mycotoxin contamination in Tanzania and in Africa in general (3). Suggestions have already been made to align legal limits for mycotoxins worldwide, as this could facilitate international trade and better protect consumers. However, EU guidelines cannot be directly applied in Tanzania due to differences in dietary habits and food security status compared to Europe. These differences in consumption behaviour also affect the level of exposure to mycotoxins. Therefore, it's essential to consider factors such as dietary habits and food security status when developing harmonized limits (66).

1.5. LC-MS/MS

Due to the increasing importance of mycotoxins in food and feed, analytical methods are essential for their detection. There are several techniques for the determination of mycotoxins, but they typically involve an extraction step followed by a purification step and finally a separation step with appropriate detection. For accurate assessment of contaminant levels in food samples, LC-MS/MS is an excellent analytical technique due to its high sensitivity and selectivity (67). It combines liquid chromatography (LC) with tandem mass spectrometry (MS/MS). LC-MS/MS is the preferred method for the

determination of mycotoxins. It involves separation of the components, followed by ionisation and separation of the ions based on their mass-to-charge ratio (m/z) (68).

1.5.1. Liquid chromatography

Liquid chromatography is an analytical technique used as a separation method. This makes it possible to distinguish components of interest from unwanted components. The method uses a solid stationary phase and a liquid mobile phase. The components to be separated should be non-volatile. In LC, the sample mixture is dissolved in a solvent (mobile phase) and pumped continuously under high pressure through a column containing chromatographic packing material. This column consists of immobilised functional groups and is called the stationary phase. The sample is transported through the column by nitrogen (N_2) carrier gas. Because of their chemical properties, each component interacts differently with the stationary phase, resulting in different flow rates for different components. The distribution of the components is based on their polarity and affinity for both phases. If a component has a higher affinity for the stationary phase, it will be retained longer on the column. If there is no or very low affinity for the stationary phase, the component will follow the mobile phase and elute earlier from the column. This means that each component has a different affinity for the stationary phase and a different specific time to elute, which is called the retention time. The components are thus separated as they flow out of the column. As the affinity for the stationary phase increases, the component stays on the column longer and therefore has a longer retention time. Figure 1.5 illustrates the equipment required to carry out an LC analysis (69,70).

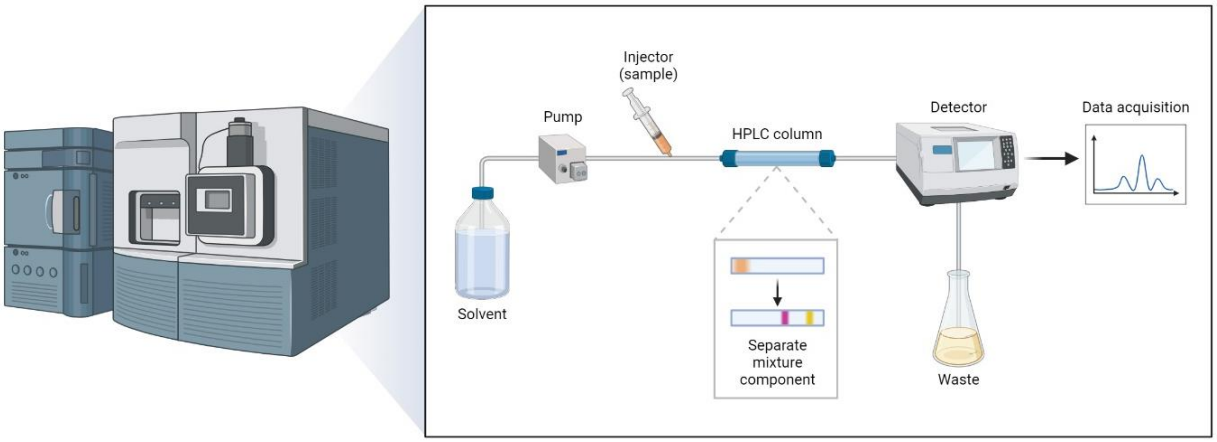

Figure 1.5. Configuration of an LC system where a sample is pumped through a column, separated, and then detected. The output is displayed as a chromatogram, with waste being collected in a flask.

Several LC techniques have been developed to achieve optimal results for each specific class of mycotoxins. The method depends on the use of various adsorbents appropriate to the physical and chemical characteristics of the toxin in question. Reversed phase chromatography, using an apolar stationary phase and a polar mobile phase, is considered to be the most common configuration. As the non-polar components interact better with the non-polar coating of the C18 column, the polar components will elute faster from the column. The way different analytes interact with the stationary phase differs due to variations in ion exchange, adsorption and polarity. These variations make it possible to separate compounds and measure the retention time of solutes through the column. This is the most commonly used method coupled to mass spectrometry (MS) due to its predictable retention and the wide range of stationary phases available (70).

1.5.2. Mass spectrometry

In LC-MS/MS, the liquid chromatography system is coupled to a mass spectrometer, which acts as the detection unit. In recent years, MS has become the standard method for the accurate and specific detection of toxins. It is used to identify and quantify molecules with high sensitivity. The method measures the m/z of the ions formed when a sample is ionised. The three main components of a mass spectrometer, as shown in Figure 1.6, are the ionisation source, the mass analyser and the detector (71).

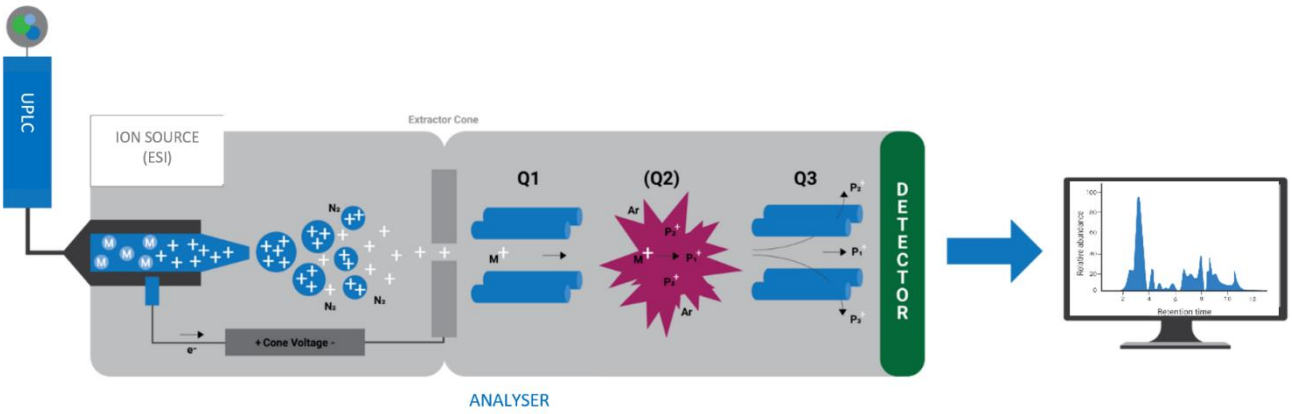

Figure 1.6. Visual representation of a triple quadrupole mass spectrometer with the ionisation source, the mass analyser and the detector being the main components (72)

MS is often coupled with a separation technique such as High-performance liquid chromatography (HPLC) and Gas chromatography (GC). After separation by LC, the effluent from the column is sent to the ionisation source of the mass spectrometer. As mycotoxins tend to be polar, electrospray ionisation (ESI) is typically used for ionisation. In this method, the mobile phase containing the analytical components is atomised into

small charged droplets by an electrospray needle or cap under high voltage. This increases the charge concentration on their surface. As the solvent continues to evaporate, the excess charge in the droplets becomes more concentrated. This causes the droplets to disintegrate, resulting in the production of gas phase ions. This is done using a nebuliser gas (N_2) and a desolvation gas (heated N_2) which together create a mist. This imparts a charge to the components: these can be positive and/or negative ions. These ions are transferred to the mass analyser in several steps, where they are separated and detected according to their m/z. The m/z is specific to each component and is used to distinguish the components from each other. Once the ions have been generated, they must be transferred from the atmospheric pressure environment to the vacuum environment of the mass filter for analysis. This is done using cones, which act as a conduction system. These cones have an attraction to charged particles, so the components to be determined must be charged before they can enter the MS (71).

For the analysis of mycotoxins, MS/MS is often used with a triple quadrupole mass spectrometer. This is valuable when a high degree of selectivity is required. The system first selects the desired precursor ions and then the desired fragments of the previously selected precursor ions. This selection process is carried out using a quadrupole mass filter, which consists of four parallel metal rods arranged in pairs facing each other. The opposite rods have the same positive or negative voltage. An alternating voltage is applied on top of these static voltages, causing the potential of the rods to change alternately between positive and negative. This creates an oscillating electric field in the quadrupole. The oscillatory motion of the ions in this electric field depends on their m/z. Ions with the right m/z value will find a so-called stabilising path and will pass through the quadrupole. Ions with other m/z values will have an unstable orbit and will collide with the rods, excluding them (73).

The first quadrupole (Q1) acts as a mass filter and this is where precursor ion selection takes place. It selects ions with a specific m/z and passes them to the collision cell. This collision cell (Q2) induces Collision Induced Dissociation (CID), which causes the selected ions to fragment into fragment ions by colliding with N_2 or argon (Ar) gas. It is referred to as Q2 of the triple quadrupole, although it is not a quadrupole. The third quadrupole (Q3) again acts as a mass filter, ensuring that only fragment ions with the desired m/z are sent to the detector. The detector, also known as the electron multiplier, is responsible for detecting the incoming ions and converting them into detectable

electrical signals. When an ion arrives at the detector, the electron is transferred to a higher energy state, releasing the electron and accelerating it towards a series of electrodes. Here the electron is multiplied by a process called secondary emission, producing a detectable current proportional to the number of ions arriving. The electrons are finally collected by a metal anode and the result is plotted on a chromatogram. The degree to which the electron multiplier performs its function affects the sensitivity of the mass spectrometer. The Xevo TQ-XS mass spectrometer uses a Stepwave ion transfer system. A Stepwave transfers the desired ions in a 'step' circuit from Q1 to Q2 and then to Q3 (70,74).

2. OBJECTIVES

Maize is a crop particularly susceptible to infestation by mycotoxigenic fungi, which produce harmful secondary metabolites (mycotoxins). As maize forms an essential staple food in Tanzania, residents are daily exposed to the potential health risks of consuming maize contaminated with mycotoxins. The lack of governmental inspections at local stores and mills, often referred to as informal markets, as well as the absence of a regulatory framework in Tanzania, results in a lack of awareness among the population regarding the extent of mycotoxin contamination in maize and the associated dangers (38).

This thesis aims to analyse the mycotoxin profile in maize flour using LC-MS/MS. A total of 90 maize flour samples were collected from three different agro-ecological zones in Tanzania and were examined for the presence of 23 mycotoxins. This thesis will focus on mycotoxins that are often found in maize according to previous research, such as AFs and FUMs. The objectives of this thesis are threefold:

(1) The simultaneous determination of multiple mycotoxin levels in maize flour samples

(2) Identifying geographical variations in multiple mycotoxin incidences among the three zones, and understanding how these variations are related to climatic conditions

(3) Investigating differences in multiple mycotoxin concentrations between undehulled and dehulled maize flour

By analysing the mycotoxin concentrations and the profile of mycotoxins in maize flour samples from Tanzania, a more thorough understanding of the extent of mycotoxin exposure in Eastern Tanzania can be obtained. In addition, an accurate assessment of the associated risks can be made. This provides insight into the safety of food products and assists in taking appropriate measures to protect public health.

3. MATERIALS AND METHODS

3.1. SAMPLING

The maize flour samples were gathered between February and March 2024 across three widespread regions in Tanzania: Dodoma, Morogoro and Dar es Salaam. Figure 3.1 provides a map of Tanzania highlighting these regions. A total of 90 maize flour samples were collected for sampling, of which 45 were undehulled and 45 were dehulled. The decision was made to collect 30 samples from each city to get a good agricultural picture of the maize flour production in Eastern Tanzania. Therefore, 30 samples from Dodoma, 30 samples from Morogoro and 30 samples from Dar es Salaam were collected. For each city, 15 places were visited in different regions to purchase 1 kg of unhulled and 1 kg of dehulled maize flour. Appendix II provides a table of the specific regions in each city where the undehulled or dehulled maize flour was collected. The maize flour was mainly bought from local mills but also from local shops and markets. The local mills bought maize from local farmers and processed it into fine maize flour for sale to local shops and markets. It is possible that several local mills processed maize from the same farm, but this is not discussed in detail because it cannot be confirmed with certainty. However, the names of the local shops, markets, mills, and providing farmers had to remain confidential.

During the sampling, it was observed that in the local mills, two types of machines were used for making maize flour. For the production of dehulled maize, the dehulling machine was first used. This dehulled maize was then applied into the milling machine to refine/pulp the maize into fine maize flour, prepared for additional analysis. Between these two processes, in some local mills the dehulled maize was additionally manually sieved (hand sorting) to be sure to remove all unnecessary parts from the maize. For the production of undehulled maize flour, only the milling machine was used as no dehulling was needed.

In some places, additional processes were added to the production, including flotation of the undehulled maize that was converted to dehulled maize. This ensures obtaining purer dehulled maize. A Sanku milling machine was also sometimes used. This refers to the Healthy Children Project where nutrients (minerals, zinc, vitamin B12, folic acid, iron) are added to the maize. As it was not possible to enter every local mill, there is no detailed information on which region uses which machines or supplemental processes. Even when maize flour was purchased from local shops and markets, nothing is known about

how the maize was manufactured. In general, local shops were the primary source of maize flour in Dodoma, as local mills only sold maize in larger quantities (5 or 10 kg or more). In Morogoro, the majority of the maize flour was obtained from the local mills. In Dar es Salaam, maize flour was acquired from all three locations.

A number of criteria were considered in the selection of the samples, including geographical representativeness, diversity of sources, transport capabilities, cost considerations and the time available in Tanzania. These criteria were used to select maize flour samples that would be valuable for further analysis and interpretation of the samples.

The three cities are located in different climatic zones of Tanzania. Climate can have a significant impact on the presence of mycotoxins in maize flour, making it interesting to examine. High temperatures and humidity, as discussed earlier, create favourable conditions for the growth of fungi such as *Aspergillus* and *Fusarium*, which produce mycotoxins. The moist climate can also increase the risk of mycotoxin contamination due to difficulties in drying and storing maize flour samples (40). Dodoma generally has a drier climate compared to Morogoro and Dar es Salaam. The presence of mountains in Morogoro creates a relatively humid climate. Dar es Salaam is considered to have a moderate climate, not as dry as Dodoma but not as wet as some other cities. These variations in climate can cause significant differences in conditions conducive to mycotoxin formation (75).

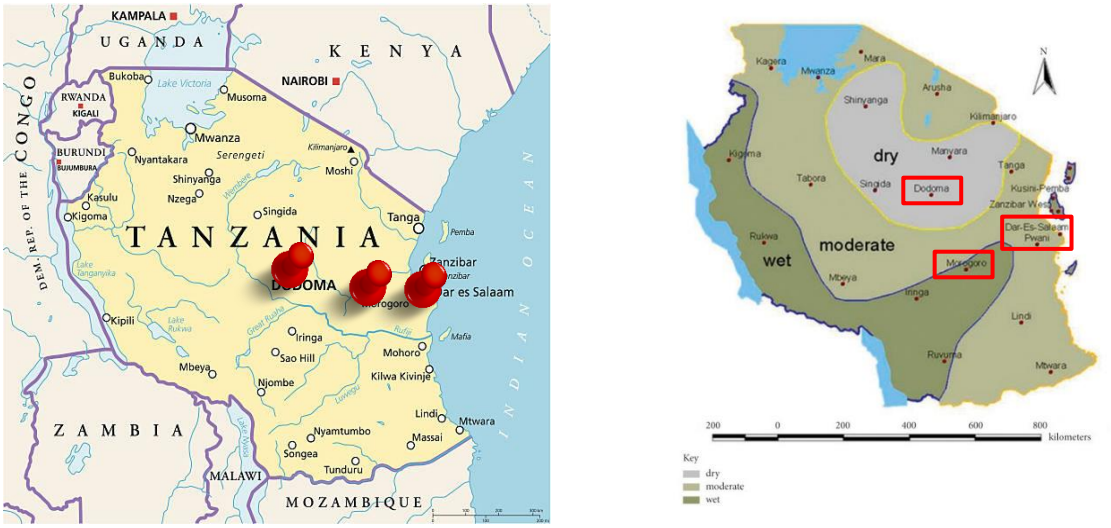

Figure 3.1. Map of Tanzania, highlighting the sampling areas Dodoma, Morogoro and Dar es Salaam and also illustrating the different climatic zones (75)

3.2. SUBSAMPLING

The samples were further processed in three times (by city and region) at the lab in Tanzania to be transported to Belgium for further analysis for the presence of mycotoxins. First, the work surface and the materials were cleaned with 70% ethanol for disinfection. Each maize flour bag (undehulled/dehulled) was opened one by one, applied to an aluminium plate, and mixed with an aluminium spoon to obtain a homogeneous distribution. The focus here was also on preventing clot formation.

A total of 30 g of each sample was weighed using a Contech analytical balance. This was then transferred into a sampling bag and labelled with the appropriate number, region, city, and D (= dona) or S (= sembe). It was ensured that air was removed from the sampling bag to minimise the risk of sample contamination. In addition, this was also done to keep the samples as representative as possible for accurate analysis and to facilitate transport. Finally, the samples were frozen to maintain stability and integrity for transport and storage of the mycotoxins in the samples. This process was repeated for the 90 samples. An Excel file was maintained to keep a record of the labelling of the samples.

3.3. REAGENTS AND INSTRUMENTS

As maize can be infected by more than one fungal species and each fungus can produce different types of mycotoxins, a multimycotoxin method has been developed, namely ANAL-18, which is outlined in the Standard Operating Procedure (SOP). ANAL-18 is a quantitative analysis that establishes a calibration curve near the analysis's cut-off value. The potential presence of one or more mycotoxins could be simultaneously confirmed and quantified using this method. This analysis was conducted at the Centre of Excellence in Mycotoxicology and Public Health in the Department of Bioanalysis, Faculty of Pharmaceutical Sciences at Ghent University. The stock and working solutions, standard mixtures, mobile phases, and other solvents described below were prepared according to the ANAL-18 method outlined in the SOP.

3.3.1. Reagents

All reagents used are detailed in Table 3.1. The reagents were of analytical grade, and the ultrapure water was sourced from a Milli-Q® system. The materials and instruments used are listed in Appendix III.

Table 3.1. List of used reagents.

Solvent	Company	Location
Acetic acid (glacial) 100%	Merck KGaA	Darmstadt, Germany
Acetonitrile (HPLC graded)	Chem-Lab NV	Zedelgem, Belgium
Ammonium acetate	Merck KGaA	Darmstadt, Germany
Disolol	Chemlab	Zedelgem, Belgium
Methanol absolute (LC-MS graded)	Biosolve Chimie SARL	Dieuze, France
Nitrogen	Central supply	
N,N-Dimethylformamide, P.A.	Acros	Geel, Belgium
n-Hexane 97%	VWR Chemicals	Louvain, Belgium
Ultra pure water purified by a Milli-Q® system	Sartorius	Göttingen, Germany

3.3.2. Stock- and work solutions of the reference components

For the reference components supplied in powder form, it was necessary to prepare a stock solution followed by one or more working solutions as required. Similarly, for the reference components supplied in solution, one or more working solutions had to be prepared as required. A list of the reference components used and their associated properties is given in Appendix IV.

For all mycotoxins, the stock solution and work solution, shown below, were prepared in methanol. An exception were the reference compounds alternariol (AOH) and alternariol methyl ether (AME), which were dissolved in dimethylformamide/methanol (40/60, v/v). The rest of the protocol remained unchanged.

3.3.2.1. Stock solution reference components of 1 mg/mL

The powder was dissolved in 1,000 µL methanol per mg and thoroughly vortexed. This solution was then transferred to a Sovirel tube and wrapped in aluminium foil to be stored in the freezer. It is important to note that this stock solution should not be stored for more than two years.

3.3.2.2. Work solutions of the reference components from 100 ng/µL

Using a calibrated pipette, 3 mL of methanol was pipetted into a Sovirel tube. Then 300 µL was removed from the Sovirel tube and replaced with 300 µL of the 1 mg/mL stock solution (described above) using a micropipette. The Sovirel tube was then vortexed and wrapped in aluminium foil for storage in the freezer. It is important to note that this work solution should not be stored for more than two years.

3.3.2.3. Work solutions of the reference components from 10 ng/µL

Using a calibrated pipette, 3 mL of methanol was pipetted into a Sovirel tube. Then

300 µL was removed from the Sovirel tube and replaced with 300 µL of the 100 ng/µL work solution (described above) using a micropipette. The Sovirel tube was then vortexed and wrapped in aluminium foil for storage in the freezer. It is important to note that this work solution should not be stored for more than two years.

3.3.3. Standard mixtures

Two different standard mixtures were used to prepare the spikes: the standard mix 'legislation' and the standard mix 'not in legislation'. The composition of both mixtures is detailed in Table 3.2 and 3.3, including the concentrations of the stock solutions of the reference components, the volumes of the stock solutions required to prepare the standard mixture and the quantities added to the spike (cut-off).

The volume stock solution of each component was pipetted into a plastic tube and evaporated at 40°C under a nitrogen stream until completely dry. The resulting dry residue was then dissolved in 1,000 µL of methanol and vortexed. The solution was transferred to a Sovirel tube and sealed in aluminium foil to be stored in the freezer. The standard mixtures were now ready to add to the spikes. The expiry date of the standard mixtures was six months.

Table 3.2. Standard mix 'legislation' as mentioned in the SOP.

Component	Concentration stock solution	Volume stock solution	Concentration in standard mix	µg/kg in spike (cut-off)
AFB1	± 20 µg/mL	100 µL	± 2 ng/µL	± 20 µg/kg
AFB2	± 20 µg/mL	100 µL	± 2 ng/µL	± 20 µg/kg
AFG1	± 20 µg/mL	100 µL	± 2 ng/µL	± 20 µg/kg
AFG2	± 20 µg/mL	100 µL	± 2 ng/µL	± 20 µg/kg
OTA	± 10 µg/mL	500 µL	± 5 ng/µL	± 50 µg/kg
DON	± 100 µg/mL	400 µL	± 40 ng/µL	± 400 µg/kg
ZEN	± 100 µg/mL	100 µL	± 10 ng/µL	± 100 µg/kg
FB1	± 50 µg/mL	800 µL	± 40 ng/µL	± 400 µg/kg
FB2	± 50 µg/mL	800 µL	± 40 ng/µL	± 400 µg/kg
T-2	± 100 µg/mL	100 µL	± 10 ng/µL	± 100 µg/kg
HT-2	± 100 µg/mL	100 µL	± 10 ng/µL	± 100 µg/kg

Table 3.3. Standard mix 'not in legislation' as mentioned in the SOP.

Component	Concentration stock or working solution	Volume stock or working solution	Concentration in standard mix	µg/kg in spike (cut-off)
FB3	± 1 mg/mL	25 µL	± 25 ng/µL	± 250 µg/kg
NIV	± 100 µg/mL	200 µL	± 20 ng/µL	± 200 µg/kg
3-ADON	± 100 µg/mL	50 µL	± 5 ng/µL	± 50 µg/kg
15-ADON	± 100 µg/mL	25 µL	± 2.5 ng/µL	± 25 µg/kg
DAS	± 10 µg/mL	50 µL	± 0.5 ng/µL	± 5 µg/kg
F-X	± 100 µg/mL	200 µL	± 20 ng/µL	± 200 µg/kg
NEO	± 100 µg/mL	100 µL	± 10 ng/µL	± 100 µg/kg
AOH	± 100 ng/µL	100 µL	± 10 ng/µL	± 100 µg/kg
AME	± 100 ng/µL	200 µL	± 20 ng/µL	± 200 µg/kg
ROQ C	± 10 ng/µL	100 µL	± 1 ng/µL	± 10 µg/kg

3.3.4. Preparation of solutions and mobile phases

3.3.4.1. Acetonitrile/water/acetic acid (79/20/1, v/v/v)

In a volumetric flask of 1,000 mL, 10 mL of acetic acid was added first. Subsequently, 200 mL of ultrapure water was gradually added. The flask was then topped up till the grade mark with acetonitrile and thoroughly homogenized. This mixture was stored at room temperature. It is important to note that the extraction solvent should not be stored for longer than three months.

3.3.4.2. Acetonitrile/acetic acid (99/1, v/v)

In a volumetric flask of 1,000 mL, 10 mL of acetic acid was added first. The flask was then topped up till the grade mark with acetonitrile and thoroughly homogenized. This mixture was stored at room temperature. It is important to note that the purification solvent should not be stored for longer than three months.

3.3.4.3. Mobile phase A

First, 10 mL of acetic acid and 50 mL of methanol were added to a 1,000 mL volumetric flask. Then, 385 mg of ammonium acetate was accurately weighed in a measuring beaker and quantitatively dissolved in a small amount of ultrapure water before being transferred to the 1,000 mL volumetric flask. After adding ultrapure water up till the grade mark, the solution was vigorously shaken. The solution was filtered using a Durapore® membrane filter and then stored at room temperature. It is important to note that this aqueous mobile phase A should not be stored for more than one week.

3.3.4.4. Mobile phase B

First, 10.00 mL of acetic acid was added to a 1,000 mL volumetric flask. Then, 385 mg of ammonium acetate was accurately weighed in a measuring beaker and quantitatively dissolved in a small amount of ultrapure water before being transferred to the 1,000 mL volumetric flask. Subsequently, 20 mL of ultrapure water was measured into a 50 mL graduated cylinder and added to the 1,000 mL volumetric flask. After adding methanol up till the grade mark, the solution was shaken vigorously. The solution was filtered using a Durapore® membrane filter and then stored at room temperature. It is important to note that this organic mobile phase B should not be stored for more than one month.

3.3.4.5. Mobile phase for injection on LC-MS/MS

In a 100 mL Duran flask, 60 mL of mobile phase A and 40 mL of mobile phase B were added. The solution was well homogenized and then stored at room temperature. It is important to note that this injection solvent should not be stored for more than one month.

3.3.4.6. Dimethylformamide/methanol (40/60, v/v)

In a 100 mL Duran flask, 20 mL of dimethylformamide and 30 mL of methanol were added. The solution was well homogenized and then stored at room temperature. It is important to note that this solution should not be stored for more than three months.

3.3.4.7. Standard mix for injection on LC-MS/MS

In an LC-MS vial, 70.2 µL of injection solvent, 11.4 µL of deepoxy-deoxynivalenol (DOM of ± 50 µg/mL), 22.8 µL of zearalanone (ZAN of ± 10 µg/mL), 22.8 µL of standard mix 'legislation' and 22.8 µL of standard mix 'not in legislation' were added successively. The vial was sealed with a cap (with a septum) and the standard mix was thoroughly mixed by vortexing. Finally, the vials were gently tapped to remove possible air bubbles.

3.4. METHODS

The quantitative analysis consists of the following steps: sample preparation, construction of a calibration curve in blank matrix, extraction, defatting, filtration and purification with a MultiSep® 226 column, evaporation and redissolving in the mobile phase, analysis by LCMS/MS, identification and quantification. These steps were performed four times as the 90 maize flour samples were divided into four runs. Figure 3.2 provides a visual representation of this quantitative analysis.

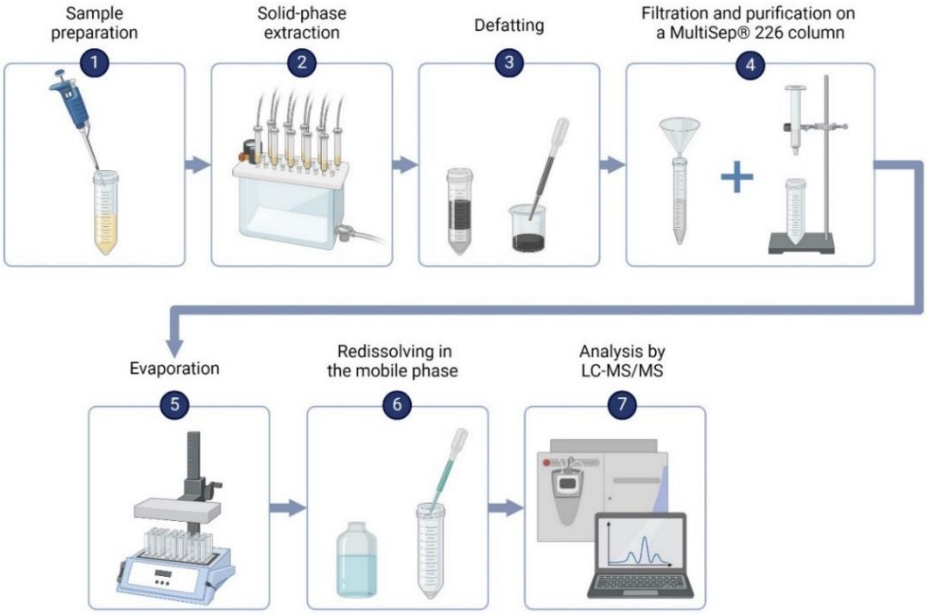

Figure 3.2. Visual representation of the quantitative analysis ANAL-18 as mentioned in the SOP.

3.4.1. Sample preparation

The quantitative analysis started with the sample preparation. Each sample bag, containing 30 g of maize flour sourced from Tanzania, was carefully homogenized. From each bag, exactly 5 g was weighed into a plastic extraction tube of 50 mL. The choice of 5 g was made because it provided sufficient material for reliable detection of mycotoxins in the samples. This process was repeated for all 24 samples in one series. Additionally, seven more samples were prepared: a blank, 5 spikes (spike 0.2x, spike 0.5x, spike 1x, spike 1.5x, spike 2x) for the calibration curve and a control spike for quality control. For these seven tubes, the blank maize flour was homogenized and 5 g was weighed in plastic extraction tubes of 50 mL. Hence, each run comprised 31 samples, each containing 5 g of maize flour. The blank used per series is illustrated in Table 3.4.

Table 3.4. The blank used per series of samples.

Series	Blanco
1	Gibe 9
2	Kitumbite 9
3	Gibe 1
4	Gibe 5

Subsequently, all samples were spiked with 100 µL of internal standard ZAN (± 10 µg/mL) and 25 µL of internal standard DOM (± 50 µg/mL) using a micropipette. The unknown samples and the blank were then sealed with a cap. To spike 0.2x, 10 µL of standard mixture 'legislation' was added using a micropipette, aiming to detect AFB1 at

very low concentrations. For spike 0.5x, spike 1x, spike 1.5x, spike 2x and control spike, standard mixture 'legislation', standard mixture 'not in legislation', and ENN B were also added using a micropipette. The exact volumes are presented in the appropriate sequence in Table 3.5. Afterwards, all samples were placed in the dark for 15 minutes, as some mycotoxins, such as aflatoxins, are sensitive to degradation by UV light. It is important to note that ZAN, DOM, standard mixture 'legislation', standard mixture 'not in legislation', and ENN B were removed from the freezer 30 minutes prior to use for acclimatization and were vortexed before use.

ZAN and DOM have similar chemical and physical properties as other mycotoxins, making them suitable internal standards. Since ZAN and DOM were added in known concentrations to the unknown samples, blank, spikes and control spike, they served as reference points for calculating the concentrations of mycotoxins in the samples. These internal standards were used to correct for any deviations between the spiked concentrations and the effectively determined concentrations of the unknown samples. Specifically, it was used to adjust for small variability during clean-up, to correct for matrix effects and to adjust for variability during analysis including injection volume, detection conditions and temperature (76).

Matrix matching was used in this quantitative analysis. The calibration standards were prepared and analysed in the same manner as the unknown samples. The aim of matrix matching was to minimise the influence of the matrix on the analytical measurement by ensuring that the calibration standards were similar to the samples in terms of matrix composition.

Table 3.5. The exact volumes that were added to the unknown samples, blank, spikes and control spike in the appropriate sequence, as mentioned in the SOP.

Sample	DOM (µL) (± 50 µg/mL)	ZAN (µL) (± 10 µg/mL)	Standard mixture legislation (µL)	Standard mixture not in legislation (µL)	ENN B (µL)
Unknown samples	25	100	-	-	-
Blank	25	100	-	-	-
Spike 0,2x	25	100	10	-	-
Spike 0,5x	25	100	25	25	20
Spike 1x	25	100	50	50	40
Spike 1,5x	25	100	75	75	60
Spike 2x	25	100	100	100	80
Control spike	25	100	50	50	40

3.4.2. Procedure

3.4.2.1. Solid-phase extraction

The first step in the quantitative analysis of mycotoxins is extraction, in this case solid-phase extraction (SPE). This is a crucial step as it ensures the removal of matrix interferences and increases concentration, making the mycotoxins easier to detect. To each maize flour sample of 5 g (unknown samples, blank, spikes and control spike) placed in a 50 mL extraction tube, 20 mL of extraction solvent acetonitrile/water/acetic acid (79/20/1, v/v/v) was added using a regulated dispenser. Each sample was vortexed for 15 s, then placed in a rack and wrapped in aluminium foil. After agitation on a vertical shaker for 1 h, the samples were centrifuged at 3,291 g for 15 min at 4,000 rpm. Meanwhile, a C18 column connected to a vacuum elution manifold was installed for each sample. All C18 columns were conditioned by adding 5 mL of the extraction solvent acetonitrile/water/acetic acid (79/20/1, v/v/v) twice. In this process, it was important to keep the columns moist. The supernatant of each sample was then transferred to the C18 columns with a plastic pasteur pipette and the eluents were collected in 25 mL volumetric flasks. Another 5 mL of extraction solvent Acetonitrile/Water/Acetic acid (79/20/1, v/v/v) was added to the residue of all samples. The samples were vortexed for 15 s, shaken on a vertical shaker for 10 min and centrifuged at 3,291 g for 15 min at 4,000 rpm. The supernatants were then transferred back to the columns and collected in the same volumetric flasks. The columns were dried for 4 min using a vacuum pump. The vacuum was released and the volumetric flasks were removed from the vacuum elution manifold. The contents of each volumetric flask were diluted to the grade mark with the extraction solvent acetonitrile/water/acetic acid (79/20/1, v/v/v) and homogenized well. Finally, the contents of each volumetric flask (25 mL) were transferred to a new 50 mL plastic extraction tube.

3.4.2.2. Defatting

In the second step, the maize samples were defatted by adding 10 mL of hexane to the new extraction tubes. This step ensures that the extracted lipids are removed from the sample. Lipids can interfere with the analysis and reduce sensitivity, so it is necessary to remove them before analysis. After the addition of hexane, the extraction tubes were shaken on a vertical shaker for 10 min and centrifuged at 3,291g for 15 min at 4,000 rpm. The top layer (hexane) was then removed in the fume hood using a plastic pasteur pipette. The remaining liquid was called fraction A and represented the defatted extract.

After defatting, the defatted extract (fraction A) was subjected to two separate cleaning steps: filtration and purification on a MultiSep® 226 column.

3.4.2.3. Filtration and purification on a MultiSep® 226 column

A folded glass filter was placed on 10 mL plastic tubes. This was followed by applying 6 mL of the defatted extract (fraction A) on each filter with a plastic pasteur pipette and it was collected in the plastic tubes. The filtered extract was identified as fraction B.

A new set of 50 mL extraction tubes was filled with 20 mL of acetonitrile/acetic acid (99/1, v/v) using a regulated dispenser. Then 10 mL of the defatted extract (fraction A) was added with a micropipette and the total (diluted extract) was homogenized. For each sample, a MultiSep®226 column was mounted on a stand, with a needle attached to the column, which directed the flow into a new 50 mL extraction tube. Then 30 mL of the previously obtained diluted extract was transferred to the MultiSep®226 columns using plastic pasteur pipettes. The eluent from each sample was collected in the new extraction tubes. The columns were then washed with 5 ml acetonitrile/acetic acid (99/1, v/v) and dried by blowing air through them three times using a 20 mL syringe. The cleaned eluent obtained was called fraction C.

3.4.2.4. Evaporation and redissolving in the mobile phase

For each sample, 2 mL of filtered extract (fraction B), which was first shaken, was added to the cleaned eluent (fraction C) using a micropipette. The samples were then evaporated at 40°C under nitrogen flow in the fume hood until completely dry. Then 150 µL of mobile phase was added to the dried residues with a micropipette to redissolve them. Each sample was thoroughly vortexed for 2 min and centrifuged at 3,291g for 5 min at 4,000 rpm. The dissolved residues were carefully transferred into centrifuge filters and centrifuged for 5 minutes at 10,000 g. Finally, the filtrate from each sample was transferred into an LC-MS vial. It was checked that each vial contained the correct volume. The vials were sealed with a cap (with a septum) and were gently tapped to remove possible air bubbles. The filtrate of spike 1x was divided into two vials, vial spike 1x and vial reinjection spike 1x.

3.4.2.5. Quality control

The standard mix and injection solvent described above were also pipetted into an LC-MS vial. The vials were sealed with a cap (with a septum) and were gently tapped to remove any air bubbles. Additionally, the standard mix was properly vortexed. A sample

list was prepared consisting of the following parts in chronological order: standard mixture - injection solvent - blank - spikes (calibration curve) - injection solvent - unknown samples - reinjection spike 1x - control spike. The samples were injected in this order on the HPLC column. This order was selected to facilitate cleaning and to minimize carry-over as much as possible. Since (re-injection) spike 1x and the control spike had the same concentrations and volumes, they had to obtain identical chromatograms. A quality control was carried out to ensure the accuracy, precision and reliability of the analytical method to ensure the accuracy of the data obtained in the analysis of mycotoxins.

3.4.2.6. LC-MS/MS analysis

LC/MS-MS was used for the simultaneous determination of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), T-2 toxin (T-2), HT-2 toxin (HT-2), nivalenol (NIV), 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), diacetoxyscirpenol (DAS), fusarenone-X (F-X), neosolaniol (NEO), alternariol (AOH), alternariol methyl ether (AME), roquefortin-C (ROQ-C), sterigmatocystin (STERIG) and enniatin B (ENN B) in the maize flour samples.

Equipment

The LC-MS/MS apparatus used for the analysis of the samples was a Waters® Acquity Ultra-high performance liquid chromatography (UPLC) system coupled to a Xevo TQ-XS triple quadrupole mass spectrometer. The Xevo TQ-XS instrument consists of a Step-Wave XS™ ion conductor that increases sensitivity. Prior to analysis, the instruments were correctly configured. The instructions for using the LC-MS/MS, along with a table detailing the precursor ions, fragment ions, cone voltage, and collision energy for each component, are summarised in Appendix V. Before running the full set of samples, the standard mix was injected first to check that the system was working properly and all the desired components were found. Once this was completed, the remaining samples were also analysed on the instrument. The software used for data collecting and processing was Quanlynx® (version 4.1) and MassLynx® (version 4.1) through the Multimyco PRO project. Furthermore, Microsoft Excel was used for additional data processing.

Separation

In this thesis, reversed-phase chromatography was chosen, utilizing a C18 column as the stationary phase, along with a mixture of two mobile phases (aqueous and organic) to run a gradient method. The composition of mobile phases A and B is given in Table 3.6. The mobile phases were injected into the LC-MS/MS in a ratio of 60/40 (mobile phase A/mobile phase B). The C18 column combined with the 60/40 ratio caused the polar components to elute first as they interacted less with the non-polar column. As the eluent became more organic, the non-polar components, which remained on the column for longer, also eluted.

Table 3.6. Composition of mobile phase A (aqueous) and mobile phase B (organic).

Mobile phase A* (v/v): aqueous phase	Mobile phase B** (v/v): organic phase
5% methanol	97% methanol
1% acetic acid	1% acetic acid
5 mM ammonium acetate	5 mM ammonium acetate

***Shelf life: one week**

****Shelf life: one month**

For the analysis the gradient elution method was used. This means that during separation, the strength of the eluent was increased by changing the composition of the mobile phase. The analysis started with 95% of mobile phase A and 5% of mobile phase B and progressed to 1% mobile phase A and 99% mobile phase B. For each sample, 10 µL was injected on the column. The flow rate used was 0.3 mL/min, the elution volume was 250 µL and the velocity was 25 µL/s. The flow rate of the mobile phase was gradually increased so that the pressure did not become too high. The temperature of the stationary phase was 40°C and the temperature of the autosampler was 20°C. Figure 3.3 gives an overview of the gradient during a run of 28 minutes.

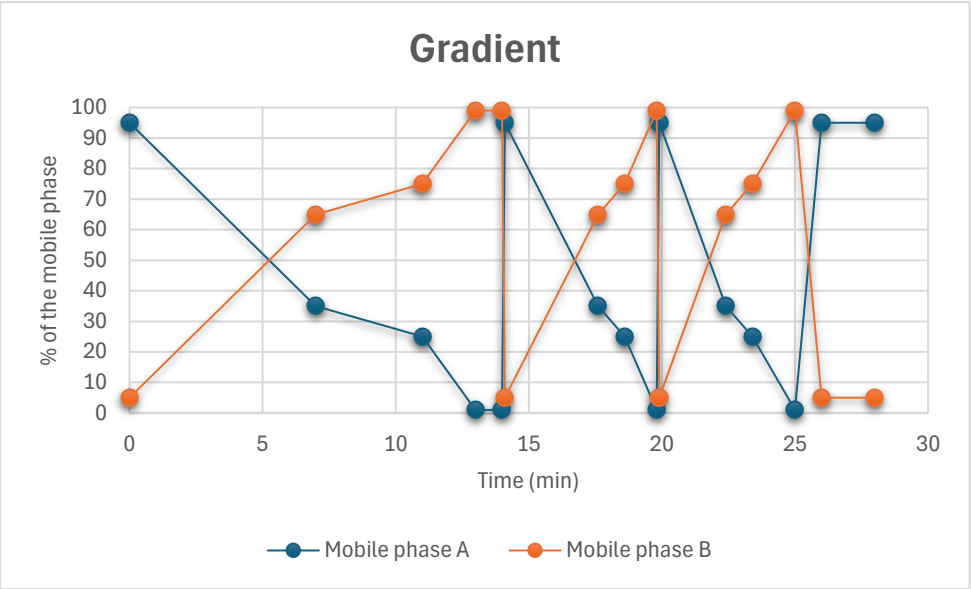

Figure 3.3: Graphical representation of the gradient elution during a run

Detection

Each component eluted from the column passed through a small capillary to the mass spectrometer for detection. The mycotoxins were determined using multiple reaction monitoring (MRM) method, meaning that multiple fragment ions could be detected and visualized simultaneously. The component-specific fragment ions were finally visualized with chromatograms.

3.4.3. Data interpretation

3.4.3.1. Integration of obtained signals

The data obtained, was transferred from the LC-MS/MS to an analysis computer via an automatic server. The software QuanLynx® and MassLynx® were used for data acquisition. To interpret the data, the appropriate layout was first selected, taking into account various parameters: name, sample text, type (standard or analyte), retention time of the analyte, area of the two peaks, response, concentration, signal-to-noise ratio of the two peaks, internal standard used and retention time and area of the internal standard. The peaks (acquired signals) were observed and peak integration was performed as necessary. This took into account a fixed retention time per component that was represented by the retention time of the spikes. While peak integration typically occurs automatically, it had to be adjusted manually in some cases.

3.4.3.2. Quantification of detected components

Subsequently, a calibration curve was constructed for each mycotoxin using the

spikes by plotting the response against the concentration of a mycotoxin in the spiked samples. The concentration range of the calibration curve was different for some mycotoxins as it was based on the expected mycotoxin concentration in the maize flour samples and the sensitivity of the method. Afterwards, it was examined whether each calibration curve had a correlation coefficient (r) > 0.95. The data was then transferred to the Excel template 'Correction calibration curve ANAL-18' for confirmation of the components.

3.4.3.3. Identification and confirmation of the results

The confirmation of the components was performed according to the guidelines of Decision 2002/657/EC. According to this Decision, there are four identification criteria that must be simultaneously fulfilled to confirm a component. The blank was also checked for contamination using these four identification criteria. Below are the identification criteria as stated in the SOP:

1. *Having at least 3 or more identification points. One fragment ions gives 1.5 identification points. At least two selected fragment ions must be present (at the same retention time and with the same peak shape)*

2. *The signal to noise ratio of each ion must be equal or more then 3. By the signal, we mean the height of the peak at half width at half maximum. By the noise, we mean the average of the signals coming from the baseline or background coming from the matrix or injection solvent, in the selected range.*

3. *The relative retention time (with regard to the internal standard) of the component in the sample must range within a margin of 2.5% of the spiked sample. For all components DOM is used as an internal standard.*

4. *The relative intensity of the selected ions, expressed as the percentage of the intensity of the most abundant ion, must correspond with those of the ions of the cut-off calibration point for semi quantitative analysis or the calibration point with a comparable concentration for quantitative analysis, within the accepted deviations presented in Table 3.7:*

Table 3.7. The relative intensity and acceptable limits of the most comparable spike, as mentioned in the SOP.

Relative intensity (% of mean peak)	Accepted limits for LC-MS/MS
> 50%	± 20%
> 20% – 50%	± 25%
> 10% - 20%	± 30%
≤ 10%	± 50%

3.4.3.4. Reporting

If these four identification criteria were met, each concentration was compared with the Limit of Detection (LOD). The LOD is defined as the lowest concentration of a component that can be reliably detected using a specific analytical method. Using the LOD, mycotoxins could be identified in the maize flour samples. If a sample had a mycotoxin concentration ≥ LOD and met the four identification criteria, it could be concluded that the mycotoxin was present in the sample. The mycotoxin concentration in this case was considered accurately measured and reliable. A sample with a mycotoxin concentration that met all identification criteria but (just) < LOD was considered suspected. This was considered left-censored data, meaning that the concentrations were below the LOD. However, this did not mean that no mycotoxins were present. The sample likely contained traces of that mycotoxin, but it could not be detected as the concentration was too low to measure accurately. If a sample with a mycotoxin concentration did not meet one or more identification criteria, it could be determined that the mycotoxin was absent from the sample. The mycotoxin concentrations ≥ LOD were then further corrected using a mycotoxin-specific correction factor. Appendix VI presents the LOD, correction factor and spike concentration of each mycotoxin.

3.4.4. Statistical analysis

First, descriptive statistics were used to summarize and describe the main characteristics of the dataset by using Microsoft Excel. Then, advanced statistics were applied to investigate differences between the three regions and between undehulled and dehulled maize flour. A Kruskal-Wallis test was conducted to determine if there were statistically significant differences in mycotoxin concentrations due to regional variations. Given that the data were not normally distributed and that there were three regions, the non-parametric Kruskal-Wallis test was preferred. Subsequently, the Dunn's post hoc test was performed to confirm the findings of the Kruskal-Wallis test. It was appropriate to conduct a Dunn's test to determine exactly which regions were different. Additionally, a Mann-Whitney U test was conducted to examine if there were significant differences in the proportions of mycotoxins between undehulled and dehulled maize flour, to assess the effect of dehulling. Given that the data were not normally distributed and that two groups were examined, the non-parametric Mann-Whitney U test was preferred. These tests were all carried out in GraphPad.

4. RESULTS

4.1. DETERMINATION OF MULTIPLE MYCOTOXIN OCCURENCE

The primary objective of this thesis was to evaluate the degree of mycotoxin contamination in maize designated for human consumption. A total of 90 maize flour samples were collected from three different agro-ecological regions in Tanzania in February and March 2024. Thirty samples were collected in each city, so a total of 30 in Dodoma, 30 in Morogoro, and 30 in Dar es Salaam. The samples were analysed using LC-MS/MS for the presence of 23 different mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEN, FB1, FB2, FB3, T-2, HT-2, NIV, 3-ADON, 15-ADON, DAS, F-X, NEO, AOH, AME, ROQ-C, STERIG, and ENN B). These mycotoxins were quantitatively analysed by comparing mass spectrometry data and retention times to standard references using a matrix-matched calibration method.

Based on concentration levels, Appendix VII presents the contamination status of the maize flour samples with the 23 aforementioned mycotoxins. The table was divided into three sections, each providing a city-specific summary of mycotoxin contamination across the samples. Additionally, it specifies the run of the analysis, the specific regions within each city where the samples were gathered, and whether the maize was undehulled or dehulled. At the bottom of the table, there is a legend with a colour code that illustrates how the table is structured. This legend follows the description provided earlier under the ‘Data interpretation’ section. If a sample had a mycotoxin concentration ≥ LOD and met the four identification criteria, it could be concluded that the mycotoxin was present in the sample, and it was coloured green. Samples with mycotoxin concentrations that met all identification criteria but were (just) < LOD were considered left-censored data, suggesting their likely presence. These suspected values were coloured orange and assigned the value LOD/2. If a sample with a mycotoxin concentration did not meet one or more identification criteria, it could be determined that the mycotoxin was absent in the sample, and it was coloured red.

According to the data presented in the table, it is evident that each sample was contaminated with at least one mycotoxin. In total, 19 secondary metabolites were found in the maize flour samples from different cities. A clear chromatogram for each detected component has been included in Appendix VIII. The most common mycotoxins were FUMs, AFB1, AFB2, DAS, DON, NIV, and ZEN. Occasionally, samples were contaminated with AFG1, AFG2, NEO, OTA and STERIG. Additionally, F-X, 3-ADON,

15-ADON, ROQ-C and ENN B were each found in only one sample. There were no samples contaminated with AOH, AME, HT-2 and T-2.

4.2. STATISTIC ANALYSIS

4.2.1. Descriptive analysis

A table was prepared for each city, providing an overview of all contaminated maize flour samples. The number of contaminated samples includes both the green and orange coloured samples, starting from a concentration ≥ LOD/2. Each table summarizes various data per mycotoxin, such as the number of contaminated samples, percentage contaminated, mean with standard deviation (SD), median, minimum and maximum concentration per mycotoxin. Subsequently, this table was visually represented using a histogram for each city, displaying the percentage of contaminated maize flour samples for the most common mycotoxins. These histograms are provided in Appendix IX. This aimed to establish a foundational level of understanding regarding the characteristics of the mycotoxin data before further analyses were conducted.

4.2.1.1. Mycotoxin contamination in Dodoma

In the maize flour samples from Dodoma, the mycotoxins FB1, FB3, and DAS were most prevalent. FB1 was found in all 30 collected samples. Following were FB3 and DAS, which were found in 28 (93.33%) and 24 (80.00%) of the samples, respectively. Other notable mycotoxin contaminations also included AFB1, FB2, DON, and AFB2, with 21 (70.00%), 22 (73.33%), 15 (50.00%), and 12 (40.00%) of the 30 samples being contaminated, respectively. A less common mycotoxin was NIV, which was found in 4 (13.33%) samples. Lower frequencies were found for ZEN and STERIG, both of which were detected in 3 (10.00%) and 2 (6.67%) samples, respectively. AFG2, AFG1, ENN B, and F-X were even less frequently detected, each in only 1 (3.33%) sample. No samples were found to be contaminated with NEO, 3-ADON, 15-ADON, AOH, HT-2, T-2, OTA, AME or ROQ-C, as the percentage of contaminated samples was 0%. This information is available in Table 4.1, which also includes the mean with SD, median, minimum and maximum concentration per mycotoxin.

Table 4.1. Overview of all contaminated maize flour samples originating from Dodoma with various data per mycotoxin: number of contaminated samples, percentage contaminated, mean with standard deviation, median, minimum and maximum concentration.

Mycotoxin	# contaminated samples	% contaminated	Mean ± SD (µg/kg)	Median (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)
F-X	1	3.33			67.87	67.87
ENN B	1	3.33			20.20	20.20
AFG1	1	3.33			1.77	1.77
AFG2	1	3.33			2.19	2.19
STERIG	2	6.67	7.08 ± 3.85	7.08	4.35	9.80
ZEN	3	10.00	106.13 ± 50.52	114.10	53.10	152.20
NIV	4	13.33	122.14 ± 149.49	55.67	33.13	344.10
AFB2	12	40.00	1.58 ± 0.62	1.40	1.40	3.52
DON	15	50.00	97.39 ± 113.93	55.44	55.44	441.30
AFB1	21	70.00	4.48 ± 5.79	1.60	1.60	23.63
FB2	22	73.33	94.90 ± 49.22	87.45	22.29	254.22
DAS	24	80.00	0.69 ± 0.29	0.61	0.61	1.73
FB3	28	93.33	57.15 ± 18.79	56.00	21.20	125.80
FB1	30	100.00	173.27 ± 119.11	137.90	29.12	650.90

4.2.1.2. Mycotoxin contamination in Morogoro

In the maize flour samples from Morogoro, fumonisins dominated as the three most common mycotoxins, ranked as FB1, FB3, and FB2. FB1 was detected in all 30 collected samples. Subsequently, FB3 and FB2 were found in 29 (96.67%) and 26 (86.67%) of the samples, respectively. Besides fumonisins, 25 (83.33%) of the samples were contaminated with AFB1, 22 (73.33%) with DON, and 16 (53.33%) with both AFB2, DAS and NIV. Furthermore, 12 (40.00%) samples showed contamination with ZEN, 9 (30.00%) with STERIG, and 6 (20.00%) with AFG1, while 5 (16.67%) samples contained AFG2. Lower frequencies were found for OTA and NEO, both detected in 2 (6.67%) samples. 15-ADON was even less frequently detected, only in 1 (3.33%) sample. None of the samples were found to be contaminated with F-X, 3-ADON, AOH, HT-2, T-2, AME, ROQ-C or ENN B, as the percentage of contaminated samples was 0%. This information is available in Table 4.2, which also includes the mean with SD, median, minimum and maximum concentration per mycotoxin.

Table 4.2. Overview of all contaminated maize flour samples originating from Morogoro with various data per mycotoxin: number of contaminated samples, percentage contaminated, mean with standard deviation, median, minimum and maximum concentration.

Mycotoxin	# contaminated samples	% contaminated	Mean ± SD (µg/kg)	Median (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)
15-ADON	1	3.33			43.60	43.60
NEO	2	6.67	7.86 ± 0.00	7.86	7.86	7.86
OTA	2	6.67	2.81 ± 0.00	2.81	2.81	2.81
AFG2	5	16.67	31.25 ± 64.99	2.19	2.19	147.51
AFG1	6	20.00	263.53 ± 435.92	19.90	1.77	766.80
STERIG	9	30.00	4.35 ± 0.00	4.35	4.35	4.35
ZEN	12	40.00	49.46 ± 42.36	37.00	16.33	132.40
NIV	16	53.33	47.05 ± 55.67	33.13	33.13	255.80
DAS	16	53.33	0.79 ± 0.51	0.61	0.61	2.30
AFB2	16	53.33	7.63 ± 23.88	3.41	1.40	97.13
DON	22	73.33	168.63 ± 170.03	1.40	55.44	579.90
AFB1	25	83.33	40.67 ± 172.50	1.60	1.60	867.41
FB2	26	86.67	208.29 ± 356.01	108.78	22.29	1,818.11
FB3	29	96.67	74.12 ± 88.92	52.30	21.20	487.60
FB1	30	100.00	500.91 ± 834.62	201.70	29.12	4,518.70

4.2.1.3. Mycotoxin contamination in Dar es Salaam

In the maize flour samples from Dar es Salaam, as well as in Dodoma, the most common mycotoxins were FB1, FB3, and DAS. FB1 was dominant, appearing in all 30 collected samples. Following were FB3 and DAS, found in 24 (80.00%) and 23 (76.67%) of the samples, respectively. Additionally, 23 (76.67%) of the samples were contaminated with AFB1, 21 (70.00%) with FB2, 19 (63.33%) with DON, 14 (46.67%) with NIV, 11 (36.67%) with AFB2, 10 (33.33%) with ZEN, and 8 (26.67%) with STERIG. Less prevalent mycotoxins were AFG1, OTA, and AFG2, occurring in 6 (20.00%), 5 (16.67%), and 3 (10.00%) of the samples, respectively. Furthermore, ROQ-C, and 3-ADON were each found in 1 (3.33%) sample. No samples were found to be contaminated with NEO, F-X, 15-ADON, AOH, HT-2, T-2 or AME, as the percentage of contaminated samples was 0%. This information is available in Table 4.3 which also includes the mean with SD, median, minimum and maximum concentration per mycotoxin.

Table 4.3. Overview of all contaminated maize flour samples originating from Dar es Salaam with various data per mycotoxin: number of contaminated samples, percentage contaminated, mean with standard deviation, median, minimum and maximum concentration.

Mycotoxin	# contaminated samples	% contaminated	Mean ± SD (µg/kg)	Median (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)
3-ADON	1	3.33			4.48	4.48
ROQ-C	1	3.33			0.99	0.99
AFG2	3	10.00	2.19 ± 0.00	2.19	2.19	2.19
OTA	5	16.67	2.81 ± 0.00	2.81	2.81	2.81
AFG1	6	20.00	15.06 ± 24.97	4.20	1.77	65.3
STERIG	8	26.67	4.35 ± 0.00	4.35	4.35	4.35
ZEN	10	33.33	26.12 ± 16.39	16.33	16.33	58.10
AFB2	11	36.67	3.19 ± 3.92	4.20	1.40	14.63
NIV	14	46.67	52.46 ± 48.93	33.13	33.13	212.00
DON	19	63.33	181.87 ± 156.27	136.90	55.44	506.30
FB2	21	70.00	187.31 ± 269.89	115.14	22.29	1,284.5
AFB1	23	76.67	12.81 ± 29.29	1.60	1.60	135.45
DAS	23	76.67	1.27 ± 1.03	0.61	0.61	3.80
FB3	24	80.00	62.76 ± 78.56	49.10	21.20	398.00
FB1	30	100.00	382.96 ± 646.02	265.30	29.12	3,536.30

4.2.1.4. Overview of the three regions regarding mycotoxin contamination

Table 4.4 provides an overview of the number of samples contaminated with various mycotoxins out of a total of 90 samples. This also includes the highest concentration found across the three cities.

Table 4.4. Overview of the mycotoxin contamination in the 90 samples with the number of contaminated samples, percentage contaminated and the maximum concentration found across the three cities.

Mycotoxin	# contaminated out of the 90 samples	% contaminated	Maximum (µg/kg)
F-X	1	1.11	67.87
15-ADON	1	1.11	43.60
3-ADON	1	1.11	4.48
ROQ-C	1	1.11	0.99
ENN B	1	1.11	20.20
NEO	2	2.22	7.86
OTA	7	7.78	2.81
AFG2	9	10.00	147.51
AFG1	13	14.44	766.80
STERIG	19	21.11	9.80
ZEN	25	27.78	152.20
NIV	34	37.78	344.10
AFB2	39	43.33	97.13
DON	56	62.22	579.90
DAS	63	70.00	3.80
AFB1	69	76.67	867.41
FB2	69	76.67	1,818.11
FB3	81	90.00	487.60
FB1	90	100.00	4,518.70

Figure 4.1 depicts the percentage of contaminated samples for the most prevalent mycotoxins in the three agro-ecological zones, aiming to provide insight into the distribution of mycotoxins across different regions. In all three cities, FB1 was the most abundant as it was present in every sample from each city. Additionally, FB2 and FB3 were significant contaminants in the maize flour samples, with Morogoro exhibiting the highest percentages. AFB1 and AFB2 were also present in the maize flour samples, with Morogoro again presenting the highest percentages. DAS also exhibited high percentages in the samples, with the highest percentages observed in Dodoma. Across the three cities, the percentages of AFG1 and AFG2 were low. For DON, NIV, ZEN, and STERIG, the highest percentages were found in Morogoro. Overall, it can be inferred that Morogoro exhibited the highest mycotoxin levels.

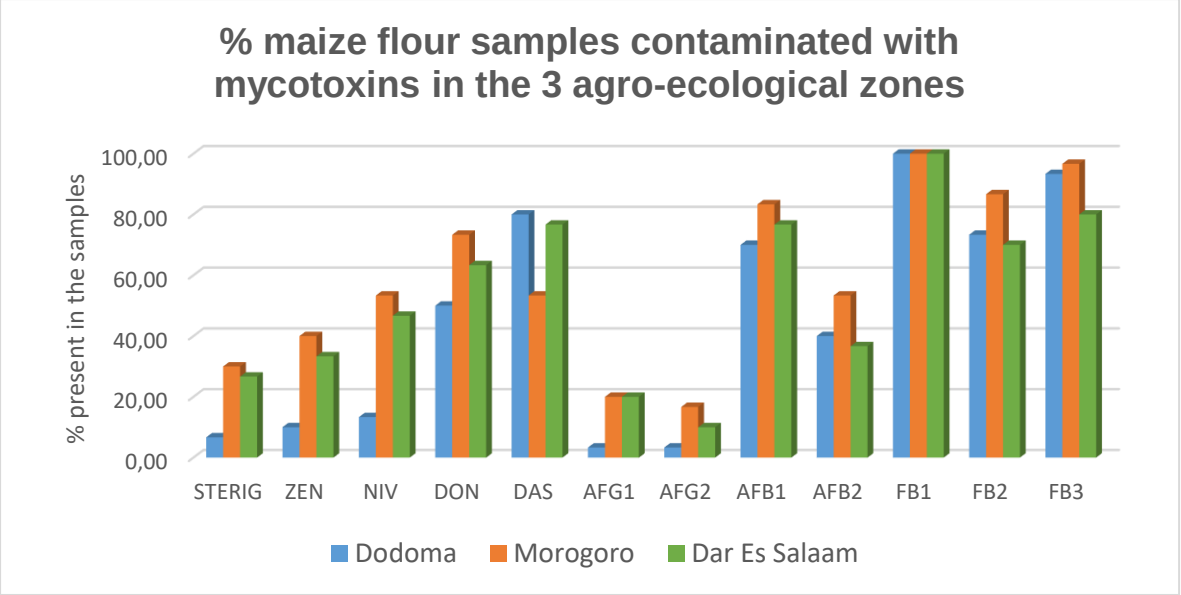

Figure 4.1. % maize flour samples contaminated with the most common mycotoxins (STERIG, ZEN, NIV, DON, DAS, AFs and FUMs) in the 3 agro-ecological zones.

It is important to consider not only the prevalence but also the contamination levels to gain a comprehensive understanding of the extent and severity of the mycotoxin contamination. While AFB1 had a high prevalence in all regions, the contamination levels in Dodoma were low, with a maximum of only 23.63 µg/kg. Similarly, although a large number of samples were contaminated with DAS in Dodoma, the contamination levels were low, with a maximum of 1.73 µg/kg. Conversely, AFG1 was found in only 6 out of 30 samples in Morogoro, but had a high contamination level with a maximum of 766.80 µg/kg. Therefore, it could be observed that a high prevalence does not always correlate with a high contamination level, and vice versa. In general, the highest concentrations

were found in the maize flour samples from Morogoro, particularly for DON, AFs and FUMs. This can be inferred from Figure 4.2.

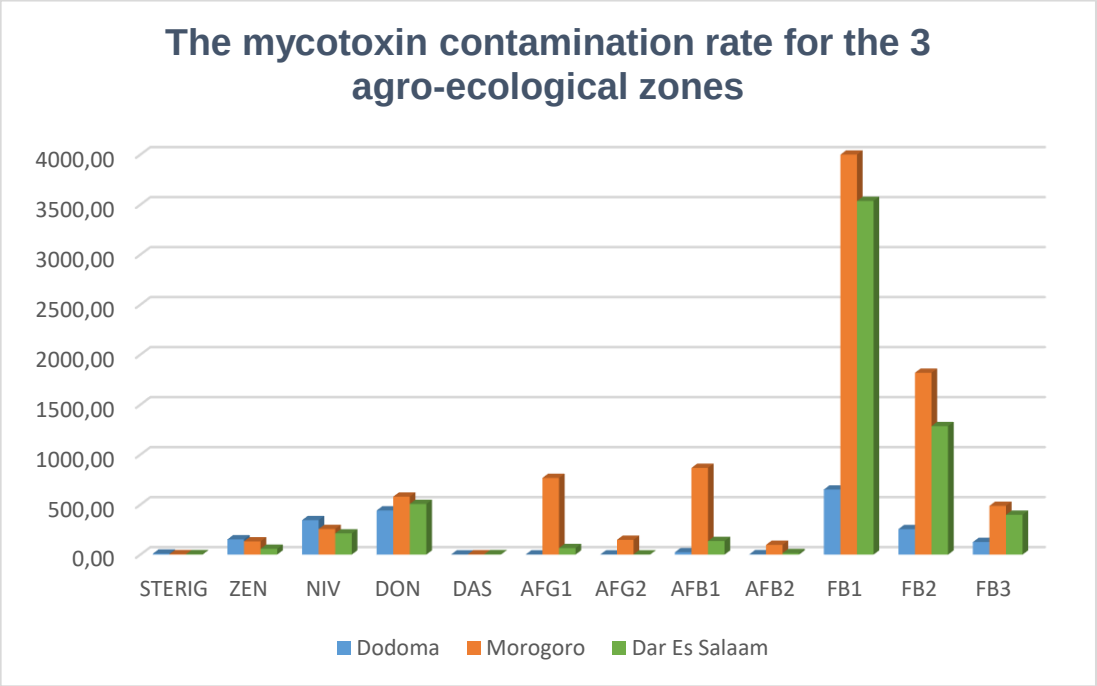

Figure 4.2. The mycotoxin contamination rate for the 3 agro-ecological zones, illustrating that Morogoro had the highest concentrations for DON, AFG1, AFG2, AFB1, AFB2, FB1, FB2, and FB3.

4.2.2. Advanced statistical analysis

4.2.2.1. Are there significant differences in mycotoxin concentration between the three agro-ecological zones?

Another aim of this thesis was to investigate whether there was a significant effect of regional variations on the mycotoxin contamination of maize flour. This was examined using a Kruskal-Wallis test and Dunn's post-hoc test to confirm the findings of the Kruskal-Wallis test.

The null hypothesis of the test posited that there was no difference among the three regions. Based on the p-value, it could be determined whether the null hypothesis should be rejected in favor of the alternative hypothesis, which proposed that there was indeed a difference. For the Kruskal-Wallis test, a p-value < significance level (0.05) was considered significant. The results of this test indicated significant differences between the regions for the mycotoxins FB1 ($p = 0.0432$), DAS ($p = 0.0330$), and ZEN ($p = 0.0261$) since $p < 0.05$. In this case, the null hypothesis could be rejected. This was considered strong evidence that the observed difference was not due to random variability in the data. On the other hand, no significant differences were observed for AFB1 ($p = 0.4948$),

AFB2 (p = 0.2321), AFG1 (p = 0.7469), AFG2 (p > 0.9999), FB2 (p = 0.3254), FB3 (p = 0.4054), DON (p = 0.1033), NIV (p = 0.1148), and STERIG (p = 0.1000) since p > 0.05. In this case, the null hypothesis could be retained, suggesting that the observed distribution could be due to random variation. For NEO, F-X, 3-ADON, 15-ADON, AOH, HT-2, T-2, OTA, AME, ROQ-C, and ENN B, no values were available as there were too few or no mycotoxin concentrations found in the samples to calculate the p-value. The test data are summarized in Table 4.5.

Table 4.5. The p-values of the Kruskal-Wallis test, examining if there is a significant effect from regional variations. The p-values indicated in bold are < 0.05 and indicate a significant effect at alpha = 0.05.

	p-value		
AFB1	0.4948		
AFB2	0.2321	NEO	-
AFG1	0.7469	F-X	-
AFG2	>0.9999	3-ADON	-
FB1	**0.0432**	15-ADON	-
FB2	0.3254	AOH	-
FB3	0.4054	HT-2	-
DAS	**0.0330**	T-2	-
DON	0.1033	OTA	-
NIV	0.1148	AME	-
STERIG	0.1000	ROQ-C	-
ZEN	**0.0261**	ENN B	-

The Dunn's post-hoc test was conducted to further investigate the differences between the regions. The results are presented in Table 4.6. The compact letter display (CLD) is a method to visually represent the results of the Dunn's test. It assigned letters next to the regions, where regions that did not significantly differ from each other were assigned the same letter. If two groups had a different letter, it indicated a significant difference between them, meaning the calculated p-value for the comparison of the regions was less than 0.05. The region with the highest mean was assigned the letter 'A'.

Regarding the FB1 content, Dodoma (B), Morogoro (A), and Dar es Salaam (AB) were determined. This indicated that Dodoma (B) had an average statistically different from Morogoro (A). Dar es Salaam (AB) had an average statistically not different from Dodoma (B) or Morogoro (A). Morogoro (A) had the highest average of FB1 in the maize flour samples. Additionally, for the DAS content, Dodoma (B), Morogoro (AB), and Dar es Salaam (A) were determined. This indicated that Dodoma (B) had an average statistically different from Dar es Salaam (A). Morogoro (AB) had an average statistically

not different from Dodoma (B) or Dar es Salaam (A). Dar es Salaam (A) had the highest average of DAS in the maize flour samples. In terms of ZEN content, Dodoma (A), Morogoro (AB), and Dar es Salaam (B) were determined. This indicated that Dodoma (A) had an average statistically different from Dar es Salaam (B). Morogoro (AB) had an average statistically not different from Dodoma (A) or Dar es Salaam (B). Dodoma (A) had the highest average of ZEN in the maize flour samples. For AFs, FB2, FB3, DON, NIV, NEO, F-X, 3-ADON, 15-ADON, AOH, HT-2, T-2, OTA, AME, ROQ-C, ENN B, and STERIG, no significant differences were found between the three zones, as all were assigned the letter 'A' per city.

Table 4.6. Compact letter display obtained with Dunn's post hoc test, where regions sharing the same letter do not significantly differ in mycotoxin content and with the significant letters indicated in bold.

	Dodoma	Morogoro	Dar es Salaam
AFB1	A	A	A
AFB2	A	A	A
AFG1	A	A	A
AFG2	A	A	A
FB1	**B**	**A**	**AB**
FB2	A	A	A
FB3	A	A	A
DAS	**B**	**AB**	**A**
DON	A	A	A
NIV	A	A	A
STERIG	A	A	A
ZEN	**A**	**AB**	**B**

4.2.2.2. Are there significant differences in mycotoxin concentration between undehulled and dehulled maize flour?

Of the 90 maize flour samples collected, 45 were undehulled and 45 were dehulled. For each mycotoxin, a Mann-Whitney U test was conducted to determine whether there was a significant difference in mycotoxin concentration between the undehulled and dehulled maize flour. The null hypothesis of the test posited that there was no difference between undehulled and dehulled maize flour. Based on the p-value, it could be decided whether to reject the null hypothesis in favor of the alternative hypothesis, which proposed that there was indeed a difference. For the Mann-Whitney U test, a p-value < significance level (0.05) was considered significant.

The results of this test indicated significant differences between undehulled and dehulled maize flour for the mycotoxins FB1 ($p < 0.0001$), FB2 ($p < 0.0001$), FB3 ($p < 0.0001$), and DON ($p = 0.0269$) since $p < 0.05$. In this case, the null hypothesis could be rejected. For all these components, higher concentrations were found for the undehulled maize flour. On the other hand, no significant differences were observed for AFB1 ($p = 0.0524$), AFB2 ($p = 0.0526$), AFG1 ($p = 0.3175$), AFG2 ($p > 0.9999$), DAS ($p = 0.0882$), NIV ($p = 0.7367$), OTA ($p = 0.7262$), STERIG ($p = 0.3684$), and ZEN ($p = 0.6169$) since $p > 0.05$. In this case, the null hypothesis could be retained. For NEO, F-X, 3-ADON, 15-ADON, AOH, HT-2, T-2, AME, ROQ-C, and ENN B, no values were available as there were too few or no mycotoxin concentrations found in the samples to calculate the p-value. The test data are summarized in Table 4.7.

Table 4.7. The p-values of the Mann-Whitney U test, examining if there is a significant effect from dehulling. The p-values indicated in bold are < 0.05 and indicate a significant effect at alpha = 0.05.

	p-value		
AFB1	0.0524		
AFB2	0.0526		
AFG1	0.3175		
AFG2	>0.9999	NEO	-
FB1	**<0.0001**	F-X	-
FB2	**<0.0001**	3-ADON	-
FB3	**<0.0001**	15-ADON	-
DAS	0.0882	AOH	-
DON	**0.0269**	HT2	-
NIV	0.7367	T2	-
OTA	0.7262	AME	-
STERIG	0.3684	ROQ-C	-
ZEN	0.6169	ENN B	-

5. DISCUSSION

This thesis investigated the variety of mycotoxins potentially present in maize flour contaminated with mycotoxigenic fungi. A total of 90 samples were collected from three agro-ecological zones in Tanzania and analysed for the presence of 23 mycotoxins using LC-MS/MS. The detection of 19 different mycotoxins in these samples highlights the diversity of fungi that colonise maize both in the field and during storage. Since each sample was contaminated with more than one mycotoxin, these findings suggest that the occurrence of single mycotoxins in maize is unusual. Recent studies across Africa also confirm that mycotoxins are more often found in combination, which can lead to synergistic or additive toxic effects, exposing the population to a higher risk (77).

5.1. DETERMINATION OF MULTIPLE MYCOTOXIN OCCURENCE

The maize flour samples exhibited the highest levels of contamination with fumonisins: 100.00% of the samples contained FB1 with a maximum of 4,518.70 µg/kg, 76.67% contained FB2 with a maximum of 1,818.11 µg/kg, and 90.00% contained FB3 with a maximum of 487.60 µg/kg. This confirmed that the fumonisins had the highest prevalence and contamination rate in the maize flour samples. Other studies also support that fumonisins are the primary pathogens in maize, specifically FB1 (78). High levels of fumonisin contamination have been reported in East Africa, including the Democratic Republic of Congo, Ethiopia, Kenya, Tanzania, and Uganda (79). A similar study by Kamala et al. (2015) investigated multiple mycotoxin concentrations in maize across three ecological zones in Tanzania and reported a lower prevalence of fumonisins (73.00% FB1 and 48.00% FB2) compared to this study, but higher concentrations (80).

In addition to fumonisins, the maize flour samples also contained aflatoxins, specifically AFB1 and AFB2. AFB1 was detected in 76.67% of the samples with a maximum of 867.41 µg/kg, indicating a high contamination rate. AFB2 was less commonly found, appearing in 43.33% of the samples with a maximum of 97.13 µg/kg. Similarly high prevalences and concentrations have also been reported in other parts of Africa (77). AFG1 and AFG2 had very low prevalences, with less than 15% contamination. Kamala et al. (2015) reported lower prevalences for aflatoxins: 50.00% AFB1, 7.00% AFB2, 5.00% AFG1, and 2.00% AFG2, but higher concentrations for AFB1 and AFB2 (80).

In Tanzania, maximum acceptable limits have been established for aflatoxins in

maize: 5 ppb for AFB1 and 10 ppb for total aflatoxins. In this study, 23.33% of the samples exceeded the 5 ppb limit for AFB1 and 17.78% for total aflatoxins. Kamala et al. (2015) reported that 28.00% of the samples exceeded the limit for AFB1 and 8% for total aflatoxins (64). In addition, a study in Ghana confirmed that 41.25% of the maize samples were contaminated with amounts above the limits of both (81). Several surveys therefore confirm that Tanzanian maize is frequently contaminated with unacceptable levels of aflatoxins. Furthermore, a limit of 2,000 ppb was set for total fumonisins. This limit was exceeded in 3.33% of the samples in this study. Kamala et al. (2015) reported that 15.00% of the samples exceeded the limit for fumonisins. It is evident that mycotoxins such as FUMs and AFs are a significant concern for food safety and public health in East Africa, given the high consumption of maize. As AFB1 and FB1 were frequently found in the samples and are classified by IARC as Group 1 and Group 2B respectively, the consumption of these mycotoxins has significant health implications. In addition, a study performed on liver cell lines showed that simultaneous exposure to AFB1 and FB1 has a synergistic effect. This means that the combined presence of these substances can increase the toxic effects, resulting in a greater risk factor than when they occur separately (82). It is therefore necessary to implement more measures to reduce exposure to these mycotoxins and control the risks to the population (3,80).

Besides FUMs and AFs, the maize flour samples also showed relatively high prevalences of DAS, DON, and NIV: 70.00% contaminated with DAS (max. 3.80 µg/kg), 62.22% with DON (max. 579.90 µg/kg), and 37.78% with NIV (max. 344.10 µg/kg). Additionally, samples were found to be contaminated with ZEN and STERIG: 27.78% contaminated with ZEN (max. 152.20 µg/kg) and 21.11% with STERIG (max. 9.80 µg/kg). A small fraction of the samples was contaminated with F-X, ENN-B, 3-ADON, 15-ADON, NEO, OTA, and ROQ-C, with less than 8.00% contamination. There was no contamination detected with AOH, HT-2, T-2, and AME. Currently, there is no legislation in Tanzania for these mycotoxins (80). In the study by Kamala et al. (2015), DON, ZEN, OTA, T-2, and HT-2 were also analysed. The prevalences of DON (63.00%) were similar to our study. Lower prevalences were reported for ZEN and OTA (10.00% and 3.00%, respectively), but higher concentrations were found. The study identified 25.00% HT-2, which was not present in this samples, and no T-2 contamination (80). The differences between the two studies could be attributed to variations in sampling methods, time and season of sampling, regions, and analytical methods used (38).

5.2. SIGNIFICANT DIFFERENCES BETWEEN THE THREE AGRO-ECOLOGICAL ZONES

An objective of this thesis was to investigate whether there was a significant effect of regional variations on the mycotoxin contamination in maize flour. The results of the Kruskal-Wallis test and Dunn's post-hoc test revealed significant regional differences ($p < 0.05$) in mycotoxin contamination of FB1, DAS and ZEN in the maize flour samples. Specifically, there was a difference between the cities Dodoma and Dar es Salaam for FB1 and ZEN, and between Dodoma and Morogoro for DAS. Further examination of the prevalence and contamination rate of mycotoxins in the samples revealed that a high prevalence did not necessarily correlate with a high contamination rate, and vice versa. This suggests that although mycotoxins can be widespread, the severity of contamination can vary, which is crucial to consider. For example, a study on mycotoxin contamination in maize in South Africa reported a high occurrence of ZEN (55.50%). However, the mean concentration of ZEN was relatively low at 39.20 µg/kg, which was below the acceptable limit of 100 µg/kg (83). In particular, Morogoro exhibited the highest prevalence rate, with samples from this city also presenting the highest mycotoxin concentrations. This was particularly noticeable for the aflatoxins and fumonisins. The higher prevalences and contamination levels in Morogoro compared to Dodoma and Dar es Salaam can be attributed to various local conditions, such as geographical and climatic factors.

Dodoma experiences a semi-arid climate due to limited rainfall, with an annual average precipitation of approximately 550 mm (84). Dar Es Salaam has a tropical coastal climate and receives more rain than Dodoma, with an annual average precipitation of 1,000 mm. Morogoro, on the other hand, has a wetter climate than the other cities, with an average annual precipitation of 1,800 mm, and is characterized by a tropical savanna climate. This can be partly attributed to the high humidity caused by its location at the eastern foot of the Uluguru Mountains, which capture moist air from the Indian Ocean. As a result, Morogoro receives more rainfall than areas further from the mountains. Additionally, the bimodal rainfall pattern contributes to the higher precipitation, with prolonged rainfall from March to May and shorter rainy periods from November to January (85,86).

During the growing season, the regular rainfall and high humidity in Morogoro can lead to moist conditions in the fields, making crops more susceptible to fungal infections. These infections can spread and result in a higher likelihood of mycotoxin production.

Additionally, the high humidity and regular rainfall can extend the growing season, meaning crops are exposed to conditions favorable for fungi for longer periods. A humid climate also poses challenges for the proper storage of maize (87). It is important to note that agricultural practices, both pre- and post-harvest, have a significant impact on mycotoxin production. Previous research indicated that practices such as drying maize on mats, sorting out damaged, discoloured, and mouldy kernels, and using synthetic insecticides during storage are linked with reduced contamination of maize with aflatoxins and fumonisins (88). These practices may have been better implemented in Dodoma and Dar es Salaam compared to Morogoro, but no definitive conclusions can be drawn as this was not studied in detail. In contrast to this study, another study in Morogoro found only 21.2% contamination in maize flour samples (89).

This study illustrated that mycotoxins are common in Africa because of the warm and humid conditions. Currently their prevalence is also becoming increasingly evident in crops in Europe and neighbouring countries. EFSA has highlighted a worrying trend of increasing mycotoxin proliferation in cereals across the EU. This is partly due to climate change, which is causing higher temperatures and humidity levels across Europe (90).

5.3. SIGNIFICANT DIFFERENCES BETWEEN UNDEHULLED AND DEHULLED MAIZE FLOUR

Another important aim of the thesis was to evaluate potential differences in multiple mycotoxin concentrations between undehulled and dehulled maize flour. The results of the Mann-Whitney U test revealed significant differences ($p < 0.05$) between undehulled and dehulled maize flour for the fumonisins and DON. These mycotoxins were significantly more prevalent in the undehulled samples. These significant differences may potentially be explained by the dehulling process. During the dehulling of maize, the outer layers of the maize kernel are removed, including parts where mould growth and mycotoxin production are more likely. This leaves the endosperm with lower mycotoxin levels. The outer layers may, for instance, be damaged by insects or microorganisms, and moisture can also more easily accumulate in these layers, promoting mould growth and mycotoxin production. This can result in lower mycotoxin levels in dehulled maize flour compared to undehulled maize flour (56,91).

In a study by Matumba et al. (2015), these findings are partially confirmed. In the study, dehulling resulted in a decrease of aflatoxins, fumonisins, and AOH, but significant

concentrations of B trichothecenes remained. The hypothesis behind this was that the B trichothecenes might have infiltrated deeper into the grains, making it necessary to remove the infected grains entirely, not just the hull (69). Sometimes higher concentrations were found in the dehulled maize flour samples, which could possibly be explained by this hypothesis, although this was generally not very pronounced. Since some local mills added additional processes before dehulling, namely hand sorting followed by dehulling, flotation/washing followed by dehulling, or a combination of these three, this may explain the variability in mycotoxin concentrations between undehulled and dehulled maize flour at different locations. According to Matumba et al. (2015), combinations of these three processes would have better effectiveness in removing mycotoxins from contaminated maize (57).

The finding that dehulling did not result in a significant difference in mycotoxin levels for some mycotoxins implies that the dehulling process alone does not provide sufficient protection against mycotoxin contamination. Additionally, it is important to note that dehulling not only reduces mycotoxins but also removes nutrients. Therefore, dehulling should not be considered the only measure for reducing mycotoxin contamination in maize. Hand sorting or flotation, combined with dehulling, might be a more efficient decontamination method according to Matumba et al. (2015) (57). Furthermore, mycotoxin binders can provide a complementary strategy as they can form physicochemical bonds with mycotoxins, neutralising their toxicity in the digestive tract (92). Although mycotoxin binders are mainly used in animal feed, there is increasing research into their application in human food. Several studies are currently evaluating the efficacy and safety of binders such as bentonite binders and activated charcoal (93).

5.4. LIMITATIONS AND STRENGTHS

A notable strength of this study is the geographical diversity of the collected samples. The maize flour samples were collected from three different zones in East Tanzania, providing deep insight into the prevalence and distribution of mycotoxins in this area. Moreover, the sampling was conducted at strategic times: at the beginning of the rainy season and later in the food processing process, after the stages of harvesting, transport to local mills, and storage were completed. This allows mycotoxin contamination to be mapped during crucial stages of the food chain, namely during conditions where mycotoxin formation is more likely to occur and close to the consumer. The combination

of geographical diversity and strategic timing significantly improves the representativeness and generalizability of the results. Additionally, it increases the reliability of the data and provides a better understanding of the influence of various environmental factors, such as climatic and geographical characteristics (94).

As already mentioned in the introduction, this study uses LC-MS/MS as the preferred method for analysing mycotoxins due to the outstanding sensitivity and selectivity of this technique. This allows for the detection and quantification of even minuscule amounts of mycotoxins, resulting in accurate and reliable results. Furthermore, LC-MS/MS enables the simultaneous detection of multiple mycotoxins in a single sample, which not only enhances efficiency but is also cost-effective. This technique thus forms another crucial pillar of this research (68,95).

While the results of this study provide valuable insights, it is important to acknowledge several limitations. A significant limitation concerns the sampling variability, which often occurs due to the heterogeneous distribution of mycotoxins in maize flour. For sampling, 1 kg of maize flour was taken from much larger quantities. If mould growth is concentrated in the centre of a bag, a surface sample may provide a distorted view of the actual concentration and distribution of the mycotoxins. A similar issue occurs with subsampling, where 30 g was taken from this 1 kg. This could result in missing relevant mycotoxins, which could affect the accuracy and completeness of the findings. This limitation highlights the need for standardised sampling and analysis protocols to ensure consistency. In addition, it would be beneficial to take samples from different parts of the same sample to reduce sampling variability (96).

As mentioned earlier, the timing of sampling plays a crucial role. Mycotoxin levels can vary depending on the season and pre- and post-harvest practices. During sampling, fungi may be present that have not yet grown sufficiently to be detected, which can result in higher mycotoxin concentrations at later sampling times. By sampling at different times, considering seasonality, growth and storage conditions, and harvest times, a more comprehensive picture of the mycotoxin burden in the samples can be obtained (97). Finally, it may be noted that although 90 samples were sufficient in this case, it is sometimes recommended to collect a larger number of samples. This is due to the fact that increasing the sample size improves the statistical power of the study, leading to more reliable and robust conclusions about mycotoxin concentrations (98).

6. CONCLUSION

Given the increasing importance of maize to the Tanzanian population, this study provides valuable insights into the occurence and distribution of mycotoxins in maize flour from three different zones in Eastern Tanzania. The results indicated that each maize flour sample was contaminated with various fungi and mycotoxins. Fumonisins and aflatoxins were the main mycotoxins in the study area. Previous studies also confirm that these are the main mycotoxins found in maize. In addition, significant amounts of DAS, DON and NIV were detected. Hence, these findings confirmed that mycotoxins can be widely co-occurring in maize (99). The presence of multiple mycotoxins exceeding the Tanzanian maximum limits raises serious concerns about the food and feed safety of maize.

The study showed significant geographical variations in mycotoxin contamination, with Morogoro exhibiting the highest prevalence and contamination rate. This is probably caused by the more humid climate of this region compared to Dodoma and Dar es Salaam. Other factors, such as farming practices, transport and storage, may also contribute to the differences between the three regions. This highlights the need to protect maize from harmful mycotoxins both before and after harvest to ensure food security and safety across Tanzania. First, additional studies are needed to determine the biophysical factors that make maize susceptible to infection by mycotoxigenic fungi. In addition, it is essential to raise awareness among farmers and food producers about mycotoxin contamination at all stages of the maize value chain to promote GAP and GMP compliance (100). Currently, the regulatory framework is insufficient to minimise mycotoxin contamination in Tanzania, indicating an urgent need for more comprehensive regulations that can be implemented by the entire population (3).

The study also reported that some mycotoxins were found in significantly higher concentrations in undehulled maize flour compared to dehulled maize flour, suggesting that the dehulling process helps to reduce mycotoxin contamination. Although this process appears to be effective, it is not sufficient to completely eliminate mycotoxin contamination. Additional techniques such as hand sorting, flotation and the use of mycotoxin binders may be appropriate (57,97).

However, future research with a larger sample size is still needed to confirm these findings and to draw more informed conclusions about the differences between regions and between undehulled and dehulled maize flour.

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8. APPENDIX

Appendix I: Maximum levels of mycotoxins

Table 8.1. Maximum levels of mycotoxins (AFB1, total aflatoxins, total fumonisins and ZEN) laid down in Commission Regulation (EU) No 2023/915 of 25/04/2023 complemented by amendments to Commission Regulation (EU) No 2024/1022 of 08/04/2024 (maximum levels of DON) and Commission Regulation (EU) No 2024/1038 of 09/04/2024 (maximum levels of T-2 and HT-2).

Mycotoxin	Maize-based food	Maximum level (µg/kg)	Regulation
AFB1	Maize and rice to be subjected to sorting or other physical treatment before placing on the market for the final consumer or use as an ingredient in food	5	COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 and repealing Regulation (EC) No 1881/2006
Total aflatoxins (AFB1, AFB2, AFG1 and AFG2)	Maize and rice to be subjected to sorting or other physical treatment before placing on the market for the final consumer or use as an ingredient in food	10	
Total fumonisins (FB1 and FB2)	Unprocessed maize grains	4,000	
	Maize placed on the market for the final consumer, milling products of maize placed on the market for the final consumer, maize-based food placed on the market for the final consumer	1,000	
	Maize-based breakfast cereals and maize-based snacks	800	
	Maize flour not placed on the market for the final consumer	2,000	
	Other milling products of maize not placed on the market for the final consumer	1,400	
	Baby food containing maize and processed maize-based food for infants and young children	200	
ZEN	Unprocessed maize grains	350	
	Maize placed on the market for the final consumer Maize-based snacks and maize-based breakfast cereals	100	
	Maize flour not placed on the market for the final consumer	300	
	Other milling products of maize not placed on the market for the final consumer	200	
	Refined maize oil	400	
DON	Unprocessed durum wheat grains and unprocessed maize grains	1,500	COMMISSION REGULATION (EU)

	Cereals placed on the market for the final consumer, maize for popping and popcorn	750	2024/1022 of 8 April 2024 amending Regulation (EU) 2023/915
	Milling products of maize placed on the market for the final consumer	750	
	Milling products of maize not placed on the market for the final consumer	1,000	
Sum of T-2 and HT-2	Unprocessed maize grains and unprocessed durum wheat grains	100	COMMISSION REGULATION (EU) 2024/1038 of 9 April 2024 amending Regulation (EU) 2023/915
	Barley, maize and durum wheat placed on the market for the final consumer	50	
	Milling products of cereals	20	
	Breakfast cereals consisting of at least 50 % of cereal bran, milling products of oat grains, milling products of maize grains, whole oat grains, barley grains, maize grains or durum wheat grains, and consisting of less than 40 % of milling products of oat grains and whole oat grains	50	
	Breakfast cereals consisting of at least 50 % of cereal bran, milling products of oat grains, milling products of maize grains, whole oat grains, barley grains, maize grains or durum wheat grains, and of at least 40 % of milling products of oat grains and whole oat grains	75	

Appendix II: List of the regions

Table 8.2. List of the specific regions in Dodoma where the samples were collected.

Dodoma	Region	# locations
1	Kizota	2
2	Kgugurnu	2
3	Mlezi	2
4	Miuji	1
5	Kihuyu	2
6	Majengo	3
7	Changumbe	3

Table 8.3. List of the specific regions in Morogoro where the samples were collected.

Morogoro	Region	# locations
1	Misufini	1
2	Kihonda Mbuyuni	1
3	Kingalu	1
4	Nunge	1
5	Mtawala	1
6	Chamwino Mwembeni	1
7	Fourways	1
8	Nanenane	1
9	Msamvu mataa	1
10	Sabasaba	1
11	Mawenzi	1
12	Mkwajuni Kichangani	1
13	Chamwino kwa Geta	1
14	Kihonda Maendeleo	1
15	Mwembe Songo	1

Table 8.4. List of the specific regions in Dar es Salaam where the samples were collected.

Dar es Salaam	Region	# locations
1	Manzese	2
2	Tandale	2
3	Tandika	2
4	Buguruni	2
5	Ilala	2
6	Kijitonyama	2
7	Mikocheni	2
8	Namanga	1

Appendix III: Materials and instruments used

Table 8.5. List of used materials.

Material	Company	Location
Adjustable dispensettes: 10 mL, 25 mL	Brand	Wertheim, Germany
Aluminium foil	Novolab	Geraardsbergen, Belgium
Centrifugal Filter Durapore® 0,22 mm	Merck KGaA	Darmstadt, Germany
Durapore® membraan filter	Millipore	Burlington, U.S.
epT.I.P.S. ® Standard/Bulk (0.5-5 mL)	Eppendorf	Hamburg, Germany
epT.I.P.S. ® Standard/Bulk (2-200 µL)	Eppendorf	Hamburg, Germany
epT.I.P.S. ® Standard/Bulk (50-1,000 µL)	Eppendorf	Hamburg, Germany
Filter system (glass, filterholder and erlenmeyer)	Egilabo	Kontich, Belgium
Glass beaker 100 mL	Egilabo	Kontich, Belgium
Glass Microfiber Filters diameter 47	Whatman Cytiva	Maidstone, United Kingdom
Glass pasteur pipettes	normax	Marina Grande, Portugal
Graduated cylinder 10 mL, 500 mL	Egilabo	Kontich, Belgium
HPLC “Screw-neck”-vials (12 x 32)	Thermo Fisher Scientific	Merelbeke, Belgium
HPLC-vial caps	Thermo Fisher Scientific	Merelbeke, Belgium
Latex disposable gloves	EcoSHIELD	Bennenkom, Netherlands
Metallic stand with buret grip		
MultiSepâ226 AflaZon+ Multifunctional column	Romer Labs	Getzersdorf, Austria
Pipette epperndorf reference 2 (10-100 µL)	Eppendorf	Hamburg, Germany
Plastic extraction tubes 50mL with screw cap	Novolab	Geraardsbergen, Belgium
Plastic Pasteur pipettes	Deltalab	Barcelona, Spain
Plastic syringes of 20 mL, Becton Dickinson	Terumo	Shibuya, Japan
Plastic tube stoppers, diameter 16	Promed	Torreglia, Italy
Schott Duranflask of 100 mL, 1 L	Novolab	Geraardsbergen, Belgium
Strata C18-column	Phenomenex	Torance, U.S.
Test tubes	Promed	Torreglia, Italy
Transferpette S Pipet (0.5-5 mL)	Brand GmbH	Wertheim, Germany
Transferpette S Pipet (100-1,000 µL)	Brand GmbH	Wertheim, Germany
Volumetric flask 25 mL en 1 L + glas stopper	Egilabo	Kontich, Belgium

Table 8.6. List of used instruments.

Instrument	Company	Location
3-16PK Refrigerated Benchtop Laboratory Centrifuge	Sigma-Aldrich	Darmstadt, Germany
Electronic analytical balance	Sartorius	Göttingen, Germany
Electronic thermostated water bath (40°C)	Grant Instruments	Royston, United Kingdom
Fridge + freezer (-20°C)	Fridge/freezer room 1.09F	
LABINCO L46 Power Mixer, vortex	Labinco BV	Breda, Netherlands
Milli-Q® system	Sartorius	Göttingen, Germany
Multifuge 3S-R Centrifuge	Heraeus	Appeldoorn, Netherlands
Quadrupole Mass Spectrometer	Waters Corporation	Etten-Leur, Netherlands
Shaker AG 6A	Agitelec	Coueron, France
TurboVap LV evaporation system	Biotage LLC	Uppsala, Sweden
Vacuum elution manifold with waterflow pump	J.T. Baker Inc	Radnon, U.S.
Xevo TQ-XS Triple	Waters Corporation	Etten-Leur, Netherlands

Appendix IV: Reference components

Table 8.7. Reference components with the corresponding characteristics.

Reference component	Characteristics
15-acetyldeoxynivalenol (15-ADON)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02014
3-acetyldeoxynivalenol (3-ADON)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02012
Aflatoxin mix (AFB1, AFB2, AFG1, AFG2)	± 20 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, 002050
Alternariol (AOH)	Sigma, 35758-0.1MG
Alternariol Monomethylether (AME)	Sigma, A3171-5MG
Deepoxy-deoxynivalenol (DOM) (= Internal standard)	± 50 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02033
Deoxynivalenol (DON)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02009
Diacetoxyscirpenol (DAS)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02037
Fumonisin B3 (FB3)	Medical Research Council
Fumonisin mix (FB1, FB2)	± 50 µg/mL in 50/50 acetonitrile/water, Biopure, Romerlabs, Food Risk managment, S02006
Fusarenon X (F-X)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02010
HT-2 toxin (HT-2)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02036
Neosolaniol (NEO)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02001
Nivalenol (NIV)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02011
Ochratoxin A (OTA)	± 10 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02023
Roquefortin C (ROQ-C)	Enzo Life Sciences, Axxora Platform, ALX-350-342- MC05
Sterigmatocystin (STERIG)	± 50 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02051
T-2 toxin (T-2)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02035
Zearalanone (ZAN) (= Internal standard)	Sigma, Z0167-1MG
Zearalenone (ZEN)	± 100 µg/mL in acetonitrile, Biopure, Romerlabs, Food Risk managment, S02029

Appendix V: Working conditions for LC-MS/MS

Table 8.8. Settings of the parameters from the XEVO TQ-XS.

Parameter	Setting
Column	Symmetry C18 column (5 µm 2,1 x 150 mm)
Guard column	Symmetry C18 column (3,5 µm 2,1 x 10 mm)
Inlet file	Multimyco-LC
Injection volume	10 µL
Run time	28 min
Tune file	Multimyco-T
MS file	Multimyco-MS
Scan mode	MRM
Ionization mode	ESI+
Ion source	ESI Turbo Spray
Cone voltage	60 V
Cone gas flow	150 L/hr
Capillairy voltage	3.30 kV
Desolvation gas	Nitrogen
Desolvation temperature	200°C
Nebulization gas	Nitrogen
Source temperature	130 °C
Nebuliser gas flow	7 bar
Collision gas	Argon (Ar)
Collision gas flow	0,16 mL /min

Table 8.9. Precursor ions, fragment ions, cone voltage and collision energy for each component from the XEVO TQ-XS.

Component	Precursor ion (m/z)	Fragment ions (m/z)	Collision energy (eV)	Cone voltage (V)
15-ADON	339,0	137.1 and 321.2	10.0 and 10.0	26.0 and 26.0
3-ADON	339,0	231.0 and 203.0	13.0 and 12.0	23.0 and 23.0
AFB1	313,0	285.0 and 241.1	24.0 and 36.0	51.0 and 51.0
AFB2	315,0	259.0 and 287.0	27.0 and 30.0	51.0 and 51.0
AFG1	329,0	243.0 and 311.0	25.0 and 20.0	44.0 and 44.0
AFG2	331,0	313.0 and 285.0	25.0 and 30.0	53.0 and 53.0
AME	272.9	258.2 and 199.3	26.0 and 30.0	57.0 and 57.0
AOH	258.9	185.1 and 213.1	30.0 and 26.0	40.0 and 40.0
DAS	384.1	307.1 and 247.1	12.0 and 14.0	21.0 and 21.0
DOM	281.1	233.1 and 215.1	19.0 and 15.0	26.0 and 26.0
DON	297.0	249.0 and 231.0	10.0 and 15.0	26.0 and 26.0
ENN B	662.0	336.3 and 594.4	37.0 and 31.0	54.0 and 54.0
FB1	722.2	334.2 and 352.2	36.0 and 29.0	56.0 and 56.0
FB2	706.1	336.2 and 354.2	40.0 and 29.0	61.0 and 61.0
FB3	706.2	354.3 and 530.2	37.0 and 31.0	54.0 and 54.0
F-X	355.0	247.1 and 137.1	20.0 and 25.0	18.0 and 18.0
HT-2	447.0	345.0 and 285.0	21.0 and 23.0	30.0 and 30.0
NEO	400.0	305.0 and 215.0	12.0 and 19.0	26.0 and 26.0
NIV	313.0	175.0 and 177.0	13.0 and 12.0	26.0 and 26.0
OTA	404.0	238.9 and 358.0	22.0 and 14.0	24.0 and 24.0
ROQ-C	390.1	193.0 and 322.0	26.0 and 21.0	40.0 and 40.0
STERIG	325.0	310.0 and 281.0	25.0 and 36.0	47.0 and 47.0
T-2	484.3	305.2 and 215.2	26.0 and 26.0	26.0 and 26.0
ZAN	321.2	303.3 and 189.1	13.0 and 19.0	26.0 and 26.0
ZEN	319.2	283.1 and 301.1	19.0 and 20.0	27.0 and 27.0

Appendix VI: LODs, correction factors and spike concentrations

Table 8.10. LOD, correction factor and spike concentration of each mycotoxin.

Mycotoxin	LOD (µg/kg)	Correction factor	µg/kg in spike (cut-off)
15-ADON	5.62	1.00	± 25 µg/kg
3-ADON	8.96	1.00	± 50 µg/kg
AFB1	3.20	1.05	± 20 µg/kg
AFB2	2.79	1.10	± 20 µg/kg
AFG1	3.53	1.00	± 20 µg/kg
AFG2	4.37	1.10	± 20 µg/kg
AME	32.48	1.10	± 200 µg/kg
AOH	21.92	1.00	± 100 µg/kg
DAS	1.22	1.15	± 5 µg/kg
DON	110.87	1.00	± 400 µg/kg
FB1	58.24	1.00	± 400 µg/kg
FB2	44.57	0.95	± 400 µg/kg
FB3	42.40	1.00	± 250 µg/kg
F-X	30.34	0.96	± 200 µg/kg
HT-2	16.89	1.10	± 100 µg/kg
NEO	15.72	1.00	± 100 µg/kg
NIV	66.26	1.00	± 200 µg/kg
OTA	6.30	1.10	± 50 µg/kg
ROQ-C	1.98	0.95	± 10 µg/kg
STERIG	8.69	1.00	± 50 µg/kg
T-2	17.16	1.00	± 100 µg/kg
ZEN	32.65	1.00	± 100 µg/kg

Appendix VII: Total contamination status with the 23 mycotoxins

Table 8.11. Contamination status of the maize flour samples with 23 mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEN, FB1, FB2, FB3, T-2, HT-2, NIV, 3-ADON, 15-ADON, DAS, F-X, NEO, AOH, AME, ROQ-C, STERIG, and ENN B) with a city-specific summary of mycotoxin concentrations, run of analysis, specific regions within each city, undehulled/dehulled and a legend with a colour code.

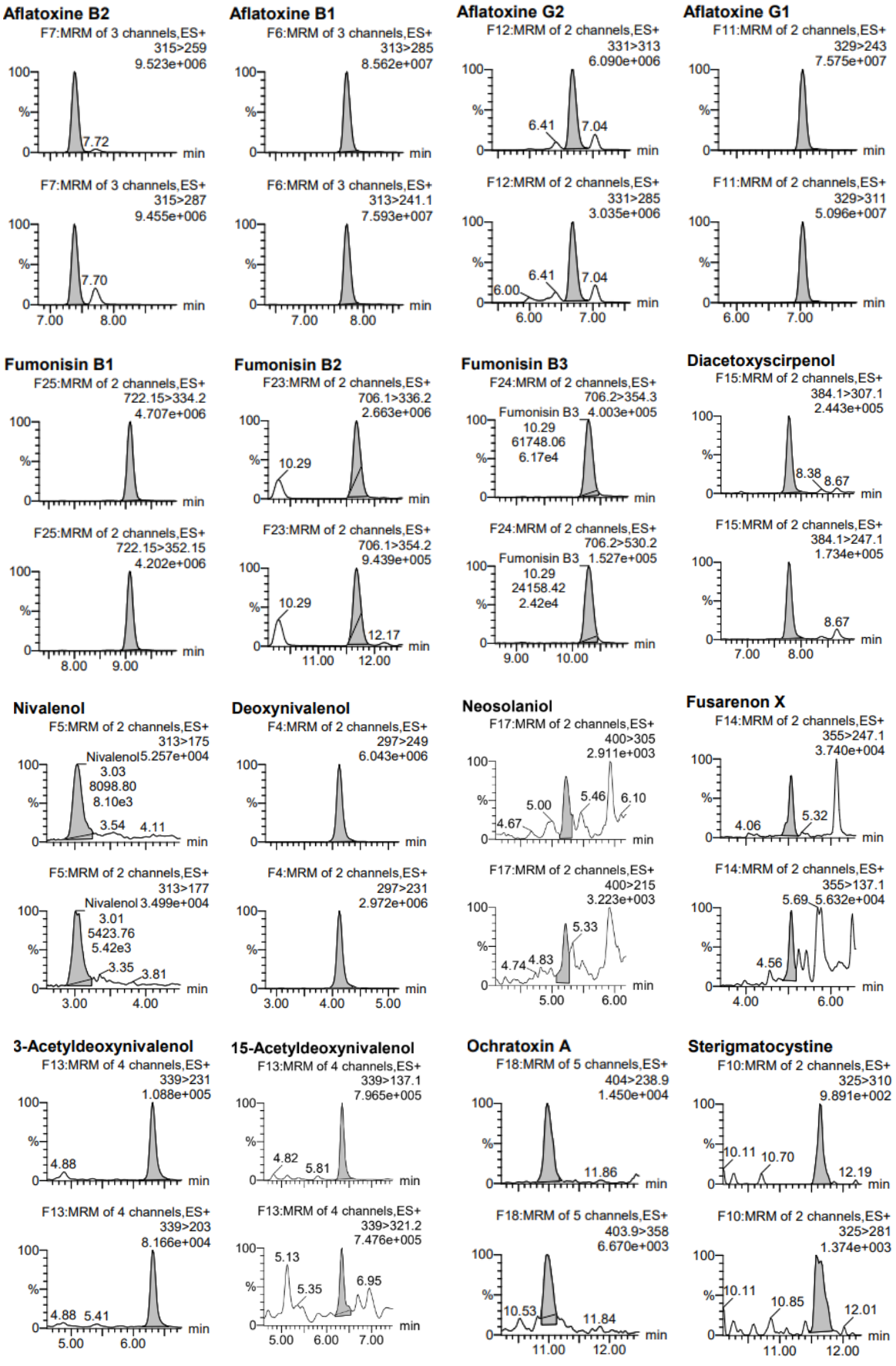
Series	**Dodoma**	AFB1	AFB2	AFG1	AFG2	FB1	FB2	FB3	DAS	DON	NIV	NEO	F-X	3-ADON	15-ADON	AOH	HT-2	T-2	OTA	AME	STERIG	ROQ-C	ZEN	ENN B
1	1 : Kizota S	1.60	1.40			97.60		48.50	1.50	55.44													53.10	
1	2 : Kizota D	1.60				176.60	92.25	59.00	0.61	441.30	78.20												114.10	
1	3 : Kizota S	1.60	1.40			92.60	60.99	47.10	0.61															
1	4 : Kizota D	8.82	1.40			296.20	143.73	78.50																
1	5 : Kgugurnu S					73.00			0.61															
1	6 : Kgugurnu D					132.70	77.24	52.20	0.61															
1	7 : Kgugurnu S	1.60				100.70		47.50	0.61	55.44														
1	8 : Kgugurnu D					201.90	101.65	62.90	0.61	55.44														
1	9 : Mlezi S	1.60				88.10		45.90		55.44														
1	10 : Mlezi D	1.60				190.50	97.38	58.60	0.61	55.44	33.13													
1	11 : Mlezi S	1.60	1.40			96.10	64.03	46.00																
1	12 : Mlezi D	1.60				267.10	137.66	68.00	0.61	55.44											9.80			
1	13 : Miuji S	1.60				137.60	75.53	54.80	0.61															20.20
1	14 : Miuji D	15.23	3.52	1.77		650.90	254.22	125.80	1.73	55.44														
1	15 : Kihuyu S	9.77	1.40			135.90	75.05	54.60	0.61															
1	16 : Kihuyu D	1.60	1.40			208.00	110.01	70.80	0.61															
1	17 : Kihuyu S	1.60				128.70		52.50	0.61	55.44														
1	18 : Kihuyu D					136.90		49.40	0.61	298.80	344.10		67.87										152.20	
1	19 : Majengo S	1.60	1.40			138.20	73.91	56.00	0.61															
1	20 : Majengo D					213.10	115.81	63.90																
1	21 : Majengo S	1.60				149.80	75.91	57.80	0.61															
1	22 : Majengo D					238.80	112.96	67.60																
1	23 : Majengo S					153.00		56.20	0.61	55.44														
1	24 : Majengo D					159.20	82.65	56.00																
2	25 : Changumbe S	8.51	1.40			29.12	22.29		0.61	55.44														
2	26 : Changumbe D	23.63	1.40		2.19	390.20	147.06	77.80	0.61	55.44														
2	27 : Changumbe S	4.20	1.40			60.10		21.20	0.61															
2	28 : Changumbe D	1.60	1.40			86.10	22.29	21.20	0.61															
2	29 : Changumbe S	1.60				107.50	42.37	42.60	0.61	55.44											4.35			
2	30 : Changumbe D					261.80	102.70	57.70	0.61	55.44	33.13													

Series	**Morogoro**	AFB1	AFB2	AFG1	AFG2	FB1	FB2	FB3	DAS	DON	NIV	NEO	F-X	3-ADON	15-ADON	AOH	HT-2	T-2	OTA	AME	STERIG	ROQ-C	ZEN	ENN B
2	1 : Misufini S	4.94	1.40			188.10	83.32	52.30	0.61	55.44														
2	2 : Misufini D	4.94	1.40			286.80	115.81	65.80	0.61	215.10	33.13										4.35		16.33	
2	3 : Kihonda Mbuyuni S	1.60	1.40			153.50		48.10	0.61	55.44														
2	4 : Kihonda Mbuyuni D	31.71	3.30	3.9	2.19	481.90	151.34	91.50	2.30	55.44	33.13								2.81					
2	5 : Kingalu S	5.88	1.40		2.19	88.40	22.29	21.20	0.61	55.44	33.13										4.35			
2	6 : Kingalu D					107.40	43.99	21.20		579.90	255.80										4.35		132.40	
2	7 : Nunge S	1.60	1.40			207.40	76.57	54.70		55.44	33.13													
2	8 : Nunge D	1.60		1.77		779.10	327.94	114.70	0.61	468.70	33.13	7.86											37.00	
2	9 : Mtawala S	1.60				29.12	22.29	21.20																
2	10 : Mtawala D	1.60				528.60	167.20	88.00	0.61	55.44	33.13													
2	11 : Chamwino Mwembeni S					141.00	50.54	42.90	0.61	501.40	33.13				43.6								108.50	
2	12 : Chamwino Mwembeni D	1.60	1.40	1.77		395.60	141.93	73.80	0.61	248.40	33.13												92.90	
2	13 : Fourways S	1.60		1.77		29.12				173.70	33.13										4.35		16.33	
2	14 : Fourways D					875.90	336.59	123.60	1.84	339.00	33.13	7.86												
2	15 : Nanenane S	4.10	1.40		2.19	189.40	62.61	48.60	0.61	55.44	33.13												16.33	
2	16 : Nanenane D	1.60	1.40			280.80	101.75	57.70	0.61	55.44	33.13												42.30	
2	17 : Msamvu mataa S	6.20	1.40			138.40	43.70	43.50	0.61	55.44	33.13										4.35			
2	18 : Msamvu mataa D	1.68	1.40			489.30	178.22	90.40	0.61	119.40	33.13										4.35		16.33	
3	19 : Sabasaba S	8.93	1.40			167.20	49.21	21.20																
3	20 : Sabasaba D	8.93	1.40			485.60	166.82	56.60	0.61	55.44														
3	21 : Mawenzi S					196.00		21.20	0.61	343.60													16.33	
3	22 : Mawenzi D					872.00	347.04	91.00																
3	23 : Mkwajuni Kichangani S	1.60				152.40	22.29	21.20																
3	24 : Mkwajuni Kichangani D	1.60				1609.20	588.34	193.50																
3	25 : Chamwino kwa Geta S	1.60				140.10	22.29	21.20		55.44														
3	26 : Chamwino kwa Geta D	1.60				4518.70	1818.11	487.60																
3	27 : Kihonda Maendeleo S	40.01	3.41	19.90	2.19	418.50	130.15	45.20													4.35			
3	28 : Kihonda Maendeleo D	867.41	97.13	766.80	147.51	881.30	322.81	89.20																
3	29 : Mwembe Songo S	11.34	1.40			137.00	22.29	21.20		55.44									2.81		4.35		16.33	
3	30 : Mwembe Songo D	1.60				59.60		21.20		55.44	33.13										4.35		49.30	

Series	Dar es Salaam	AFB1	AFB2	AFG1	AFG2	FB1	FB2	FB3	DAS	DON	NIV	NEO	F-X	3-ADON	15-ADON	AOH	HT-2	T-2	OTA	AME	STERIG	ROQ-C	ZEN	ENN B
3	1 : Manzese S	1.60				29.12			0.61															
3	2 : Manzese D	5.36	1.40			631.30	204.44	65.30	0.61	55.44														
3	3 : Manzese S	1.60				29.12			0.61	55.44											4.35		16.33	
3	4 : Manzese D	1.60				343.60	157.80	21.20	0.61	291.90														
3	5 : Tandale S	1.60				29.12			0.61	55.44														
3	6 : Tandale D					29.12				305.70	33.13												16.33	
3	7 : Tandale S	1.60				113.10	22.29	21.20	0.61	55.44														
3	8 : Tandale D	3.78				513.60	182.21	49.20	1.96	183.80												0.99		
3	9 : Tandika S	1.60				312.30	104.88	21.20													4.35			
3	10 : Tandika D	51.35	1.40	65.30	2.19	3536.30	1284.50	398.00											2.81		4.35			
3	11 : Tandika S	1.60		1.77		145.50	22.29	21.20	0.61										2.81					
3	12 : Tandika D	1.60				252.00	86.07	21.20	2.30	55.44													16.33	
4	13 : Buguruni S					74.50		21.20	0.61	55.44	33.13			4.48										
4	14 : Buguruni D					593.10	210.43	95.90	1.96	498.20	33.13												39.10	
4	15 : Buguruni S	1.60				317.30	103.08	64.10																
4	16 : Buguruni D					278.60	115.14	49.00	0.61		33.13													
4	17 : Ilala S	1.60	1.40			354.80	112.77	70.40	3.45		33.13													
4	18 : Ilala D	135.45	14.63	13.10	2.19	141.80	53.87	21.20	0.61															
4	19 : Ilala S	1.60	1.40			178.20	22.29	21.20	2.42	172.50	33.13													
4	20 : Ilala D	30.03	3.74			343.90	132.34	55.90	1.50	411.20	33.13												16.33	
4	21 : Kijitonyama S					29.12		21.20	0.61	272.50	70.20										4.35		49.70	
4	22 : Kijitonyama D					29.12				55.44	87.80								2.81		4.35		16.33	
4	23 : Kijitonyama S	1.60	1.40			108.50	22.29	21.20													4.35			
4	24 : Kijitonyama D	15.20	3.52	3.90	2.19	406.80	137.47	69.60	0.61	55.44													16.33	
4	25 : Mikocheni S	1.60				29.12			0.61	177.50	212.00										4.35		58.10	
4	26 : Mikocheni D	1.60	1.40	4.50		309.10	118.66	51.00	0.61	136.90	33.13								2.81					
4	27 : Mikocheni S					129.70		21.20	0.61	55.44	33.13												16.33	
4	28 : Mikocheni D	1.60				1063.90	438.52	145.30	3.80		33.13													
4	29 : Namanga S	17.96	3.41	1.77		327.40	111.82	53.20											2.81		4.35			
4	30 : Namanga D	11.55	1.40			809.70	290.42	106.10	2.76	506.30	33.13													

Present	Confirmed: meets all criteria and above LOD
Confirmed but below LOD	Suspected: contains traces because meets criteria, but below LOD
Not present	Not confirmed: does not meet 1 or more criteria
S = sembe = dehulled	
D = dona = undehulled	

Appendix VIII: Chromatograms of the detected components



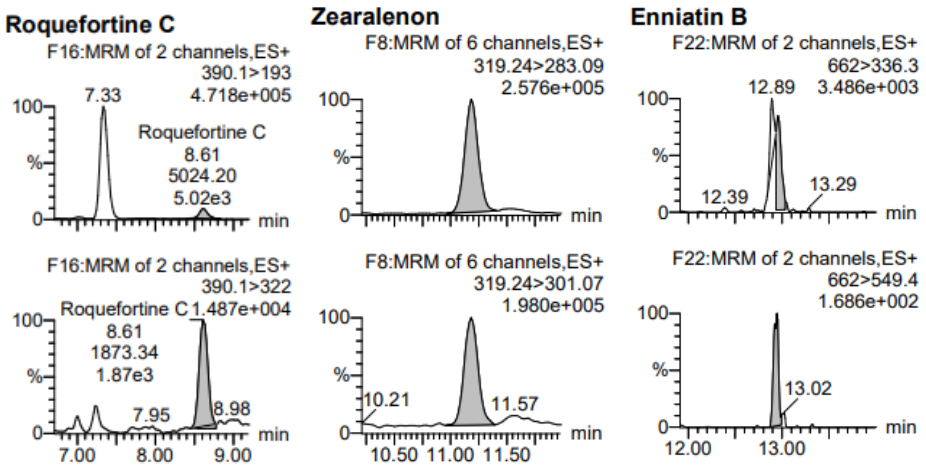


Figure 8.2. One clear chromatogram of the 19 mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEN, FB1, FB2, FB3, NIV, 3-ADON, 15-ADON, DAS, F-X, NEO, ROQ-C, STERIG, and ENN B) found in the maize flour samples using LC-MS/MS.

Appendix IX: Descriptive analysis represented by histograms

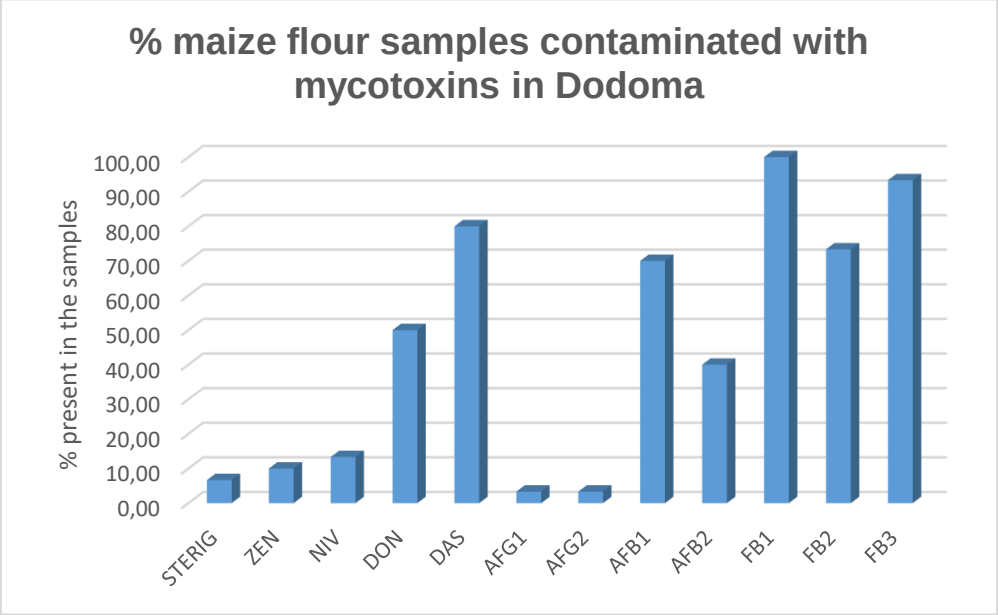


Figure 8.2. Histogram presenting the percentage of contaminated maize flour samples in Dodoma for the most common mycotoxins (STERIG, ZEN, NIV, DON, DAS, AFs and FUMs).

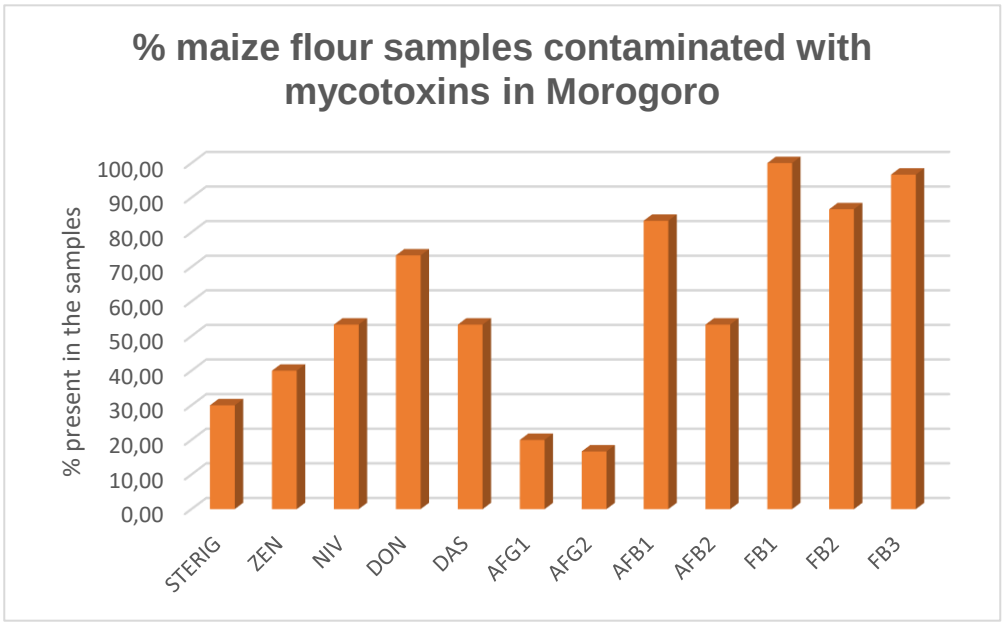


Figure 8.3. Histogram presenting the percentage of contaminated maize flour samples in Morogoro for the most common mycotoxins (STERIG, ZEN, NIV, DON, DAS, AFs and FUMs).

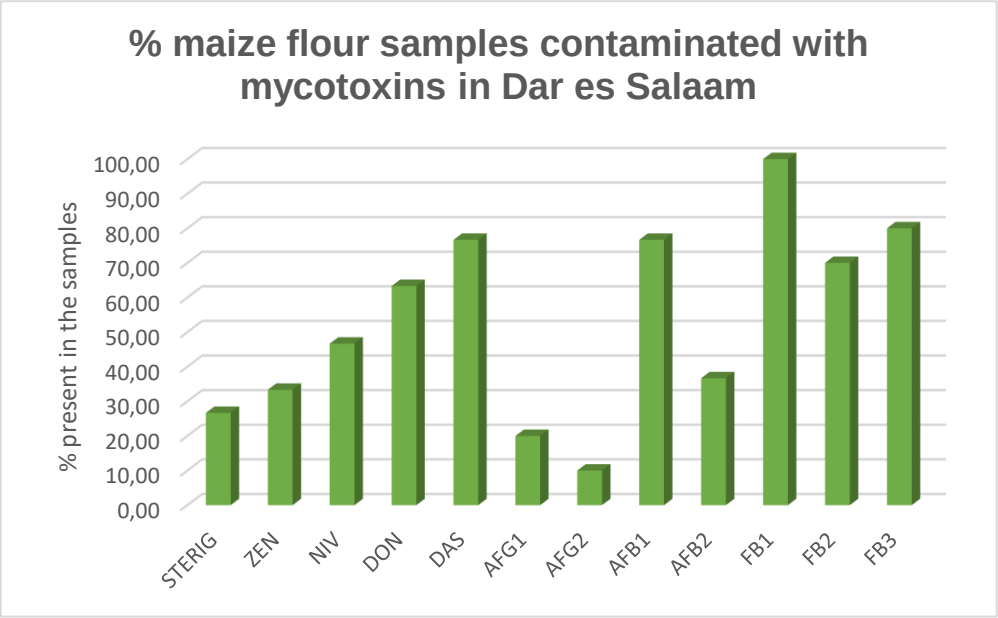


Figure 8.4. Histogram presenting the percentage of contaminated maize flour samples in Dar es Salaam for the most common mycotoxins (STERIG, ZEN, NIV, DON, DAS, AFs and FUMs.

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