

**AFLATOXICOLOGICAL RISK OF HOME-MADE COMPLEMENTARY FOOD
FORMULAE AND CAREGIVERS' COMPETENCY FOR MITIGATION: THE CASE
OF AMURU AND NWOYA DISTRICTS, UGANDA**

BY

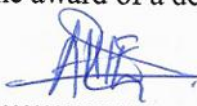
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**A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND
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GULU UNIVERSITY**

OCTOBER, 2025

DECLARATION

I **Eunice Achiro**, hereby declare that this dissertation is my original work and has never been submitted for the award of a degree in any higher institution of learning.

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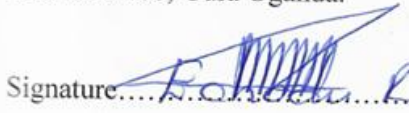
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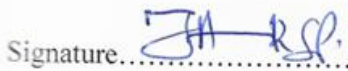
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DEDICATION

I dedicate this book to my parents, the late Lt. Col. Charles Odur Alele and the late Mrs. Sr. Margaret Atim Odur, who set a strong family foundation that enabled me to achieve all in my education without much hindrance, even in their absence. Also this book is dedicated to my children Odongo Meshach Bob, Odongo Shadrach Bob, Odongo Michelle Fokwesiza, and Odongo Laetitia Sheryln who motivate me to work hard.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB ₁	: Aflatoxin B ₁
ANOVA	: Analysis of Variance
CAC	: Codex Alimentarius Commission
CFF	: Complementary Food Formulae
DALYs	: Disability Adjusted Life Years
DNA	: Deoxyribonucleic Acid
<i>E. coli</i>	: <i>Escherichia Coli</i>
EAC	: East African Community
EC	: European Commission
EDI	: Estimated Daily Intake
ELISA	: Enzyme-Linked Immunosorbent Assay
FAO	: Food and Agriculture Organization
FBI	: Food-borne Illnesses
FDA	: Food Drug Authority
FGD	: Focus Group Discussions
FHP	: Food Hygiene Practices
FSA	: Food Safety Attitude
FSK	: Food Safety Knowledge
GAP	: Good Agricultural Practices
GHP	: Good Hygiene Practices
GMP	: Good Manufacturing Practices
GUREC	: Gulu University Research and Ethical Committee
HACCP	: Hazard Analysis Critical Control Point
HBV	: Hepatitis B Virus
IARC	: International Agency for Research on Cancer
IDPs	: Internally Displaced Peoples' Camps
KAP	: Knowledge, Attitude, and Practices
Kcal	: Kilo calories
Kg-1bw day ⁻¹	: Per Kilogram per body weight per day

MMSSC	:	Malted Millet Sesame Soy Composite
MOH	:	Ministry of Health
PMTDI	:	Provisional Maximum Tolerable Dietary Intake
Ppb	:	Parts per billion
SARS	:	Severe Acute Respiratory Syndrome
SPSS	:	Statistical Package for Social Scientists
SSA	:	Sub - Saharan Africa
TAF	:	Total aflatoxin
UBOS	:	Uganda Bureau of Statistics
UNCST	:	Uganda National Council of Science and Technology
UNICEF	:	United Nations Children's Fund
USD	:	United States Dollars
US-FDA	:	United States- Food and Drug Authority
WFP	:	World Food Programme
WHO	:	World Health Organisation

ABSTRACT

The safety of home-made complementary foods is one of the critical factors that impact negatively on nutrition outcomes among children in rural areas in low- and middle-income countries including Uganda. This study examined the aflatoxicological safety of a home-made Malted Millet Sesame Soy composite (MMSSC) complementary food and caregiver's competency for mitigation in Acholi sub-region of Uganda. Specifically, the study: (i) examined along the processing points of the MMSSC across wet and dry season, the prevalence of aflatoxigenic *Aspergillus* species, aflatoxin contamination, and exposure of children to aflatoxin; and (ii) cross-sectionally assessed caregivers' food safety knowledge (FSK), attitude (FSA), and hygiene practices (FHP) and influence of caregivers' competence on contamination levels. Descriptive statistics and multivariate binary logistic regression was used to analyze quantitative data and thematic content analysis for qualitative data. Pearson Chi-square was used to compare the prevalence of *Aspergillus* species and one-way analysis of variance (ANOVA) and Duncan's post-ANOVA test for mean comparison of aflatoxin concentrations. The *t*-test was used to assess differences in means and pair-wise correlation analysis to test for the significance of the correlation. Overall prevalence of *Aspergillus* species was low (7.3%) but was highest in the wet than dry season by 39 %. All raw materials and processed products (100 %) had detectable levels of total aflatoxin (TAF: $0.578\mu\text{gkg}^{-1}$ - $1.187\mu\text{gkg}^{-1}$) and aflatoxin B₁(AFB₁: $0.221\mu\text{gkg}^{-1}$ - $0.649\mu\text{gkg}^{-1}$) irrespective of the processing point, and in all cases, contamination was below the European Commission (EC) maximum tolerable levels for TAF ($0.4\mu\text{gkg}^{-1}$) and AFB₁ ($0.1\mu\text{gkg}^{-1}$). The average exposure of children ($15.7 \pm 12.6\text{ ngkg}^{-1}\text{bwday}^{-1}$) was much higher than the Provisional Maximum Tolerable Dietary Intake for aflatoxin ($0.4\text{ngkg}^{-1}\text{bwday}^{-1}$ - $1.0\text{ngkg}^{-1}\text{bwday}^{-1}$). In terms of competency, most caregivers had sufficient FSK (74.1%) and positive FSA (68.1%) but only 17.6% practiced FH largely due to inadequate sanitation facilities and high work load. FSK and FHP were associated (positively) with aflatoxin contamination and exposure of children dependent on the processing point and season ($P \leq 0.05$). The highest aflatoxin contamination and exposure were in households that had insufficient FSK and poor FHP. This study has demonstrated that home-made MMSSC is aflatoxicologically unsafe. Thus, a concerted effort is needed to improve FSK and FHP to minimize aflatoxin contamination of MMSSC and exposure of children.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Food safety challenges especially for weaning children (6-23 months) pose threats to children's health (Sheth & Dwivedi 2006; Theurich *et al.*, 2020). Moreover, adequate nutrients and their utilization at this phase after exclusive breastfeeding play an important role in the trajectory of growth and development of the children. Contamination of complementary food formulae by pathogenic microorganisms may play a major role in causing short-term and long-term illness (Scallan *et al.*, 2015; Malik *et al.*, 2021; Beshiru & Igbiosa, 2023). On the introduction of complementary foods at half a year and onwards, cases of the various burdens of diseases including diarrheal and non-communicable diseases have been registered. Contamination can occur from production to consumption (in the raw material, during processing, storage, preparation, and after preparation (Kaaya & Warren, 2005, Sheth & Dwivedi, 2006, Islam *et al.*, 2012). To avoid contamination of complementary foods, they should be safely prepared and stored (Grummer-Strawn & Rogers, 2021).

Food contamination by pathogenic microorganisms is a serious public health challenge globally (Hald *et al.*, 2016). Universally, pathogenic microorganisms and chemical substances account for more than 250 diseases that lead to death ranging from diarrhea to cancer (Scallan *et al.*, 2015; Acheson 1999; Newell *et al.*, 2010). An accurate estimate of the burden of food-borne diseases is still a global challenge. This is because data showing trends of occurrence of food-borne diseases is underestimated and limited to a few high-income countries and fewer pathogens due to inadequate diagnostic tools (Olumide 2016; Pires *et al.*, 2021; Davedow *et al.*, 2022). Microbial food contamination is more pronounced in low-income and middle-income regions of the world largely due to poor hygiene (Baluka *et al.*, 2015). Poverty and lack of awareness have been pointed out as one of the main contributing factors to poor hygiene that leads to exposure to food-borne pathogens (Okoth & Ohingo, 2005). In addition, the lack of proper implementation of the hazard analysis critical control points (HACCP) measures during food production has been reported to account for food contamination (Lund & O'Brien, 2014; Lamuka, 2014; Olumide, 2016a; Odeyemi, 2016b). Sub-Saharan Africa (SSA) is a breeding ground for the outbreak and

spread of foodborne diseases (Kaferstein, 2003; Kunadu *et al.*, 2016). The challenge is exacerbated by high-temperature changes in the tropical environment that favor the proliferation of food-borne pathogens, especially at production and storage points (Tirado *et al.*, 2010; Lone & Malik, 2021).

Microorganisms likely to contaminate locally formulated complementary foods are *Salmonella typhimurium*, *Campylobacter jejuni* and, *E. coli*, *Listeria monocytogenes*, *Shigella* spp (serogroups: - *S. sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*), *Vibrio* spp, *Norovirus*, *Hepatitis A*, *rotaviruses*, newly emerging viruses such as severe acute respiratory syndrome (SARS), enterotoxigenic *E.coli*, *Bacillus cereus*, *Clostridium botulinum*, *Staphylococcus aureus* and toxigenic fungi (*A. flavus*, *A. parasiticus*, and *A. fumigatus*) and many parasitic food-borne pathogens such as *Ascaris*, *Cryptosporidia* and *Trichinella*) among others (Kaaya & Warren, 2005; Sheth & Dwivedi, 2006; Newell *et al.*, 2010; Islam *et al.*, 2012; Anyogu *et al.*, 2021).

Although industrially formulated complementary food formulae are less prone to microbial contamination due to controlled production conditions (Kaaya and Warren, 2005; Magdovitz *et al.*, 2020; Kamboj *et al.*, 2020), they are relatively expensive and cannot easily be afforded by resource-poor rural communities (Akinola *et al.*, 2014; Osendarp *et al.*, 2016). In Uganda, such communities are located in the post-conflict areas such as Amuru and Nwoya districts that are in the secondary adjustment phase (after emergencies) when war-affected communities returned to their original homes after the official closure of internally displaced peoples' (IDPs') camps in 2006 (Nassanga *et al.*, 2018). Under these circumstances, relief aid was reduced and formally displaced communities started carrying out their livelihood activities. As part of the adjustments, households started using locally available foods for their nutritional needs. The key area of nutritional adjustments is the local production of formulated complementary foods for weaning children using locally available raw materials (Kounnavong *et al.*, 2011; Nassanga *et al.*, 2018). However, such products may be more prone to microbial contamination due to household uncontrolled storage conditions. This may cause food-borne diseases when consumed and consequently affect the health and nutritional well-being of the children.

Generally, the burden of food-borne diseases in Africa accounts for over 91 million ($\frac{1}{3}$) of the global toll of death. The most affected are the immuno-suppressed people, the elderly, and children mainly those under five (WHO, 2015). This scenario is critical because food is a basic human need therefore, people are predisposed to the risk of infection and intoxication if safety is not assured (Tauilo *et al.*, 2008; Gallo *et al.*, 2020; Marat-Mendes *et al.*, 2021). In low- and middle-income countries, most food-borne disease outbreaks are underreported (Olumide, 2016). Although there is limited data on the food disease outbreaks in many low- and middle-income countries, especially in Africa, there are reported incidences of food-borne diseases. For instance, a previous study from East Africa indicates that Kenya had experienced several recurrences of acute aflatoxicosis in humans summing up to 317 cases, and 125 deaths (Wagacha & Muthomi, 2008).

In Uganda, cereals and grains, the main ingredients for complementary food production have aflatoxin contamination higher than the FDA/WHO and EAC recommendation of 20ppb and 10 ppb respectively (Kaaya & Warren, 2005; Echodu *et al.*, 2019a; Lukwago *et al.*, 2019). Regardless of the empirical proof of the harmful effect of microbial contaminants on human health, human exposure, and prevention have not been prioritized in resource-poor rural households (Asiki *et al.*, 2014). Moreover, even within the overburdened low and middle-income countries, exposure rates to toxigenic microorganisms are higher in rural populations than in urban populations because of poor sanitary practices (Atherstone *et al.*, 2014).

A study by the Ugandan Ministry of Health and the World Health Organization (MOH/WHO) in 2015 revealed that about 1.3 million Ugandans are diagnosed with food-borne diseases annually. The report indicates that 14 % of all diseases treated yearly are due to food contamination (WHO, 2015). Socio-economically, food-borne pathogens cause illnesses leading to threatened human health, reduced productivity, and increased cost of treatment hence escalating poverty and disrupting the national economy in the long run (Fung *et al.*, 2018). Among the human population, children especially children (6-23 months old) are among the most vulnerable to food-borne infection/ intoxication when calculated as $\text{mgkg}^{-1}\text{bodyweight}^{-1}$ since all their body systems are underdeveloped (Cohen *et al.*, 1994; Piccinelli *et al.*, 2010; Vasco & Alvito, 2011; Ross, 2015). In Africa, children mainly those under-five account for $\frac{1}{2}$ of the global food-

borne related death and diarrheal diseases contribute 70% of the reported death. In Uganda, 40% of the food-borne disease burden is on children below five years old (WHO, 2015). Moreover, good health during the first 1000 days is crucial for human and economic development (Hlaing *et al.*, 2016). This growth phase in children, therefore, is a significant critical window for preventing the threat of food-borne illnesses in children.

The unanswered question, however, is whether the local production predisposes locally made complementary food formulae to the risk of microbial contamination specifically the toxigenic pathogens such as *Aspergillus species* and related toxins. This is because aflatoxin cannot be inactivated at typical home cooking temperatures of 75 - 120 °C although, most complementary food formulae are heat-treated, (Coe and Spiro, 2022). There is need to determine the TAF and AFB₁ contamination levels, related exposure of children (6-23 months old) to total aflatoxin and aflatoxin B₁, caregivers competence and the influence of competence on aflatoxin levels.

It is important to answer the above question because if the safety of locally produced complementary food formulae is not guaranteed then proper health and nutritional outcomes among children cannot be reached (Newell *et al.*, 2010). This is because the vital elements to achieving good nutrition outcomes among weaning children (6-23 months old) are safe and nutritious complementary foods (Osendarp *et al.*, 2016). Progress and tangible achievements have been made in the area of nutritional quality (Akinola *et al.*, 2014 & Hlaing *et al.*, 2016) even though safety, a critical element of food utilization that significantly affects nutrition outcomes in children has received limited attention (Olumide, 2016). Successes in developing locally formulated complementary food composite may not achieve the expected nutrition outcome if safety elements are not adequately addressed. Therefore, complementary food formulae should not only be nutritious but also safe (WHO, 2015; Warnatzsch *et al.*, 2020).

Attempts to reduce the burden of food-borne diseases require monitoring food-borne pathogens in the production-consumption continuum while encouraging improved and incorporating Hazard Analysis Critical Control Point (HACCP) principles, a structured approach to food safety (Newell *et al.*, 2010).

To ensure food safety, sufficient food safety knowledge, a positive attitude towards good agricultural practices and good hygiene practices are required right from initial production at pre- and postharvest levels. This entailed adherence to good agronomic practices, proper postharvest management, and good hygiene practices (Udomkun *et al.*, 2018; Gonçalves *et al.*, 2019; Godswill Awuchi *et al.*, 2020; Jallow *et al.*, 2021; Abdeta & Milki, 2023). Studies have linked positive attitude, sufficient knowledge, and appropriate practices to safe food (Cissé, 2019; Abdulrahman *et al.*, 2020; Namubiru *et al.*, 2022; Abdeta & Milki, 2023). However, most of these studies were done in formal settings where production is done under controlled conditions.

Additionally, the association between KAP and aflatoxin contamination levels and related exposure levels was not indicated especially in rural settings. Therefore, to bridge the knowledge gap, a critical need remained to examine food safety KAP of caregivers of weaning children and its association with aflatoxin contamination and their exposure in such settings. As a mitigation measure to reduce exposure of weaning children to aflatoxins, this study determined the association of the KAP of caregivers of weaning children and the level of aflatoxin contamination of home-made complementary food formulae along the processing point across the wet and dry seasons of an agricultural year and resultant exposure. Thus, there is a need to ascertain the KAP of the caregivers and establish the microbial safety of locally formulated complementary food composite to realize good nutrition outcomes. There are various methods to assess the KAP and microbial and subsequent toxins contamination but, this study employed the Health Belief Model, molecular and, immunological techniques to establish the KAP and microbial and subsequent toxins contamination respectively.

1.2 Statement of the problem

Improper complementary feeding is one of the critical factors that contribute to poor nutrition outcomes among children 6-59 months old in low- and middle-income countries including Uganda. In the case of Uganda, the challenge is illustrated by the two National Demographic Health statistics carried out in 2016 and 2022 that show a consistently higher prevalence of stunting (29% and 26%), wasting (4% and 3%) and underweight (11% and 10%) respectively among children 6-59 months old based on WHO Z score of < -2 SD to < -3 SD from the median

of the reference population (WHO, 2006). The challenge is more prominent in rural areas where standardized industrially produced complementary food products are largely inaccessible. Thus, households in such localities largely use locally made plant-based complementary food formulae. However, the safety of locally formulated complementary food formulae has been identified as a critical issue of concern due to improper adherence to hygiene and sanitary practices in rural areas (Mshida *et al.*, 2020; Teshome *et al.*, 2022). Occurrence of *Aspergillus* species and subsequent mycotoxin contamination of dry plant-based food materials that constitute the principal components of locally produced home-based complementary food formulae (Echodu *et al.*, 2019; Lukwago *et al.*, 2019) is a serious safety element in rural areas. This is due to the potential of aflatoxins to cause liver cancer, impaired growth and stunting in children (Milki and Abdeta, 2023). Whereas complementary food products are usually heat-treated before feeding children, aflatoxins can withstand heat up to 150°C and above and cannot be inactivated at typical home cooking temperatures (75 - 120 °C), (Pankaj *et al.*, 2018; Coe and Spiro., 2022). Alowo *et al.*(2018), in cooperation with caregivers in Acholi sub-region developed the Malted Millet Sesame Soy Composite (MMSSC) targeting complementary feeding of children in rural areas and the product was well accepted by the community. However, aflatoxigenic safety of the product was not ascertained and thus it remains questionable as to whether or not, the product is safe from aflatoxins and cannot act as a dietary aflatoxin exposure route to children. In addition, caregiver competency in terms of food safety knowledge (FSK), attitude (FSA) and hygienic practices (FHP) for safety assurance of the MMSSC is also largely unknown. Understanding the identified knowledge gap is essential for designing strategies to assure aflatoxigenic safety of MMSSC to ensure that the food product contributes effectively to achieving better complementary feeding outcomes among children.

1.3 General objective

The overall objective of the study was to ascertain the aflatoxicological safety of homemade complementary food composite among households in Amuru and Nwoya districts of northern Uganda.

1.4 Specific objectives

The specific objectives of this study were to:

- 1) Determine the prevalence of *Aspergillus* species in the Malted Millet-Sesame-Soy Complementary Foods Composite (MMSSC) at three process points (raw materials, freshly processed products, and stored processed products) across the wet and dry seasons.
- 2) Determine the contamination levels of total aflatoxin and Aflatoxin B₁ at three process points (the raw materials, freshly processed products, and stored processed products) across the wet and dry seasons and assess the children's exposure to them.
- 3) Assess the status of knowledge, attitude, and practices (KAP) of caregivers of children (6-23 months regarding the safety of locally formulated complementary food composite.
- 4) Determine the influence of caregivers' KAP on aflatoxin contamination at three process points (raw materials, freshly processed products, and stored processed products) across the wet and dry seasons and children's exposure.

1.5 Research questions

The following research questions directed the study: -

- 1) What is the prevalence of *Aspergillus* species at three process points (raw materials, freshly processed products, and stored processed products (MMSSC)?
- 2) What are the contamination levels of total aflatoxin and aflatoxin B₁ in the raw materials, freshly processed products (MMSSC), and stored processed products (MMSSC) across the wet and dry seasons?
- 3) What are the exposure levels of the children (6-23 months) to total aflatoxin and aflatoxin resulting from consumption of locally formulated MMSSC?
- 4) What is the status of food safety knowledge, attitudes, and practices of caregivers of children (6-23 months) in Amuru and Nwoya districts of northern Uganda regarding locally formulated complementary food formulae?

- 5) What are the predictors of food safety knowledge, attitudes, and practices among caregivers of children (6-23 months) in the Amuru and Nwoya districts of northern Uganda?
- 6) What is the influence of caregivers' KAP on the contamination level of aflatoxin contamination at three process points (raw materials, freshly processed products, and stored processed products) across the wet and dry seasons and the related exposure?

1.6 Justification of the study

Food safety is an important element in ensuring good nutrition outcomes from using complementary food formulae. This can be achieved through adequate food safety KAP (Kaferstein, 2003, Okoth & Ohingo, 2005; Kunadu *et al.*, 2016) and implementing proper hazard analysis critical control point (HACCP) principles during food production (Lund & O'Brien, 2014; Lamuka, 2014; Olumide, 2016a; Odeyemi, 2016b). However, this requires information on caregivers' KAP and the prevalence of disease-causing microorganisms such as *Aspergillus* species and their toxins that persist in processing conditions that the production of complementary food goes through. Examining caregivers' KAP, the prevalence of *Aspergillus* species and subsequent aflatoxin is thus important for tracking sources of contamination. Even though many studies have determined the prevalence of *Aspergillus* species and aflatoxin contamination in complementary foods (Adejumo *et al.*, 2013; Kamala *et al.*, 2016; Aron *et al.*, 2017), species prevalence and aflatoxin concentrations in the food chain, especially at processing points are not well understood and yet this is a vital element in the control of microbial contaminants in food. Therefore, there was a need to study the prevalence of *Aspergillus* species, determine the aflatoxin concentrations along the processing points across the wet and dry seasons, and consequently determine the exposure of children (6-23 months) to toxins produced. Further, studies have attributed inadequate knowledge and self-practices as the main contributing factors to exposure to food-borne pathogens, particularly in a rural home setting where food production is mainly done (Kaferstein, 2003, Okoth & Ohingo, 2005; Kunadu *et al.*, 2016). Thus, determining the KAP of the caregivers of children (6-23 months) towards microbial food safety was essential in this study to ascertain the factors that predict their status.

1.7 The significance of the study

Information on the prevalence of *Aspergillus* species, aflatoxin contaminations, and related toxins at processing points of locally made Malted Millet Sesame Soy Composite (MMSSC) alongside exposure of the children would be beneficial to the caregivers, composite producers for tracking sources of contamination and control contamination and minimize exposure of the children to aflatoxins and hence ensures good nutrition outcomes from locally formulated food formulae.

In addition, examining the FSK, FSA, and FHP and subsequently assessing the influence of caregivers' competency on aflatoxin contamination and exposure of the children through consumption of MMSSC is vital towards achieving microbial safety of locally formulated complementary food composite. This is useful in understanding the contribution of food safety culture of the caregivers to microbial safety risks that locally formulated complementary food pose to weaning children hence minimizing their exposure.

As indicated in Appendix 8, the guidelines for producing complementary food formulae focused largely on industrial production using controlled conditions. The results of this study, therefore, add to the information on food safety risk management. This could provide information for to develop an HACCP plan to use in local production of complementary food formulae in a rural setting and enhance the safety of locally formulated complementary food formulae. In addition, determining toxins concentrations and exposure of children (6-23 months) to toxins produced by toxigenic microorganisms will provide evidence to justify the control of food-borne pathogens. Ultimately, the results of this study would be of use to inform policy on locally formulated food products to protect children from adverse exposure to aflatoxin during complementary feeding.

1.8 Conceptual framework

The independent variables are the socio-economic factors (sex, age, occupation and level of education of caregiver, food safety training, decision on household income), and technical factors (Seasonality and processing point) that influence microbial contamination of locally

made MMSSC. Intermediate factors include food safety knowledge, experience, and attitude whose levels are influenced by the independent variables. The last sets of variables are the dependent variables related to the observed outcomes of the interaction between the independent and intermediate variables. These include microbial contaminants and related toxin and exposure of weaning children.

The conceptual frameworks present the inter-relationship between these variables with each inter-relationship reflecting a specific objective that was investigated in this study. The the first and second objectives determined the prevalence of *Aspergillus* species and the concentrations of total aflatoxin and Aflatoxin B₁ respectively at three process points (the raw materials, freshly processed products, and stored processed products) of locally made MMSSC across the wet and dry season and assess the children exposure to them. The key assumption for the two objectives is that an understanding of the prevalence of toxigenic *Aspergillus spp* and the level of aflatoxin contamination may contribute to their control along the production continuum and may reduce TAF and AFB₁ contamination by prioritizing critical processing points based on the season.

The objective third and fourth objectives assessed the knowledge, attitude, and practices (KAP) of caregivers of children (3-23 months old) regarding the microbial safety of locally formulated complementary food composite and KAP influence on contamination levels. In these objectives, the assumption was that social demographic factors may influence food safety knowledge and attitude which inturn influence contamination levels. An understanding of the predictors of food safety knowledge and attitude may help in improving food hygiene practices among caregivers and hence controlling the contamination.

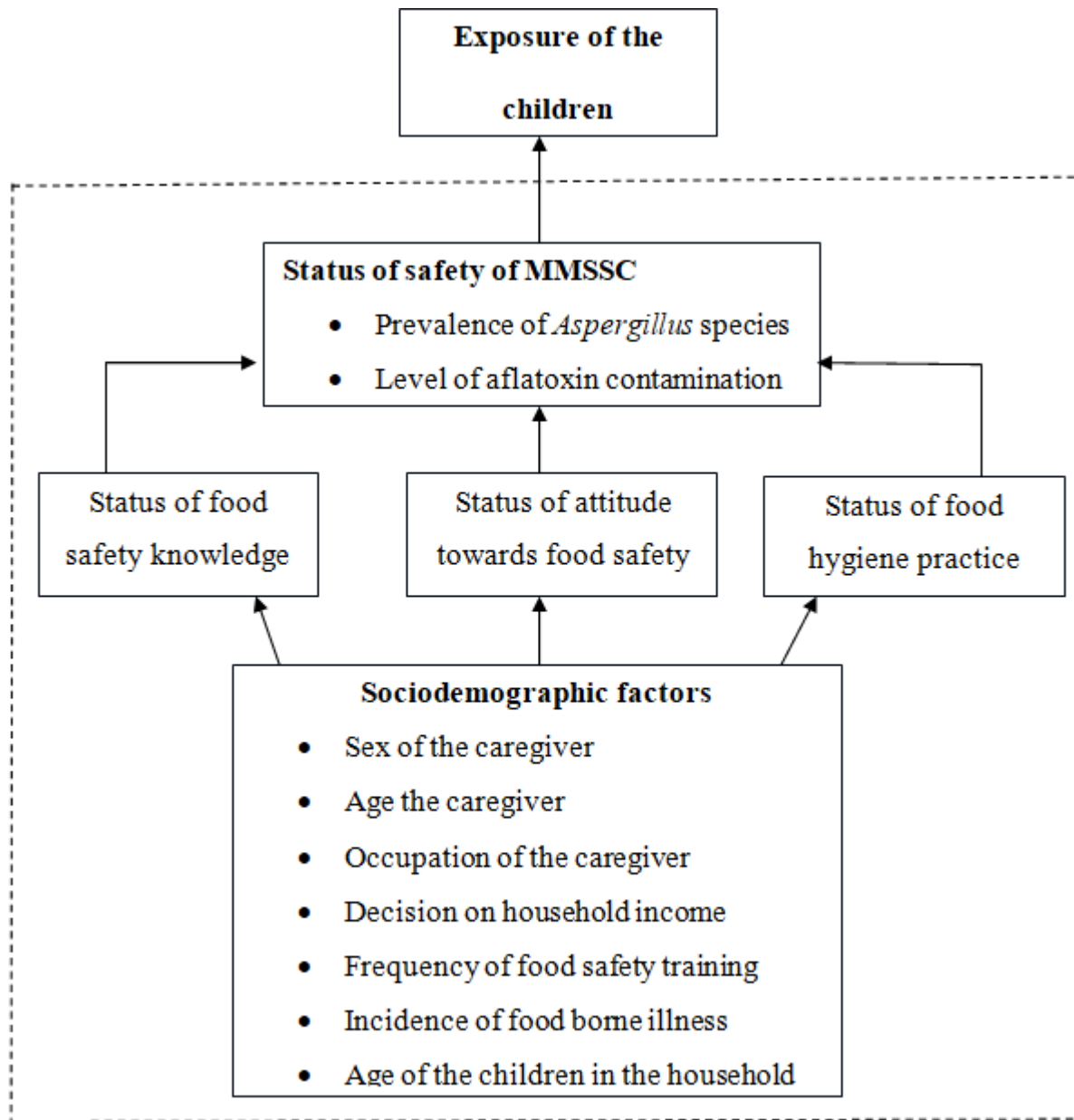


Figure 1: Study conceptual framework based on literature(Guloba *et al.*,2018, Lukwago *et al.*, 2019, Grosshagauer *et al.*, 2020, Aworh, 2021 and Atube *et al.*, 2022)

1.9 Scope of the study

The study was done in the Amuru and Nwoya districts in the Acholi Sub-region of Uganda from 2018 to 2019. The two districts were selected to follow up on a study done by Alowo *et al.* (2018). These districts were purposively chosen based on their high rates of under-nutrition in the Acholi sub-region of Uganda according to the World Health Organization (WHO) classification of the prevalence of malnutrition (UBOS, 2016). Alowo *et al* (2018) therefore, locally produced a model complementary food formula (MMSSC) that was readily accepted and used. However, the safety of the product was not ascertained. Thus, this study sought to evaluate the aflatoxicological risk of homemade complementary food formulae and caregivers' competency for mitigation of aflatoxin contamination and related exposure through the consumption of complementary foods that are locally produced.

CHAPTER TWO: LITERATURE REVIEW

2.1 Microbial safety of complementary food formulae

Although breast milk is best for infants, it cannot support optimal growth even from a well-nourished mother at six months, (Eidelman, 2012; Victora *et al.*, 2016; Allen & Hampel, 2020; Silfverd *et al.*, 2023). Therefore, complementary feeding should thus be introduced at 6 months to support optimal growth requirements (Sihag *et al.*, 2015; WHO, 2023). Complementary food formulae are follow-on food and other food products having more than one food commodity ingredient makeup designated for infants and young children (International Commission on Microbiological Specifications for Foods, 1986; Lutter *et al.*, 2021). Based on the availability as well as affordability of the primary commodity, complementary food formulae are categorized as plant and animal products (Taylor *et al.*, 2010; Asare *et al.*, 2022; Simeone *et al.*, 2022). Several standardized quality complementary food formulae are available in the local and international markets (Lutter *et al.*, 2021; Faber *et al.*, 2022). However, they are expensive and resource-poor communities in low- and middle-income countries can not afford them.

As pointed out in the literature, nutritious cereal-based Complementary Food Formulae (CFF) can be relatively affordable and accepted by low-income populations in rural communities (Sihag *et al.*, 2015). In such countries, the main source of nutrients for young children is plant-based and may not adequately provide safe and nutritious food (Mensah & Tomkins, 2003). These could be due to the presence of contaminants that may be biological, chemical, or physical and anti-nutritive factors such as tannins, lectins, inhibitory enzymes, and phytates that may interfere with the bioavailability of the nutrients (Mensah & Tomkins, 2003; Masuke *et al.*, 2021; Oke *et al.*, 2022). Empirically, poor breastfeeding and complementary practices may predispose many infants and young children to short and long-term health effects leading to poor nutrition outcomes (Lutter, 2003; Palmieri *et al.*, 2018). However, safe and nutritious foods are children's right and are essential for fulfilling children's optimal health status and the overall growth and well-being of the weaning children (WHO/UNICEF, 2003).

The inability of rural communities to feed weaning children on standardized complementary food formulae (CFF) has compelled many rural communities to feed children on CFF made from locally available materials (Nassanga *et al.*, 2018).

Locally made CFF is widely utilized by rural communities because they are relatively cheap and are made from locally available raw materials (Amagloh *et al.*, 2012; Desalegn, *et al.*, 2015). However, pathogenic microorganisms and associated toxins have been isolated from several raw materials and processed formulations of locally produced complementary food formulae (Kaaya & Warren, 2005; Lewis *et al.*, 2005; Oh *et al.*, 2012; Achiro *et al.*, 2023). Such situations pose major food safety concerns as production safety guidelines adopted for use in rural households are not readily available even though empirical evidence indicates that depending only on the hygienic conditions observed during processing may pose a safety risk since some food contaminants may persist the usual good hygiene practices and survive processing condition (McHugh *et al.*, 2017). For instance, MMSSC is accepted by the rural communities in the Amuru and Nwoya districts of Uganda and is being produced from within the household using locally available raw materials (millet, sesame, and soybean) (Nassanga *et al.*, 2018 and Alowo *et al.*, 2018). Moreover, the FSK, FSA, and FHP of the caregivers of weaning children that are vital factors and affect the safety of weaning food are not well understood. Studies have addressed the KAP of the producers of such products, although, these are mainly limited to the final products. Therefore, the gap remains to address the KAP along the processing points across the seasons.

2.2 Food safety knowledge, attitude and food hygiene practices (KAP) of weaning foods among caregivers of children (6-23 months)

Food safety KAP may be key in influencing the food safety of weaning foods/complementary foods. Literature affirms that KAP influences the safety of foods since they may directly affect practices that influence food contamination (Kunaduet *et al.*, 2016; Cunha *et al.* 2019; Kwol *et al.*, 2020; Gallo *et al.*, 2020; Tuglo *et al.*, 2021). For instance, food hygiene practices and postharvest practices have a linkage to control of food contamination and safe foods (Aron *et al.*, 2017; Echodu *et al.*, 2019; Ojuri *et al.*, 2019; Mollay *et al.*, 2021; Hoffmans *et al.*, 2022;

Namubiru *et al.*, 2022). Several studies have linked positive attitude, sufficient knowledge, and appropriate practices to safe food (Cissé, 2019; Abdulrahman *et al.*, 2020; Namubiru *et al.*, 2022; Abdeta & Milki, 2023). In particular, caregivers' KAP is linked to the safety of complementary foods (Rahman *et al.*, 2022; Niare *et al.*, 2023).

Furthermore, Socio-demographic factors are key variables that moderate the influence of food safety KAP (Abid *et al.*, 2022). KAP is affected by a multifaceted interaction between socio-demographic variables (Zyoud *et al.*, 2019; Al-Kandari *et al.*, 2019). Literature points out that social demographic variables such as education level attained, income earned, work experience, and the number of training on food safety significantly associate with KAP (Niare *et al.*, 2023). Other studies affirmed that KAP is affected by age, gender, level of education, and income levels (Zyoud *et al.*, 2019; da Vitória *et al.*, 2021; Haidari *et al.*, 2022). These studies have mostly been done in formal settings, not informal rural settings where production is done under uncontrolled conditions. Besides, these studies have not explained the disparity between KAP and contamination levels, particularly aflatoxin contamination and related exposure levels of weaning children in rural settings. Therefore, the information gap exists on the influence of the status of microbial food safety KAP of caregivers on aflatoxin contamination levels and exposure of weaning children in such settings. This study thus, determined the influence of the status of KAP of caregivers of weaning children on the level of aflatoxin contamination and related exposure levels across the wet and dry seasons as a mitigation measure to reduce exposure of weaning children to aflatoxins.

Complementary food formulae have a special function in the diet of children (6-23 months) because they are the major source of nutrients before the children are weaned (Mensah & Tomkins, 2003). Therefore, caretakers of children (6-23 months) need to be knowledgeable about food safety issues right from production to consumption at household levels (Brewer & Prestat 1994). The general food safety concerns that may influence consumers are based on several risk factors such as natural contaminants like microorganisms and their toxins, veterinary drug residues, heavy metals, agro-chemical residues, and unsafe packaging materials among others (Uyttendaele *et al.*, 2016; Madilo *et al.*, 2024).

Additionally, other factors that may significantly influence consumers' behaviors and choice toward food safety include food chemical issues (e.g. hormones and food additives), health issues (e.g. food nutrient imbalance), food regulatory issues (e.g. food inspection and labels), and deceptive practices e.g. weight-loss or gain diets (Meijer *et al.*, 2021; Núñez, 2021; Visciano, & Schirone, 2021).

Food safety factors such as improper handling practices from production to consumption have been recognized as critical issue to be assessed to prevent food-borne illnesses. Thus, consumer education regarding safe food handling practices in the domestic environment has been advocated as a key strategy to control food contamination (Bolek, 2020; Ncube, *et al.*, 2020). Understanding the behaviors of the caregivers and assuring the education of the caregiver regarding the risk of unsafe food handling practices is, therefore, an essential element for the prevention of illnesses associated with foods. This is because the handling of food including storage largely takes place in the domestic environment, especially at home (Kamboj *et al.*, 2020; Cardoso *et al.*, 2021). Thus, caregivers' behaviors at home may reflect their knowledge or beliefs regarding food safety (Meysenburg *et al.*, 2014).

Improper practices of food handling commonly reported are:- utilizing foods from unsafe sources; insufficient cooking; serving contaminated foods; cooling foods and inappropriate heating and re-heating foods allowing too much time lapse (Abuga *et al.*, 2017). It is therefore thought that caregivers' awareness, attitude, and practices towards safe food remain under consideration if “food safety” is completely defined and to determine the improper behaviors and beliefs of the caregivers that impact regular exposure to associated hazards on children.

Microbial-safe food awareness of the caregivers' of vulnerable populations is important since they are more susceptible to microbial infections and intoxication because their body systems are underdeveloped (Vasco & Alvito, 2011). Strategizing ways to ensure the safety of locally made food formulae necessitates prioritization for children's optimum well-being. This should be done while considering the nutrients in them hence warranting rural communities broadly benefit from locally made complementary foods. And so, the caregivers will have available safe nutritious food to ensure the optimal nutritional status of the children. If this is not done, only a

tiny fraction of the community who can afford standardized quality commercial complementary food will benefit from complementary feeding.

Despite this scientific fact, the caregiver's KAP regarding the safety status of complementary food in the local domestic environment is insufficiently known. Therefore, a study on the awareness, perception, and practices of the caregiver of the vulnerable subpopulation needs to be prioritized to educate local communities regarding food hygiene practices from production to consumption in the domestic environment. The Knowledge, Attitude, and Practice (KAP) model was used. The study explored respondents' behavior concerning food safety issues of complementary food formulae pointing out what the respondents know about (K), their feelings (A), and what they do about it (P) (Dong, 2015).

2.3 Microbial contaminants associated with complementary food formulae

Although WHO believes that safe food saves lives (WHO, 2015), empirical evidence demonstrates that commonly used local complementary foods have microbial contaminants greater than the permissible limit for Aerobic Plate Count (APC) of 4log CFU/g (Grosshagauer *et al.*, 2020). This may indicate that such complementary foods contain bacterial and fungal concentrations above the permissible international standard and resulting from poor food production, processing, and food handling due to inadequate or poorly enforced regulatory standards (Ezirigwe, 2018; Lukwago *et al.*, 2019). In the tropics, the challenge is compounded by a favorable tropical climate that enhances the proliferation of microorganisms and their occurring toxins (WHO, 2015; Aworh, 2021). In this regard, the WHO reported that microbial diseases related to food are still key in causing death and morbidity globally mainly in less affluent countries (WHO, 2015). Therefore, the estimation of the load of illnesses due to food consumption is reported as Disability Adjusted Life Years (DALYs) (WHO, 2015).

In Uganda, 5700 per 100,000 cases of foodborne illnesses may be associated with enteric pathogens (*Vibrio cholerae*, dysentery *Brucella spp*, hepatitis E virus, and invasive nontyphoidal and typhoidal *salmonella*, toxin-producing *Escherichia coli* (*E-coli*), *Enterohaemorrhagic E-*

coli, *Campylobacter* *ssp*, *Listeria monocytogenes*, *Shigella* spp, *Vibrio cholerae*, and toxigenic fungi (WHO, 2015).

While there is much information on food microbial contamination, little is known about the critical control points from primary production to consumption, especially for food designated for the vulnerable populace. Moreover, the cornerstone to ensuring safe and quality food sources depends on detecting contaminants along the food chain (Ge & Meng, 2009). Accordingly, it was paramount to prioritize the identification of microbial contaminants, specifically *Aspergillus* species and subsequent total aflatoxin and aflatoxin B₁ at a processing point of complementary food formulae. Assessing the contaminants at these points may improve understanding of the microbial contaminants and apply risk management strategies of locally made food products.

2.4 Genus *Aspergillus*

The population of organisms adapts to the changing environment based on the total number of genetic characteristics in the genetic makeup of a species (Pauls *et al.*, 2013). Genus *Aspergillus* is a fungal species that adapt to the tropical climate and consists of about 250 recognized species distinctively characterized by aspergillum, spore-bearing structure (Paterson & Lima, 2017; Nepal *et al.*, 2019). According to information available at the National Center for Biotechnology Information (NCBI), *Aspergillus* species have similar genome sizes ranging from 29 to 40 Mbp (de Vries 2017). Specifically, the genome size of *A. flavus* ranged from 35 to 36 Mbp; *A. parasiticus* is 30 Mb; *A. fumigatus* 28 to 30 Mbp and *A. niger* is 40 Mbp (Denning 2002; Paola, 2016; Ellena *et al.*, 2021).

Genus *Aspergillus* has many sections. Some members produce sexual states (teleomorphs) containing constant cleistothecial ascomycetes with well-arranged ascospores in dehiscent asci (Klich, 2007). But, section *flavi* contains species such as *Aspergillus flavus* and *Aspergillus parasiticus* that are economically important fungi producing aflatoxin and fewer aflatoxin producers includes *Aspergillus nomius*, *Aspergillus pseudotamarii*, *Aspergillus bombyosis*, and *Aspergillus parvisclerotigenus* (Ehrlich *et al.*, 2007; Levin, 2012). According to Frisvad *et al.* (2005), *Aspergillus ochraceoroseus*, *Aspergillus rambellii*, *Emericella venezuelensis*, and

Emericella astellata are the four species that produce aflatoxin but do not belong to section *flavi*. Amongst the aflatoxin-producing fungal species, *Aspergillus flavus* is the major species responsible for aflatoxin production and is the second leading cause of aspergillosis in immunocompromised human patients (Ivanova *et al.*, 2003).

Additionally, aflatoxin contamination is accountable for crop losses internationally, for instance, peanut and corn industries in the United States annually register between \$25 and \$58 million and \$ 280 million losses respectively due to aflatoxin contamination (Lamb & Sternitzke, 1996; Klich, 2007; Nierman *et al.*, 2015). In Sub-Saharan Africa where Uganda falls, 38% of global agricultural losses are due to aflatoxin totaling economic loss of USD 450 million (Madala *et al.*, 2018).

2.5 Identification of *Aspergillus* species

Several techniques are used for identifying fungal species. These include morphological identification involving culturing of fungal spores on selective media, whereby the spores are inoculated into the media such as Czapek yeast extract agar, malt extract agar, potatoes dextrose agar, and Rose Bengal agar (Heo *et al.*, 2019; Sohrabi & Taghizadeh, 2018). The macro-morphological analyses are then done involving measuring colony diameter, and determining the colony color, elevation, and texture (Díaz *et al.*, 2015; Tan *et al.*, 2021; Wati *et al.* 2023). Conversely, micro-morphological analyses involved examining conidiophores under the microscope (Tan *et al.*, 2021).

Another technique employed in the fungal identification is molecular analyses involving extracting genomic DNA from harvested mycelia and then amplifying using the internal transcribed spacer (ITS) region of ribosomal DNA using the universal ITS1 and ITS4 or ITS 1 and ITS 2 primers (Roohani *et al.*, 2018). The amplicons can then be purified, sequenced, and blasted to obtain the nucleotide sequences (Heo *et al.*, 2019). The extracts are amplified using universal ITS1 and ITS4 or ITS 1 and ITS 2 primers and species-specific primers (AL-Ezzy and Abdulameer 2021). Morphological and molecular identification methods are usually employed concurrently since the prior may not usually distinguish the vegetative form reliably.

Morphological analyses can be used as a screening technique (Ruibal *et al.*, 2008; Dave and Pal 2015; Roohani *et al.*, 2018) preceding PCR and sequencing analyses.

2.6 Contamination of complementary foods with Aflatoxins

Mycotoxins are toxic bi-products of fungal metabolism that cause food contamination in the field during primary production and at storage stage (Omotayo *et al.*, 2019). Mycotoxins such as aflatoxin have both direct and indirect negative impacts on the global economy. Directly, aflatoxin contamination of crop products negatively impacts a country's economy by lowering the produce quality leading to low sales and even product rejection both in the local and international markets (Pal, 2018). Indirectly when livestock and humans feed on aflatoxin-contaminated feed or food, they are harmed by the metabolites that may result in morbidity, mortality, and secretion of the bio-transformed metabolites into their milk, meat, and eggs (Pal *et al.*, 2021).

Furthermore, literature indicates that feed business owners and farmers are usually into legal friction as a result of selling contaminated feeds (Kebede & Tamiru, 2018). These significantly lead to national economic loss. As observed in Canada and the United States of America, USD 5 million losses are registered due to mycotoxin contamination (Madala *et al.*, 2017). While 38% of global agricultural losses due to aflatoxin are registered in Sub-Saharan Africa totaling a greater economic loss of USD 450 million (Madala *et al.*, 2017).

The most common mycotoxins of concern for food safety worldwide are aflatoxins (Kumar *et al.*, 2017; Singh & Mehta, 2020). Primary aflatoxins identified so far include:-Aflatoxin B₁ (AFB₁), aflatoxin B₂ (AF B₂), aflatoxin G₁ (AF G₁), and aflatoxin G₂ (AF G₂) (Negash, 2018). Two secondary types that are a result of the mammalian metabolism of AFB₁ and AFB₂ are aflatoxin M₁ (AFM₁) and aflatoxin M₂ (AFM₂), respectively, and are usually secreted in milk (Singh & Mehta, 2020).

Of all the six (6) aflatoxin types, AFB₁ was originally considered to be the most toxic and placed among group 1 carcinogens by the International Agency for Research on Cancer (IARC) (Ostry

et al., 2017). However, it was later found that when AFB₂, AFG₁, AFG₂, AFM₁, and AFM₂ coexist, the mixture becomes more toxic than the individual toxins, and as such, the mixture has also been included among group 1 carcinogens (Marchese *et al.*, 2018). Considering the fact that locally formulated complementary food utilized in local settings in low-income countries is largely plant-based, contamination by AFM₁ and AFM₂ and subsequent exposure of children to a mixture of AFB₂, AFG₁, AFG₂, AFM₁, and AFM₂ would be unlikely. Therefore, AFB₁ remains the most toxic fungal contaminant of plant-based complementary foods in this context.

Several human health conditions including hepatocellular carcinoma, aflatoxicosis, haemorrhage, hepatotocosis, nephroptosis, mutagenesis, immuno-suppression, birth defects, and stunting have been sought to relate to consumption of aflatoxin contaminated foods (Ismail *et al.*, 2018; Ali *et al.*, 2020). Considering multiplicity of adverse negative effects of aflatoxin exposure, various regulatory bodies such as the EU, US-FDA, and EAC have put in place acceptable contamination levels in food with a range of 0.1 to 20 µg kg⁻¹ (Sirma *et al.*, 2018). To further guarantee safety, a PMTDI of 1.0 ng kg⁻¹bw day⁻¹ and 0.4 ng kg⁻¹bw day⁻¹ for children and adults who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively have been adopted (dos Santos *et al.*, 2018; Boni *et al.*, 2021).

2.7 Assessment of exposure to microbiological hazard such as aflatoxin

Assessing exposure to microbial hazards is done either singly, quantitatively, qualitatively, or both by evaluating probable ingestion of the microbial hazard in food that is potentially risky and may compromise health when exposed to (WHO/FAO, 2008). According to Lammerding & Fazil (2000), all the information about specific groups mostly the vulnerable categories such as infants, young children, expecting mothers, elderly, and the immune-compromised populations should be included in exposure assessment. This is because they are more susceptible to infections and intoxication and have different eating habits and exposure levels than other groups of the population.

Contaminated foods may be risky when people and livestock consume them. Food contaminants may also compromise the quality and quantity of foods and feeds. However, levels of

contamination may be kept reasonably low through best practices such as Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP), and Good Hygiene Practices (GHP) in addition to appropriate risk assessment (CAC, 2015). Based on empirical evidence, therefore, aflatoxin B₁ and its principle excretion 4- hydroxylated form (AFM₁) have been classified as IARC as class 1 possible human carcinogen respectively (Kuiper-Goodman, 1990). Because of the adverse health conditions associated with aflatoxin exposure, various regulatory bodies such as the EU, US-FDA, and EAC put in place acceptable values for aflatoxin contamination levels in foods with a range of 0.1 to 20 µg kg⁻¹ (Sirma *et al.*, 2018).

As well, a PMTDI of 1.0 ng kg⁻¹bw day⁻¹ and 0.4 ng kg⁻¹bw day⁻¹ for children and adults who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively have been adopted to guarantee safety (dos Santos *et al.*, 2018; Boni *et al.*, 2021). In particular, the EU limit for processed cereal-based foods and baby foods for infants and young children is 0.10 µg/kg for AFB₁ and 0.025 µg/kg for AFM₁ (EC, 2006; Raiola *et al.*, 2015).

Lammerding & Fazil, (2000) reported that assessment of exposure to microbiological agents includes the magnitude of definite or predictable human exposure. The basis for exposure assessments is the probable level of food contamination by specific hazard, dietary information, and the specificity of units of food consumed, i.e., the portion size. In the assessment, incidence, and contamination levels by hazards for some time are factors that should be considered. Additionally, the frequency of consumption is another factor for consideration (WHO/FAO, 2008) and should be related to socio-demographic factors depending on the community of interest.

Also, since food handler may cause cross-contamination, they may be included in addition to time and temperature management (Ncube *et al.*, 2020). However, the mentioned factors may be influenced by the nature of the hazard, the food ecosystem, and the primary level of contamination (Lammerding & Fazil, 2000).

Further, different seasons of cultivation and location, hygiene, and food safety control procedures along the value chain should be taken into account (Lammerding & Fazil, 2000;

WHO/FAO, 2008 and WHO, 2015). Since biological hazards are dynamic, these attempts may help in controlling the levels of the hazards in foods (Lammerding & Fazil, 2000).

Regardless of scientific evidence, little is done in the area of complementary foods used by rural communities. Moreover, a 13-year review (2005–2018) of mycotoxin contamination in foods consumed in Uganda shows that the foods utilized by rural populations are from potentially aflatoxin-contaminated raw materials (Kaaya & Warren, 2005; Lukwago *et al.*, 2019). Therefore, when the vulnerable subpopulation (children aged 6 to 23 months) is exposed to aflatoxin, the nutritional outcome from complementary food may not be realized. Thus, assessing exposure deserves attention.

CHAPTER THREE: GENERAL MATERIALS AND METHODS

3.1 Study area

The study was carried out in the Acholi sub-region of Uganda in two districts (Amuru and Nwoya). Amuru and Nwoya are rural districts that were part of the Gulu district before the establishment by the Parliament of Uganda in 2006 and 2010 respectively. Amuru district location in Uganda lies in 02050'N 33005'E coordinates. Amuru district is bordered by Adjumani district to the north, South Sudan and Lamwo district to the northeast, Gulu district to the east, Nwoya district to the south, Nebbi district to the southwest, and Arua to the west (Nassanga *et al.*, 2018). Amuru district has a total area of 3626 Km² comprising six sub-counties, 32 parishes, and 67 villages with a population estimate of 178,800 by 2012. conversely, Nwoya district location in Uganda lies in (02038'N 32000'E) coordinates bordering Amuru district to the north, Gulu district to the northeast, Oyam district to the east, Kiryandongo district to the southeast, Buliisa district to the southwest and Nebbi district to the west (Nassanga *et al.*, 2018). Nwoya district covers a total area of 4736 km² composed of 5 sub-counties, 25 parishes, and 63 villages with a population estimate of 54,000 by 2012.

Specifically, in Amuru district, the two sub-counties where the study was conducted were Pabbo and Lamogi. The parishes were Gaya and Lacor, and the villages were Pukwany and Pukure respectively. In Nwoya district, the two sub-counties where the study was conducted were Kochgoma and Alero. The parishes were Panyabongo and Kal, and the villages were Kal A1 and Lalar respectively.

The two districts were selected to follow up on a study by Alowo *et al.* (2018). These districts were among the districts where undernutrition was more prevalent in the Acholi sub-region (UBOS, 2016). Based on World Health Organization (WHO) classification, the two districts had serious stunting (36.5 and 32.3 respectively) and poor underweight (15.0 and 11.3 respectively), (Alowo *et al.*, 2018). Therefore, locally made MMSSC was readily accepted.

These districts have one livelihood zone, the agriculture livelihood zone with less livestock farming but is dominated by crop farming. Since 2013 there has been a plan for commercial extraction of crude oil in Nwoya. Rain distribution pattern in these areas is bimodal with two Agricultural seasons from March to June and August to November. The main production season is the rainy from late March to the end of November (Atube *et al.*, 2022). The main food crops are sesame, millet, sorghum, beans, cassava, sweet potatoes, and groundnuts while rice, maize, groundnuts, beans, sesame, and to a lesser extent cassava and millet are grown for cash.

Location of study sites in Amuru and Nwoya districts, northern Uganda

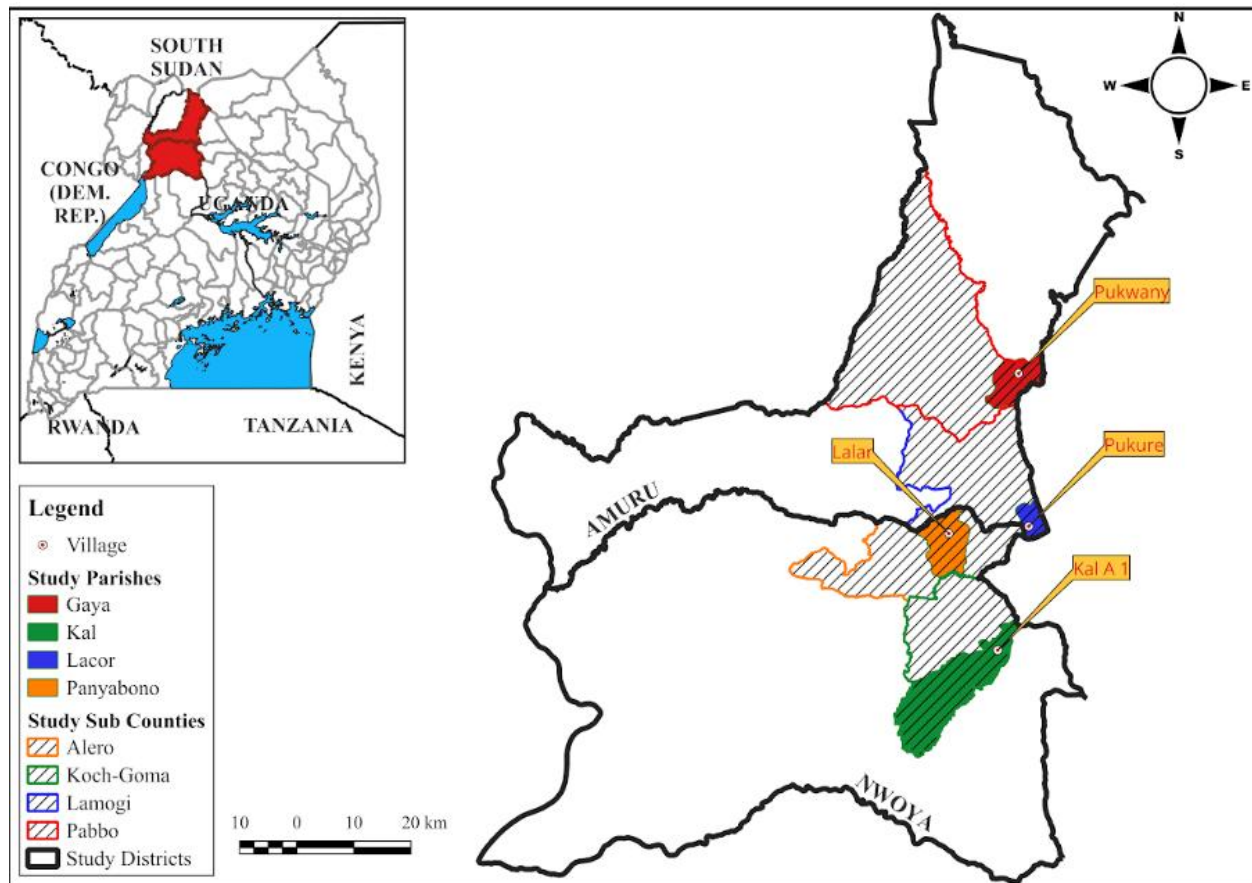


Figure 2: Map of the study area

3.2 Research design, sample size and sampling procedure, data collection and data analysis

The study employed both qualitative and quantitative research designs. In qualitative design, the KAP model involved 8 FGD in each district comprising 8-12 participants, (parents or caregivers of a child aged 6-23 months) (Dong, 2015). In the quantitative design, factorial experimental design was done using a single-factor design at three levels, 1. Raw material (Millet, Sesame, and Soybean), 2. Freshly processed composite, and 3. Processed stored products (30 days). This design was employed to determine the prevalence of genus *Aspergillus* and the concentrations (μgkg^{-1}) of total aflatoxin and aflatoxin B₁ at the above-mentioned three processing points across the wet and dry seasons.

3.3 Study design

This study adopted a cross-sectional design involving a survey using in-depth household interviews, focus group discussions (FGDs), and experiments. The study was conducted in the Acholi sub-region of Uganda, in Amuru (02°50'N33°05'E) and Nwoya (02°38'N 32°00'E) districts, Northern Uganda, between March and June 2019. The two districts were selected to follow up on a study by Alowo *et al.* (2018). The study population consisted of caregivers (mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father) of children (6–23 months).

3.4 Sample size estimation

The total number of participants was determined using a standard table advanced by Krejcie and Morgan, (1970) presented in appendix 10. Food samples were collected from all the caregivers who participated in the study.

3.5 Sampling procedure

A total of 180 participants in the previous study conducted by Alowo *et al.* (2018) were purposely recruited for this study. They included all caregivers who were trained on local

production of MMSSC from two sub-counties per district in the Nwoya and Amuru districts of northern Uganda. From the two districts, two sub-counties per district and one parish per sub-county were purposively selected. In each parish, one village was then selected.

The inclusion criteria were a caregiver who received training on local production of MMSSC and was willing to participate in the study. The training of the caregivers lasted for 82 days and aimed at equipping them with good hygiene and good manufacturing practices to produce safe complimentary food composite in a domestic environment. The training content included using safe water and raw materials for making the composite, hand washing by the caregivers and the child, hand washing, keeping the utensils and environment clean, thorough cooking, and keeping cooked food covered in a safe place. The training was done in the local dialect (Acholi). Figure 8 shows the detailed porridge production process using three ingredients, millet (*Eleusine coracana*), sesame (*Sesamum indicum*), and soybeans (*Glycine max*).

Overall, 363 foods were purposely sampled and picked from all the caregivers of children trained to prepare locally made MMSSC complementary food composite who were recruited in the study. The wet season samples (208) were collected in June 2019. The dry season samples (155) were collected in early March 2019. Wet season samples included millet (n = 46), sesame (n = 41), soybean (n = 45), freshly processed composite (n = 32), and composite stored for 30 days (n = 44). Consequently, samples for the dry season consisted of millet (n = 46), sesame (n = 35), soybean (n=1), and freshly processed composite (n = 30) composite stored for 30 days (n = 43).

3.5 Data collection

A modified pretested questionnaire with provision for socio-demographic characteristics of the household adopted from FAO (2014) and Marías and Glasauer (2014) was used to assess the KAP of the caregivers. To control the general bias when answering according to Dunsch *et al.* (2018), 10 food safety knowledge questions were positively and negatively framed. Also, six positively and negatively framed questions measured attitude on a 3-point Likert scale (0=disagree, 1=neither agree nor disagree, and 2=agree) (Anand & Puri, 2013).

Caregivers' self-reported practices on the other hand were assessed by asking caregivers to articulately express how they handle food, especially food for complementary feeding from postharvest to consumption. To supplement the information obtained from household interviews, the community's perspective on the safety of MMSSC and food safety in general was obtained using the pretested FGDs guide based on health belief model constructs (Pourtaheri *et al.*, 2018; Rosenstock, 1974; Rosenstock *et al.*, 1988). Before household interviews and FGDs, modified semi-structured questionnaires and FGD guide adopted from WHO (2010) and Marías and Glasauer (2014) were pretested among 30 caregivers of weaning children attending postnatal care at Laroo Health Center III in Gulu district to ensure accurate, clear, and consistent interpretation of questions.

Ambiguous and invalid answers were checked by analyzing responses. Results of the first pretest showed that respondents found difficulties interpreting questions such as, “What is the source of birth?” This question was revised to “What is the place of birth?” Additionally, participants did not answer “What is the estimated household income allocated to food?” This question was split into two questions: “What is the estimated household income in a month?” and “What is the estimated amount spent on food?” The checked and validated pretested tools were then re-pretested among 30 caregivers of weaning children in Unyama Village, Gulu District (a similar distant community), within 1 week. From the second pretest, respondents did not have difficulties and the responses conformed to the study objective. However, results from the second pretest showed that most (97%) respondents failed to estimate the money spent on food in a month. Therefore, this question was excluded from the data collection instrument.

Trained research assistants (6) with an understanding of nutritional studies and fluently spoke English and Acholi dialect administered study questionnaires and conducted FGDs in the local dialect (Acholi). The study instruments were written in English and translated into the local dialect (Acholi). To guarantee confidentiality and prevent any interference, other members of the household and members of the neighboring household were not allowed to participate or be present during the interview and FGDs. To ensure the originality of information, FGD consisted of 8 to 10 participants (caregivers) who had not participated in the interviews. As affirmed by

Guest *et al.* (2006) and McCafferty (2004), a total of eight FGDs were conducted essentially higher than the lowest number (6) of focus group discussions required to saturate the data.

Based on the survey information generated from the household interviews and FGDs representative samples were collected using sterile metallic probes based on procedures set by the Food and Agriculture of the United Nations set techniques sampling for mycotoxin analyses (FAO, 2013). Using a sterile sealable sample bag, 2 sample replicates of 250g were collected, and labeled with a sample identification number, location, and date. Samples were then transported at 4°C in cool box to the Gulu University multifunctional laboratory for further processing as described by Altaher *et al.*, (2017). Samples were finely ground using a laboratory grinder (KA M20 Batch Mill / Grinder, 20,000 rpm, Max. Vol 250ml, 1603603), and the sample holder was cleaned and disinfected by 70% ethanol after every grinding process to avoid cross-contamination. One hundred grams was removed from each sample in another sterile sample bag, labeled, and stored at 4°C until use.

3.6 Laboratory analyses

3.6.1 Culturing of *Aspergillus* species

Isolating and identifying *Aspergillus* species viable fungal count was carried out to estimate the number of fungi per sample as described by Abdulrahman *et al.* (2020). Briefly, the sample stock solution was produced by weighing 10 g of each sample using a weighing balance (ENTRIS224/Sartorius, Germany) in a sterile bottle comprising 90 ml of sterile diluents (peptone water), then homogenized. The stock solution was diluted tenfold, and 1 ml from each sample was dispensed into a sterile Petri dish labeled with the sample number and date. Aseptically, sterile cooled potato dextrose agar (PDA), OXOID Himedia incorporated with 0.06 g/L of chloramphenicol was poured into the plate and swirled for proper distribution between the media and the inoculums. Cultures were then incubated for 4-7 days at ambient temperature. Every colony was sub-cultured on sterile plates in duplicate and incubated. The cultures were examined macroscopically based on colonial appearance, pigmentation, shape, and margin. Fungal cultures were confirmed by the molecular method using universal primers for detecting

Aspergillus, and *Aspergillus* spp were confirmed by species-specific polymerase chain reaction (PCR) assays (Table 1).

3.6.2 Molecular Identification of *Aspergillus* species by PCR assay

3.6.3 DNA Extraction

A modified protocol previously carried out by by (Passoneet *al.*, 2010; Wokorachet *al.* (2021) was followed. Briefly, the mycelia were scraped and put inside the 1.5 ml Eppendorf tubes and run in a Geno grinder for 5 min. On to the 1.5 ml Eppendorf tubes having ground mycelia, was added 450 μ L of preheated CTAB buffer and vortexed and incubation was done using a water bath (65°C) for 10 min with occasional mixing. Then 450 μ L of chloroform: isoamyl alcohol (3:1) was added to the homogenized sample and inverted twice to mix, and then centrifuged at 15,000 rpm for 10 minutes. A 400 μ L from each upper aqueous layer was transferred into 1.5 ml sterile Eppendorf tubes. Precipitation of Deoxyribonucleic acid (DNA) was done by adding 450 μ l of chilled isopropanol (stored at -20 °C), and the tubes were then slowly inverted five times to mix. To the mixture in each tube, 315 μ L of sodium Acetate solution was added and placed in ice for 10 min.

The precipitated DNA in the tubes was centrifuged at 15,000 rpm for 15 min to form pellets. The supernatant was decanted, and to the pellet 200 μ L of 70% ethanol was added and centrifuged at 4,000 rpm for 10 min. The supernatant was decanted, and the pellet air dried for 1 hr. The dry pellets were suspended in 50 μ L of elution buffer, and their concentration was measured spectrophotometrically using a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE, USA) and stored at -20°C until use. The DNA extract quality was evaluated on 1.5% agarose gel and visualized under a UV transilluminator.

3.6.4 *Aspergillus* species-specific DNA Amplification

Diverse *Aspergillus* species (*A. flavus*, *A. parasiticus*, *A. fumigatus*, and *A. niger*) were detected using species-specific PCR assays with specific primer pairs and annealing temperatures (Table 1). The DNA amplification was performed in a 10 μ l reaction volume with 0.5 μ mol L⁻¹ of each

forward and reverse primer, 1µl of DNA template, 3 µl nuclease-free PCR water, 5 µl of premix containing 2 µl of 10 x PCR buffer, 1.5mmolL⁻¹ MgCl₂, 80µmolL⁻¹ of each dNTP and 1 unit of Taq polymerase (Bioneer MBI fermentas, India) in a mastercycler thermal cycler (Eppendorf, Hamburg, Germany). Primer temperatures and time for 35 cycles were 95°C for initial denaturation for 5 min, final denaturation at 95°C for 1 min, initial extension temperature was 72 °C for 40 sec, and final extension temperature was 72 °C for 7min (Table 1). The Universal ITS1 and ITS4, ITS1 and ITS2, and species-specific primer pairs and nucleotide sequences used in the study are in Table 1.

Table 1: Universal ITS 1 and ITS 4 and species-specific primers and nucleotide sequence used in the study

Target organism/ chemotype	Species specific primer pair	Primer sequence (5'→3')	Annealing Temperature (°C)
<i>ITS 1</i>		5' - TCCGTAGGTGAACCTGCGG - 3'	55
<i>ITS 4</i>		5' - TCCTCCGCTTATTGATATGC - 3'	
<i>A. flavus</i>	Forward, ASPU Reverse Af2r	5' -ACTACCGATTGAATGGCTCG -3' 5' - TTCACTAGATCAGACAGAGT-3'	58
<i>A. niger</i>	Forward ASPU Reverse Nilr	5' - ACTACCGATTGAATGGCTCG-3' 5'- ACGCTTTCAGACAGTGTTCG-3'	55
<i>A. parasiticus</i>	Forward PARAQ2 Reverse FLAVIQ1	5' - GTCGTCCCCTCTCCGG - 3' 5' - GAAAAAATGGTTGTTTTGCG - 3'.	53.5
<i>A. fumigatus</i>	Forward ASPU Reverse Af3r	5'- ACTACCGATTGAATGGCTCG-3' 5' CATACTTTCAGAACAGCGTTCA-3'	54.8

3.6.5 Gel Electrophoresis and Visualization of Amplicons

The amplified PCR product (5 µl) was viewed by loading it in the well of a 1.5% ethidium bromide-stained agarose gel. The product was loaded with a marker (100 -1000 bps) and visualized in the gel documentation system. The positive samples appear as bright bands.

3.6.6 Aflatoxin extraction and analysis

Extraction was done following the manufacturer's instructions (TAF Assay- low matrix and AFB1 Assay- low matrix HELICA Biosystems, Inc 2019). Concisely, 50% methanol extraction

solvent was prepared by adding 50ml analytical grade methanol to 50ml double distilled water. Twenty grams (20g) of finely ground samples were extracted by adding it to 100 ml of 50% methanol mixed for 10 minutes and centrifuged at 3500 rpm for 5 minutes; the supernatant was collected and preceded the ELISA assay. The resultant ratio of the sample to the extraction solvent was then 1:5 (w/v).

Analyses of TAF and AFB1 were done by low matrix competitive solid phase inhibition, a competitive enzyme-linked immunoassay intended to quantitatively analyze AFB1, B2, G1, and G2 in grains, nuts, cottonseeds, cereals, and other commodities including animal feeds. The detection limit was < 1 ppb. But, the limit of detection (LOD) is commodity-specific and should be measured for each commodity. Concisely sample extracts were analyzed as described by the manufacturer's protocol. the manufacturer's spreadsheet, the dose-response curve was constructed by the mean of OD values The optical density (OD) of samples in a microwell was read at a 450nm filter using a micro-titer ELISA plate reader (Thermo-fisher 357-Ty, Multiskan-FC, Shangai, China) and then entered into the Excel Program. Using stated as a percentage of the OD of zero standards (0.0) against the aflatoxin content of the standard (%B/Bo). Whereby %B is the percentage binding for each standard and sample while Bo is the zero standards set as 100 percentage binding.

As described in the extraction protocol, the samples were diluted in a 5:1 ratio during extraction. Aflatoxin concentrations were multiplied by 5 to indicate parts per billion (ppb) of the samples. The LOD was used for determining the method's sensitivity by calculating the mean concentration of 10 blank samples plus three standard deviations. The limit of quantification (LOQ) was calculated as the mean value plus 10 standard deviations. The limit of LOD and LOQ values were estimated to be 0.021 and 0.069 $\mu\text{g kg}^{-1}$, respectively. Concentrations of both the TAF and AFB in the samples were measured by interpolation of the standard curve by constructing a dose-response curve using the average of OD values expressed as a percentage (%B/B0) of the OD of the zero (0.0) standard against the aflatoxin content of the standard.

The unknown TAF and AFB1 concentrations were measured by interpolation from the standard curve. The concentrations of the standards were labeled on the vial (0.0, 0.02, 0.05, 0.1, 0.2, and

0.4). However, the sample was diluted at a 5:1 ratio by extraction solvent as instructed in the extraction procedure. The level of aflatoxin shown by the standard was multiplied by 5 to indicate the ng per gram (ppb) of the samples. Linear regression and coefficient of determination (R^2) were calculated to assess the linearity of the calibration curve using six points of the standard curve. The R^2 values for all the curves (R^2 0.989 - 0.996) were within acceptable values.

3.6.7 Estimation of TAF and AFB₁ dietary intake

In this study, deterministic methods were performed by combining normalized MSSC daily intake per body weight data. Whereby, the MSSC intake data was collected using a modified food frequency questionnaire, and 24 hr. recall for three consecutive days. The average was used as previously described by Huong *et al.* (2019). Children's weights were measured using a precise weighing balance. Exposure of individual children to TAF and AFB₁ was assessed as Estimated Daily Intake, EDI ($\text{ng kg}^{-1}\text{bw day}^{-1}$) = (TAF or AFB₁ concentration x consumption/day/body weight) (Huong *et al.*, 2019).

3.7 Statistical analysis

Statistical Package for Social Sciences (SPSS) version 27 was used to statistically analyze the data. The prevalence of the different *Aspergillus* species along the processing points was determined by calculating the number of *Aspergillus* species detected and dividing it by the total number of *Aspergillus* species that were recovered and then multiplied by 100. Data was analyzed using descriptive statistics and expressed as frequencies and percentages. Pearson Chi-square was used to compare the prevalence of *Aspergillus* species across the processing point. The *P*-values less than 0.05 were regarded as significantly different.

Additionally, to determine the prevalence of TAF and AFB₁ concentrations along the processing points across the wet and dry seasons, descriptive statistics and inferential statistics were used for data analysis. Specifically, means, standard deviation, standard error, and median were analyzed for the dependent variables. The significance of the means was tested using a one-way analysis

of variance (ANOVA), and Duncan's post-ANOVA test for mean comparison. The t-test was used to assess differences in mean for EDI TAF and EDI AFB₁ between stored and processed products. A pair-wise correlation analysis was also performed to test for the significance of the correlation between aflatoxin exposure indicators; age and weight.

To determine KAP, for the case of knowledge questions, correct answers were scored 1, wrong answers, and in scenarios where the respondent did not know, a score of 0 was awarded. An attitude score of 0, 1, and 2 was given for disagreeing, neither agree nor disagree, and agree, respectively. Conversely, the reverse scores were given to negatively framed questions. This implied that 0, 1, and 2 were given for agreeing, neither agree nor disagree, and disagree, respectively (Anand & Puri, 2013). Knowledge and attitude for each caregiver were calculated by summing up scores on each question, and the overall score ranked as sufficient or insufficient knowledge and positive or negative attitude, respectively. The cutoff for sufficient knowledge and a positive attitude was 70% based on previously published studies (Tamang *et al.*, 2020).

Descriptive statistics (frequency, percentages, mean, and standard deviation) where applicable were used to summarize socioeconomic demographic characteristics and knowledge, attitude, and practices (KAP) data. To establish the socioeconomic and demographic predictors of sufficient knowledge and positive attitude, Pearson correlation analysis was run before regression analysis to eliminate highly correlated variables ($r > .7$) (Dormann *et al.*, 2013). Multivariate binary logistic regression was performed using Equation (1):

$$Y_i = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_{17} X_{17} + \mu(i)$$

Y_i is the binary dependent variable representing the status of knowledge and attitude; α is the regression constant; β is the regression coefficient; X_1 to X_{17} are the independent variables; and μ is the error term.

Factors selected to run the multivariate binary logistic regression are shown in Table 12. Binary multivariate logistic regression analysis was performed to establish the socio-demographic predictors of sufficient knowledge and positive attitude. Significant statistical differences were considered at a 95% confidence level ($P \leq .05$). FGD data were transcribed, coded, and organized

into common themes based on the health belief model constructs (Pourtaheri *et al.*,2018) and then analyzed using qualitative content analysis (Cho & Lee,2014).

Further, the influence of the status of KAP on the level of aflatoxin contamination and the exposure of the weaning children to aflatoxin was assessed using the t-test. Whereby, a pairwise comparison of the different levels of aflatoxin as well as exposure by the KAP categories was done. Specifically, the t-test was used to test whether the mean level of aflatoxin contamination and mean exposure at a given point/product was significantly different for households with sufficient knowledge and those with insufficient knowledge, those with a positive attitude, and those with a negative attitude and those that practice food hygiene and those that did not.

3.8 Ethical Clearance

This study was reviewed and approved by the Gulu University Research Ethical Committee (GUREC 02719) and the Uganda National Council of Science and Technology (SS 4958). All participants (legal guardians) provided informed consent to participate in the study. All participants (legal guardians) provided informed consent for the publication of their images. Permission was sought from caregivers (legal guardians) to collect weight and exposure data of the children.

CHAPTER FOUR: Prevalence of *Aspergillus* species at process points of locally made complementary food formulae in northern Uganda across dry and wet seasons

4.1 Introduction

Locally made complementary food formulae composed of malted millet (*Eleusine coracana*), sesame (*Sesamum indicum*), and soy-bean (*Glycine max*) are produced in northern Uganda; mainly in Amuru and Nwoya districts (Alowo *et al.*, 2018). These are ingredients in most processed complementary foods in Uganda. They are also important food and cash crops. Yet, studies show that these crops can be infected by toxigenic fungi such as the genus *Aspergillus* (Echodu *et al.*, 2019; Wokorach, *et al.*, 2021).

Universally, about 143 different fungal genera have been isolated from cereals (Reddy and Sathyanarayana, 2001). Out of these, the main three genera that produce mycotoxin include: - *Aspergillus*, *Penicillium*, and *Fusarium* (Hassan *et al.*, 2018; Moretti *et al.*, 2017). Genus *Aspergillus* is one of the most important fungal genera that contribute to economic loss and public health challenges affecting both animals and humans (Mahmoud 2016). This genus consists of both non-aflatoxigenic and aflatoxigenic fungi. The non-aflatoxigenic ones include *A. oryzae*, *A. sojae*, and *A. tamari* while aflatoxigenic ones include *A. flavus*, *A. parasiticus*, and *A. nomius* that are placed in the *flavi* section (Acur *et al.*, 2020).

The aflatoxigenic *Aspergillus* species are known to produce the most important mycotoxin of concern called aflatoxin and specifically aflatoxin B₁ which is globally spread in most foods such as grains, cereals, spices among others (Bhat, 2003; Wayne, 2007). The toxins produced are secondary metabolites secreted as defense, and cell signaling such as virulence (Valiante, 2017; Ismail and Papenbrock, 2015). These metabolites are heat resistant under normal processing conditions including cooking temperatures although temperatures above 150°C effectively reduce mycotoxins (Ryu *et al.*, 2008). Humans and animals are exposed through ingestion and inhalation of these metabolites and, the effect can either be acute or chronic illness (Mohsenzadeh, 2016). However, food processing has been shown to play a role in controlling

aflatoxins in agricultural products (Liu *et al.*, 2016; Rajasekaran *et al.*, 2017; Thakur *et al.*, 2022).

In contrast, *A. fumigatus* has also been known to cause aspergillosis i.e., ear infection. They are widely distributed in the environment ranging from soil, and aquatic environment. Warm and cold climatic conditions in addition to salty substrates support their proliferation (Klich, 2002, Abtebr1 2004; Jan-Gar and Cotty 2010; Butinai *et al.*, 2011; Taang *et al.*, 2012; Bongugli, 2015). Genus *Aspergillus*, therefore, is a problem in the agricultural production chain of various crops (Bayuan, 2002; Horn 2003; Jarrcir, 2012; Abdel-Azeem., 2020).

Section *Nigri* of genus *Aspergillus* conversely is important in food industries for fermentation and production of useful organic acids and enzymes that are hydrolytic although literature shows that *A. niger* and *A. carbonnanus* can produce ochratoxin (Refai, 2014; Cairns *et al.*, 2021). Nevertheless, *A. niger* has been known to secrete RNase that has anti-carcinogenic chrematistics and solubilization of heavy metal sulfides (Abdel-Azeem, 2020. Conversely, *A. fumigatus* has been known to cause aspergillosis; and ear infections (AL-Ezzy and Abdulameer, 2021).

Although the aflatoxigenic group is significant to public health due to their ability to produce aflatoxins, there is still a paucity of information on their prevalence along the processing chain of locally made complementary food composite. The current study sought to find out the prevalence of *Aspergillus* species contaminating locally made malted millet-millet-sesame-soy composite at processing points across wet and dry seasons. Samples were collected from caregivers trained in local production of weaning food in northern Uganda.

4.2 Materials and Methods

4.2.1 Study area

The study was conducted in the Acholi sub-region in Amuru (02°50'N 33°05'E) and Nwoya (02°38'N 32°00'E) districts, Northern Uganda. The two districts were selected to follow up on a study conducted by Alowo *et al.*, (2018). The study was conducted among caregivers

(mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father) of children (6-23 months).

4.2.2 Samples size

Overall, 363 foods were purposely sampled and picked from all the caregivers of children trained to prepare locally made MMSSC complementary food composite who were recruited in the study. The wet season samples (208) were collected in June 2019. The dry season samples (155) were collected in early March 2019. Wet season samples included millet (n = 46), sesame (n = 41), soybean (n = 45), freshly processed composite (n = 32), and composite stored for 30 days (n = 44). Consequently, samples for the dry season consisted of millet (n = 46), sesame (n = 35), soybean (n=1), and freshly processed composite (n = 30) composite stored for 30 days (n = 43).

4.2.3 Samples collection

A representative sample was collected using sterile metallic probes based on procedures set by the Food and Agriculture of the United Nations techniques sampling for mycotoxin analyses (FAO, 2013). Using a sterile sealable sample bag, 2 sample replicates of 250g were collected, and labeled with a sample identification number, location, and date. Samples were then transported at 4°C in a cool box to the laboratory for further processing as described by Altaher *et al.*, (2017). Samples were finely ground using a laboratory grinder (KA M20 Batch Mill / Grinder, 20,000 rpm, Max. Vol 250ml, 1603603), and the sample holder was cleaned and disinfected by 70% ethanol after every grinding process to avoid cross-contamination. One hundred grams was removed from each sample in another sterile sample bag, labeled, and stored at 4°C until further analyses.

4.2.4 Culturing of *Aspergillus* species

Isolation and identification of *Aspergillus* species was done according to Abdulrahman *et al.*(2020), whereby,viable fungal count was carried out to estimate the number of fungi per sample. Briefly, the sample stock solution was produced by weighing 10 g of each sample using

a weighing balance in a sterile bottle containing 90 ml of sterile peptone water and homogenized. The stock was diluted tenfold and 1 ml from each sample was dispensed into a sterile petri dish labeled with sample number and date. Aseptically, sterile cooled potato dextrose agar (PDA), OXOID CMO139 incorporated with 0.06 g/L of chloramphenicol was poured into the plate and swirled for proper distribution between the media and the inoculum. The plates were then incubated for 4-7 days at ambient temperature. Every colony was sub-cultured on sterile plates in duplicate and incubated. The cultures were examined macroscopically based on colonial appearance, pigmentation, shape, and margin. Fungal cultures were confirmed by the molecular method using universal primers for the detection of *Aspergillus* and *Aspergillus* species were further confirmed by species-specific polymerase chain reaction (PCR) assays (Table 1).

4.2.5 Molecular Identification of *Aspergillus* species by Polymerase Chain Reaction (PCR) assay

4.2.5.1 Deoxyribonucleic Acid (DNA) Extraction

A modified protocol previously described by Wokorach *et al.* (2021) was followed. Briefly, the mycelia were scraped and put inside the 1.5 ml Eppendorf tubes and run in a Geno grinder for 5 min. On to the 1.5 ml Eppendorf tubes containing the ground mycelia, was added 450 µl of preheated CTAB buffer and vortexed. The mixture was incubated in a water bath set at 65°C for 10 min with occasional mixing. Then 450 µl of chloroform: isoamyl alcohol (3:1) was added to the homogenate and inverted twice to mix. The mixture was centrifuged at 15,000 rpm for 10 minutes. A 400 µl from each upper aqueous layer were transferred into a 1.5 ml sterile Eppendorf tubes. The precipitation of deoxyribonucleic acid was done by addition of 450 µl of chilled isopropanol (stored at -20 °C) and the tubes were then slowly inverted five times to mix. To the mixture in each tube, 315 µl of sodium Acetate solution was added and placed in ice for 10 min. Centrifugation of precipitated DNA in the tubes at 15,000 rpm was done for 15 min to form pellets. The supernatant was decanted and to the pellet 200 µl of 70% ethanol was added and centrifugation done at 4,000 rpm for 10 min. The supernatant was decanted, and the pellet air dried for 1 hr. The dry pellets were suspended in 50 µl of elution buffer and its concentration

was measured spectrophotometrically. The DNA extracts were evaluated on 1.5% agarose then observed under Ultra Violet transilluminator and stored at -20°C until use.

4.2.5.2 *Aspergillus* species – specific DNA amplification

Molecular detection of *Aspergillus* species (*A. flavus*, *A. parasiticus*, *A. fumigatus*, and *A. niger*) was carried out using species-specific PCR assays with specific primer pairs and annealing temperatures (Table 1). The DNA amplification was performed in a 10 µl reaction volume with 0.5 µmol L⁻¹ of each forward and reverse primer, 1 µl of DNA template, 3 µl nuclease-free PCR water, 5 µl of premix containing 2 µl of 10 x PCR buffer, 1.5 mmol L⁻¹ MgCl₂, 80 µmol L⁻¹ of each dNTP and 1 unit of Taq polymerase (Bioneer MBI fermentas, india) in a mastercycler thermal cycler (Eppendorf, Hamburg, Germany). Primer temperatures and time for 35 cycles were 95°C for initial denaturation for 5 min, final denaturation at 95°C for 1 min, and initial extension temperature was 72 °C for 40 sec and final extension temperature was 72 °C for 7min. The specific primer annealing temperatures are shown in Table 1.

4.2.5.3 Gel Electrophoresis and observation of the amplicons

Amplicons (5 µl) were run in a 1.5% ethidium bromide-stained agarose gel electrophoresis. A DNA ladder with a range of 100-1500 bps was loaded and visualized in the gel documentation system as bright bands.

4.3 Statistical analysis

The prevalence of different *Aspergillus* species along the processing points across two seasons was calculated by dividing the specific *Aspergillus* species detected by the total number of *Aspergillus* species that were recovered and then multiplied by 100. Using SPSS version 21, data was analyzed using descriptive statistics and expressed as frequencies and percentages. Pearson Chi-square was used to compare the prevalence of *Aspergillus* species across the processing point. The *P*-values less than 0.05 were regarded as significantly different.

4.4 Results

4.4.1 The labelled bands on a gel map after electrophoresis indicating positive samples

Figure 3, 4, 5 and 6 below shows a gel map indicating the positive and negative samples and genome size of different species of *Aspergillus* species using DNA ladder of 1500bp.

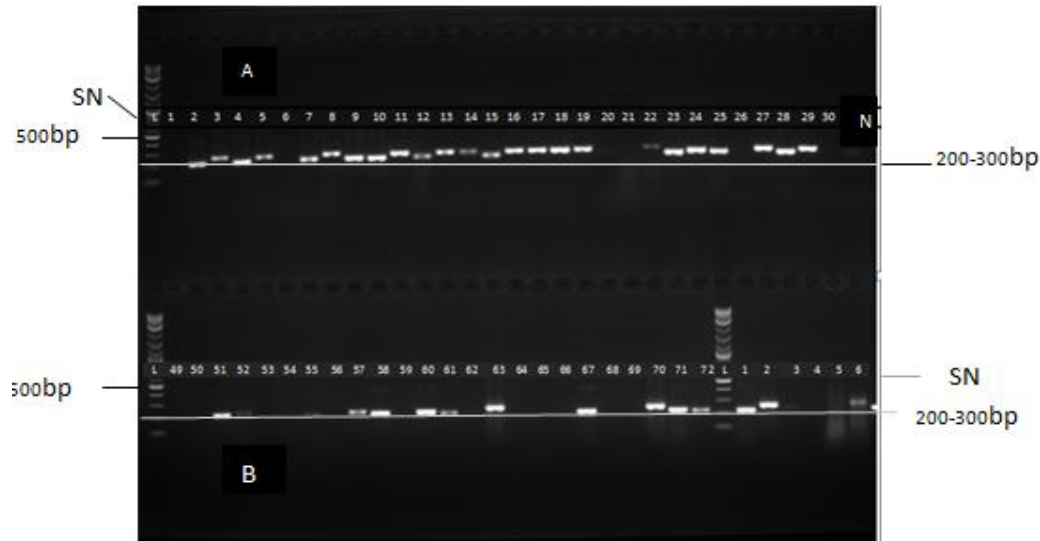


Figure 3: Gel 1Upper (A) and Lower (B) showing PCR amplicons for the ITS 1 and 4 for general fungal species with a product size of 200-300bp. L is 1500bp DNA ladder, SN is the sample number and N is the negative control. The empty wells indicate negative samples.

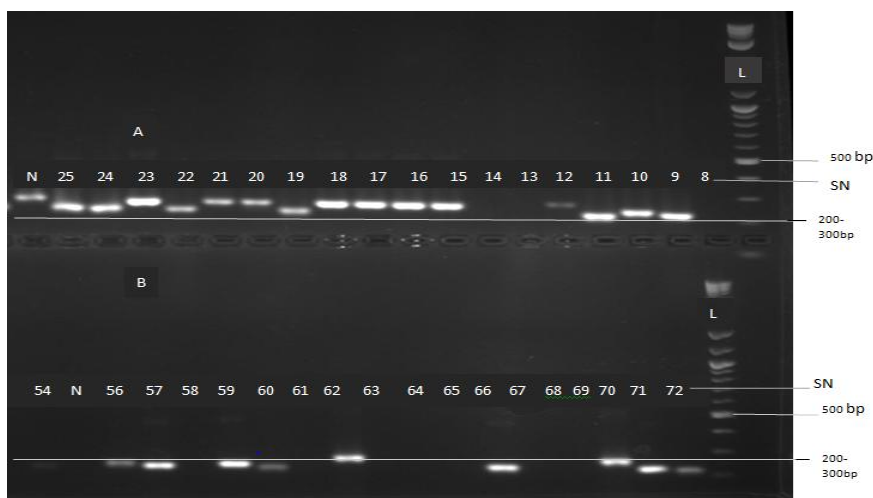


Figure 4: Gel 2, Upper (A) and Lower (B) showing PCR amplicons for the ITS 1 and 2 for general fungal species with a product size of 200-300bp. L is 1500bp DNA ladder, SN is the sample number and N is the negative control. The empty wells indicate negative samples.

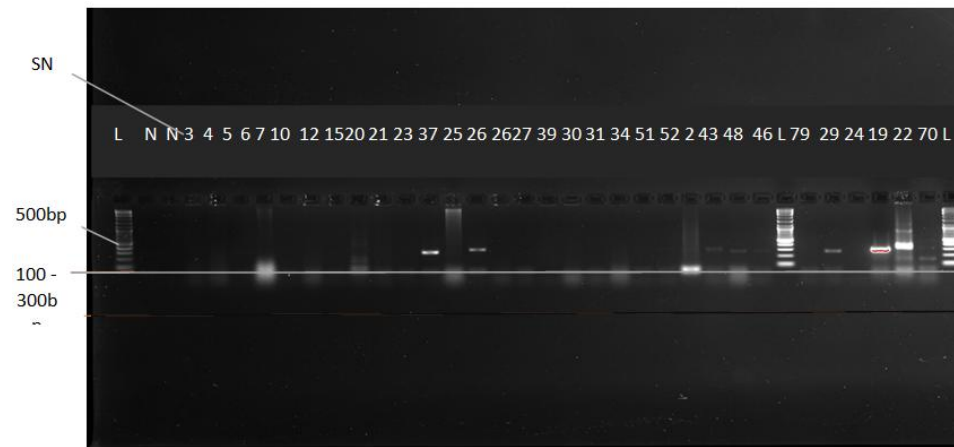


Figure 3: Gel 3 showing positive PCR amplicons for *A. niger* with a product size of 100-300bp. L is 1500bp DNA ladder, SN is the sample number and N is the negative control. The empty wells indicate negative samples.

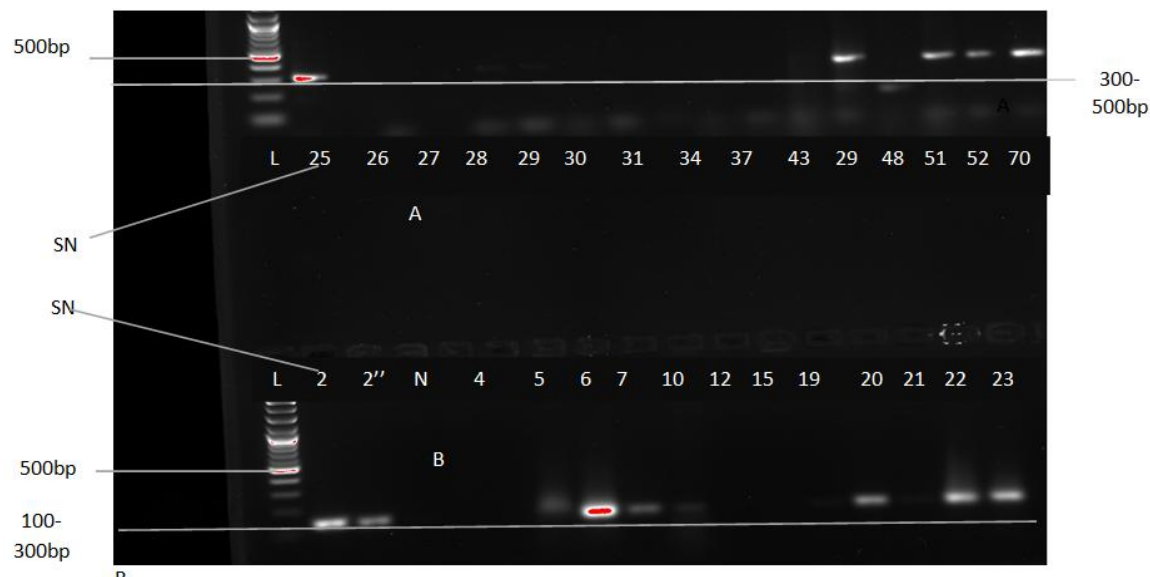


Figure 4: Gel 4 showing positive PCR amplicons with upper gel A for *A. flavus* with a product size of 300-500bp and the lower well (B) is *A. parasiticus* with a product size of 100-300bp. L is 1500bp DNA ladder, SN is the sample number and N is the negative control. The empty wells indicate negative samples

4.4.2 Overall prevalence of *Aspergillus* species along processing points across the wet and dry season

Overall (Table 2), less than a quarter of the samples along the processing points were contaminated with *Aspergillus* species. Millet had the highest contamination followed by stored processed products, soybean, freshly processed products, and least in sesame. In the wet season, soybean had the highest contamination followed by freshly processed products, millet, and least in processed stored products and sesame (Table 3). During the dry season, the most contamination was observed in millet followed by stored processed products, and sesame and none was detected in the freshly processed products (Table 4). However, there was no significant difference (Pearson Chi-square = 5.195, *P*-value = 0.268) in the *Aspergillus* species' contamination along the processing points and across the two seasons.

Table 2: Overall prevalence of *Aspergillus* species along processing points across both the wet and dry seasons

Processing points	<i>Aspergillus</i> species detected		<i>Aspergillus</i> species not detected	
	Frequency	%	Frequency	%
Overall	32	7.3	408	92.7
Raw material (soybean)	6	6.7	84	93.3
Raw material (sesame)	3	3.5	83	96.5
Raw material (millet)	12	11.8	90	88.2
Freshly processed products	4	5.7	66	94.3
Stored processed products	7	7.6	85	92.4

Table 3: Overall prevalence of *Aspergillus* species along processing points in the wet season

Processing points	<i>Aspergillus</i> species detected		species not detected	
	Frequency	%	Frequency	%
Overall	14	6.4	206	93.6
Raw material (soybean)	6	13.3	39	86.7
Raw material (sesame)	1	2.3	42	97.7
Raw material (millet)	2	3.9	49	96.1
Freshly processed products	4	11.4	31	88.6
Stored processed products	1	2.2	45	97.8

Table 4: Prevalence of *Aspergillus* species along processing points across dry season

Processing points	<i>Aspergillus</i> species detected		<i>Aspergillus</i> species not detected	
	Frequency	%	Frequency	%
Overall	18	10.2	158	89.8
Raw material (soybean)	*	*	*	*
Raw material (sesame)	2	4.7	41	95.3
Raw material (millet)	10	19.6	41	80.4
Freshly processed products	0	0.0	35	100.0
Stored processed products	6	13.0	40	87.0

*Samples not included in the statistical analysis due to limited sample number.

4.4.3 Overall specific *Aspergillus* species detected along the processing points across wet and dry season

Generally, *A. parasiticus* was the most prevalent followed by *A. flavus*, *A. niger*, and *A. fumigatus* was the least detected across the two seasons (Table 5). The contamination spread along the processing points. *A. flavus* and *A. parasiticus* were more frequently detected in millet and soybean. *A. niger* was more frequent in millet and stored processed products, Table 6. *A. fumigatus* was only detected in millet and sesame. In the wet season, soybeans had the highest contamination by *A. flavus* followed by millet and freshly processed products. The least number of *A. flavus* contaminations was in sesame and stored products. Millet and processed stored products had the highest frequency of *A. niger*, *A. fumigatus* in sesame and millet in the dry season.

Table 5: Overall prevalence of specific *Aspergillus* species detected across wet and dry season

Species	Overall (n = 40)		Wet season (n=21)		Dry season (n=19)	
	Frequency	%	Frequency	%	Frequency	%
<i>A. flavus</i>	13	32.5	9	42.9	4	21.1
<i>A. parasiticus</i>	15	37.5	7	33.3	8	42.1

<i>A. niger</i>	10	25.0	5	23.8	5	26.3
<i>A. fumigatus</i>	2	5.0	0	0.0	2	10.5

Table 6: Prevalence of specific *Aspergillus* species detected along the processing points across wet and dry season

Species	Overall (n=40)		Soybeans		Sesame		Millet		FP-MMSSC		PS-MMSSC	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
<i>A. flavus</i>	4	44.4	1	33.3	5	35.7	2	28.6	1	14.3		
<i>A. parasiticus</i>	4	44.4	0	0.0	5	35.7	3	42.9	3	42.9		
<i>A. niger</i>	1	11.1	1	33.3	3	21.5	2	28.6	3	42.9		
<i>A. fumigatus</i>	0	0.0	1	3.33	1	7.1	0	0.0	0	0.0		
Wet season												
<i>A. flavus</i>	4	44.4	1	100.0	2	66.7	2	28.6	1	100.0		
<i>A. parasiticus</i>	4	44.4	0	0.0	0	0.0	3	42.9	0	0.0		
<i>A. niger</i>	1	11.1	0	0.0	1	33.0	2	28.6	0	0.0		
<i>A. fumigatus</i>	0	0.0	0	0.0	0	0.0	0.0	0.0	0	0.0		
Dry season												
<i>A. flavus</i>	0	0.0	0	0.0	3	27.3	0	0.0	1	16.7		
<i>A. parasiticus</i>	0	0.0	0	0.0	5	45.4	0	0.0	3	50.0		
<i>A. niger</i>	0	0.0	1	50.0	2	18.2	0	0.0	2	33.3		
<i>A. fumigatus</i>	0	0.0	1	50.0	1	9.1	0	0.0	0	0.0		

4.5 Discussion

The observed low contamination could have been because the caregivers were trained on best practices to produce local complementary food formulae. Millet had the highest contamination followed by stored processed products, soybean, freshly processed products, and least in sesame. This could have resulted from poor handling of raw materials at the pre and postharvest levels (Gonçalves *et al.*, 2019). Structural characteristics of small grains could have contributed to the high contamination in millet since sorting as a strategy to control contamination may not be adequately effected in small grains (Boudergue *et al.* 2017; Marshall *et al.*, 2020). The certainty that millet seems to contain soluble sugars such as glucose, maltose, fructose, sucrose raffinose, and stachyose that favor biosynthesis of aflatoxin B₁, the presence of aflatoxigenic may also make it prone to aflatoxin B₁ contamination (Liu *et al.* 2016; Singh and Sinha 2013). Contrarily, literature reports lower contamination of small grains than large grains due to their structure which does not support colonization and subsequent aflatoxin production by the fungal species (Akello *et al.*, 2021; Ajmal *et al.*, 2022; Achiro, *et al.*, 2023).

Generally, throughout the processing points across the wet and dry seasons, less than a quarter of the total samples were contaminated with *Aspergillus* species. The study noted the highest contamination in soybean and freshly processed products in the wet season. In the dry season, the high prevalence was observed in millet and stored processed products though, seasonal variation and processing point had no significant statistical influence on *Aspergillus* species contamination, the current study noticed soybean had the highest contamination in the wet season followed by freshly processed products, millet, and least in processed stored products and sesame. The high prevalence of *Aspergillus spp* in the soybean could have resulted from favorable environmental conditions, and structural and chemical properties that favor the colonization of *Aspergillus* species (Khan, *et al.*, 2021; Ajmalet *al.* 2022).

During the dry season, the most contamination was observed in millet followed by stored processed products, and sesame, and none was detected in the freshly processed products. Recent studies reported high aflatoxigenic fungal contamination during dry season especially due to

poor storage conditions that favor their growth and aflatoxin production (Akello *et al.* 2021; Ajmal *et al.* 2022; Achiro *et al.* 2023). To ensure the safety of stored products therefore, both the biotic (such as insect invasions) and abiotic factors (temperature, carbondioxide, oxygen, and humidity) should be controlled appropriately. For instance, water content should be lowered to 94 g/kg and temperature kept at 30 - 40 °C if possible, relative humidity should not exceed 70%, and carbondioxide and oxygen should be lowered. The field soil cadmium and iron are known to control the proliferation of fungi and subsequent toxin production should be increased to optimum levels, (Khan *et al.*, 2021).

Main aflatoxin-producing and non-aflatoxigenic *Aspergillus* species; *A. flavus*, *A. parasiticus*, *A. niger*, and *A. fumigatus* respectively were recovered in relatively low frequencies from raw materials (Millet, sesame, and soybean), freshly processed products and stored processed products cross the wet and dry season. This could be explained by other reports indicating that small grains are less susceptible to fungal contamination compared to large grains such as maize and groundnuts (Njoroge 2018; Akello *et al.*, 2021; Gichohi-Wainaina *et al.*, 2022). The reports showed that millet is less contaminated with aflatoxigenic fungi. This disagrees with our finding indicating that millet was the most contaminated. However when the same samples were analyzed for aflatoxin contamination, millet and processed products had the least aflatoxin concentrations (Achiro *et al.*, 2023b). The interaction between *A. flavus* and *A. niger* that were more prevalent in millet could have caused this since literature points out that the interaction between the two organisms has been associated with a reduction in aflatoxin contamination (Xing *et al.*, 2017; Ting *et al.*, 2020). These studies attest to the reported interaction whereby, *A. flavus* and *A. niger* were more frequently detected in millet.

The current study showed variation in the mycobiome along processing points across the wet and dry seasons. Generally, *A. parasiticus* was more frequently detected followed by *A. flavus*, *A. niger*, and *A. fumigatus* was the least detected across the two seasons. Similarly, other studies have observed a high prevalence of *A. flavus* and *A. parasiticus* in cereals compared to other *Aspergillus* species (Dangwa and Dhlamini, 2014; Alkuwari *et al.*, 2022). This indicates aflatoxin contamination since these microorganisms are the major producers of aflatoxin (Ting *et al.*, 2020). This scenario does not only threaten food safety and food and nutrition security as a

whole of communities especially rural settings who depend on cereals as staple foods, but also negatively affect trade both at the local and international levels.

The study indicates that contamination spread along the processing points with *A. flavus* and *A. parasiticus* more frequently detected in millet and soybean. *A. niger* on the other hand was more frequent in millet and stored processed products. The presence of *A. niger* in millet samples could have contributed to the counteraction of aflatoxin production by *A. flavus* thus leading to the observed low concentrations of aflatoxin in the millet samples (Achiro *et al.*, 2023b). And, *A. fumigatus* was only detected in millet and sesame. Though cross-contamination has frequently been reported during processing due to poor hygiene and manufacturing practices, food handlers are usually trained on proper food handling practices but the knowledge gained is usually not translated into good practices due to barriers such as inadequate sanitary facilities (Nassanga *et al.*, 2018; Achiro *et al.*, 2023a).

About season, there was a seasonal variation of specific *Aspergillus* species contamination whereby, in the wet season soybean had the highest number of *A. flavus* contamination followed by millet and freshly processed products and the least number of *A. flavus* contaminations was observed in sesame and stored products. Comparably, sesame has been recorded to be contaminated with potential aflatoxigenic *A. flavus* (Ajmal *et al.*, 2022). Although sesame like other oily seeds has oily components that support the growth of aflatoxigenic *Aspergillus* (Liu *et al.*, 2016; Rajasekaran *et al.*, 2017), it was among one of the least contaminated. This could be explained by the fact that an array of intrinsic and extrinsic factors contributes to contamination by *Aspergillus* species (Jallow & Li, 2021). Millet and processed stored products on the other hand had the highest frequency of *A. niger*. Earlier, the high prevalence of *A. niger* has got a comparative advantage of positively competing with *A. flavus* hence minimizing aflatoxin production (Xing *et al.*, 2017). This positive impact on food safety calls for further investigations to determine the safe concentrations of the inhibitors for maximum reduction of aflatoxin occurrence. On the other hand, a high prevalence of *A. fumigatus* was only detected in sesame and millet in the dry season. This agrees with other studies that indicate the occurrence of *A. fumigatus* in millet (Bamkefa *et al.*, 2020).

4.6 Conclusion and recommendation

The study indicated low contamination of locally made complementary food composite with *Aspergillus* species along the processing points across both the wet and dry seasons. The highest contamination was observed in millet and stored processed products, and the least contamination was in freshly processed products and sesame.

The current study revealed that contamination spread along the processing points. *A. flavus* and *A. parasiticus* were more in millet and soybean, and *A. niger* was more frequent in millet. Hence, more effort is needed to reduce cross-contamination along the processing points of locally made MMSSC while prioritizing raw materials such as millet, soybean, and stored processed products across the wet and dry seasons.

Further study should determine the total aflatoxin and aflatoxin B₁ concentrations alongside gene sequence variability of aflatoxigenic *Aspergillus spp.* Control and reduction of contamination of locally made complementary food composite by genus *Aspergillus* requires a multi-sectoral approach involving producers, regulating bodies, policymakers, food producers, and researchers.

CHAPTER FIVE: Prevalence of aflatoxin along processing points of locally made complementary food formulae in northern Uganda: safety and children's exposure across seasons

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5.1 Introduction

Proper and adequate complementary feeding is critical for good nutritional outcomes in children aged 6-23 months. At this stage of child growth, breast milk is inadequate in quantity to support optimal growth even if a child's mother is well-nourished. Based on this challenge, the World Health Organization (WHO) and Ministry of Health (MOH) authorities in various countries have put in place guidelines for implementing safe complementary feeding (Koletzko *et al.* 2020).

Globally, several certified companies produce and market standardized complementary foods designed to achieve good nutrition outcomes in children (Padarath, *et al.*, 2020). However, such industrially produced complementary food products are expensive and are usually inaccessible to rural communities, particularly in low-income countries. Households in low-income countries mainly use locally made plant-based complementary food formulae. Safety is a critical issue of concern with locally formulated complementary food in low-income countries due to low adherence to sanitary practices (Mshida *et al.*, 2020; Teshome *et al.*, 2022). One of the concerns is the issue of mycotoxin contamination due to the high prevalence of mycotoxins in dry plant-based food materials that constitute part of the diet and are the principal constituents of the complementary food formulae in such countries (Echodu *et al.*, 2019; Lukwago *et al.*, 2019).

Mycotoxins are toxic bi-products of fungal metabolism that contaminate food along the production continuum (Omotayo *et al.*, 2019). Aflatoxin has direct and indirect negative impacts on the global economy. Directly, aflatoxin contamination of crop products negatively impacts a country's economy by lowering the quality leading to low sales and even rejecting the product in the local and international markets (Pal, 2018). Indirectly, when livestock and humans feed on aflatoxin-contaminated feed or food, they are harmed by the metabolites that may result in

morbidity, mortality, and secretion of the bio-transformed metabolites into their milk, meat, and eggs (Pal *et al.*, 2021). Additionally, the report indicates that feed business owners and farmers are usually into legal friction as a result of selling contaminated feeds (Kebede & Tamiru, 2018). These significantly lead to national economic loss. For instance, in Canada and the United States USD 5 million losses is registered due to mycotoxin contamination (Madala *et al.*, 2017). Globally, 38% of losses occurring in agriculture may be due to aflatoxin contamination and are mostly registered in Sub-Saharan Africa economic loss of USD 450 million.

The most common mycotoxins of concern for food safety worldwide are aflatoxins (Kumar *et al.*, 2017; Singh & Mehta, 2020). Primary categories of aflatoxin have so far identified, i.e., AFB₁, aflatoxin B₂ (AF B₂), aflatoxin G₁ (AF G₁), and aflatoxin G₂ (AF G₂) (Negash, 2018). Two secondary types that are a result of the mammalian metabolism of AFB₁ and AFB₂ are aflatoxin M₁ (AFM₁) and aflatoxin M₂ (AFM₂), respectively, and are usually secreted in milk (Singh & Mehta, 2020; Negash, 2018).

Of all the six (6) aflatoxin types, AFB₁ was considered to be the most toxic and placed among group 1 carcinogens by the International Agency for Research on Cancer (IARC) since it form a carcinogenic adducts when it attaches to the DNA (Ostry *et al.*, 2017). However, it was found that when AFB₂, AFG₁, AFG₂, AFM₁, and AFM₂ coexist, the mixture becomes more toxic than the individual toxins, and as such, the mixture is in group 1 carcinogens (Marchese *et al.*, 2018). Because locally formulated complementary food utilized in local settings in low-income countries is plant-based, contamination by AFM₁ and AFM₂ and subsequent exposure of children to a mixture of AFB₂, AFG₁, AFG₂, AFM₁, and AFM₂ would be unlikely. Therefore, AFB₁ remains the most toxic fungal contaminant of plant-based complementary foods in this context.

Several human health conditions as hepatocellular carcinoma, aflatoxicosis, haemorrhage, hepatococosis, nephroptosis, mutagenesis, immunosuppression, birth defects, and stunting are associated with aflatoxin exposure (Ali *et al.*, 2020). Given the multiplicity of adverse health conditions associated with aflatoxin exposure, various regulatory bodies such as the EU, US-FDA, and EAC have acceptable limits for aflatoxin contamination levels in food from 0.1 to 20 µgkg⁻¹, (Sirma *et al.*, 2018). To further guarantee safety, a PMTDI of 1.0 ng kg⁻¹bwday⁻¹ and 0.4 ng kg⁻¹bwday⁻¹ for children and adults who are not exposed to Hepatitis B Virus (HBV) and those infected with HBV are adopted (dos Santos *et al.*, 2018; Boni *et al.*, 2021).

Previous studies have examined aflatoxin contamination of locally processed complementary foods and exposure of children in low-income countries (Alamu *et al.*, 2018; Eshete *et al.*, 2021; Mollay *et al.*, 2022; Wacoo *et al.*, 2020; Wacoo *et al.*, 2020). A critical examination of those studies indicates that results are based on data derived from already prepared complementary food. However, limited information exists on the contamination status of the raw materials for preparing weaning food formulae as well as contamination status along the processing-storage pathway. The certainty that contamination of foods by aflatoxin can occur at any point along the production-consumption continuum (Granados-Chinchilla *et al.*, 2017) makes it difficult to identify critical points at which contamination of locally formulated complementary food formula can occur. Thus, this lacuna needed to be addressed.

Results of exposure assessment already available provide indications of the risks of aflatoxin exposure through feeding children on locally formulated food composite, such information does not provide a comprehensive indication of the exposure risks because of a lack of information on critical handling aspects such as the length of storage of the food product once prepared. The aim of this study was to compressively examine seasonal variation in aflatoxin contamination along the processing-storage-consumption continuum of MMSSC, a model product developed previously by Alowo *et al.* (2018) and exposure of children aged 6-23 months old to aflatoxin as a function of product, storage and season was also examined. The seasonality of Northern Uganda, Amuru, and Nwoya districts where the study was carried out is bimodal, wet and dry seasons. The wet periods range from the end of March to the beginning of November, and the dry season is from the end of November to early March.

5.2 Materials and methods

5.2.1 Study area and population

The study was conducted in the Acholi sub-region in Amuru (02°50'N 33°05'E) and Nwoya (02°38'N 32°00'E) districts, Northern Uganda which is recovering from 20 years of war. The two districts were selected to follow up on a study conducted by Alowo *et al.* (2018). The study population consisted of caregivers (mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father) of children (3-23 months).

5.2.2 Samples size

A total of 363 samples were collected in 2019. The wet season samples (208) were collected in June 2019, and the dry season samples (155) were collected in early March 2019. The samples were purposively collected from all the caregivers of children who were trained in the preparation of locally made MMSSC complementary food composite. Wet season samples included millet (n = 46), sesame (n = 41), soybean (n = 45), freshly processed composite (n = 32), and composite stored for 30 days (n = 44). Consequently, samples for the dry season consisted of millet (n = 46), sesame (n = 35), soybean (n=1), and freshly processed composite (n = 30) composite stored for 30 days (n = 43).

5.2.3 Sample collection

Samples were collected into a sterile sealable sample bag. Two sample replicates of 250 g each were collected and labeled with a sample identification number, location, and date. Samples were transported to the laboratory at 4°C in a cool box for further processing. Samples were finely ground using a laboratory grinder (KA M20 Batch Mill / Grinder, 20,000 rpm, Max. Vol 250ml, 1603603), and the sample holder was cleaned and disinfected with 70% ethanol after every grinding process to avoid cross-contamination. One hundred grams (100 g) were removed from each sample in another sterile sample bag, labeled, and stored at 4°C until further analyses.

5.2.4 Aflatoxin extraction and analysis

Extraction was done following the manufacturer's instructions (TAF Assay- low matrix and AFB₁ Assay- low matrix HELICA Biosystems, Inc 2019). Concisely, 50% methanol extraction solvent was prepared by adding 50ml analytical grade methanol to 50ml double distilled water. Twenty grams (20g) of finely ground samples were extracted by adding it to 100 ml of 50% methanol mixed for 10 minutes and centrifuged at 3500 rpm for 5 minutes. The supernatant was collected and preceded the ELISA assay. The resultant ratio of the sample to the extraction solvent was then 1:5 (w/v).

Analyses of TAF and AFB₁ were done by low matrix competitive solid phase inhibition, a competitive enzyme-linked immunosorbent assay intended to quantitatively analyze AFB₁, B₂, G₁, and G₂ in grains, nuts, cottonseeds, cereals, and other commodities including animal feeds.

The detection limit was < 1 ppb. But then, the limit of detection (LOD) is commodity-specific and should be measured for each different commodity. Sample extracts were analyzed as described by the manufacturer's protocol. The optical density (OD) of each sample in a micro-well was read at a 450nm filter using a micro-titer ELISA plate reader (Thermo-fisher 357-Ty, Multiskan-FC, Shanghai, China) and then entered into Excel Program. Using the manufacturer's spreadsheet, the dose-response curve was constructed by the mean of OD values stated as a percentage of the OD of zero standards (0.0) against the aflatoxin content of the standard (%B/Bo). %B is the percentage binding for each standard and sample while Bo is the zero standards set as 100 percentage binding.

As described in the extraction protocol, the samples were diluted in a 5:1 ratio during extraction. Therefore, the concentrations of aflatoxin were multiplied by 5 to indicate parts per billion (ppb) of the samples. The LOD used to determine the sensitivity of the method was determined by calculating the mean concentration of 10 blank samples plus three standard deviations. The limit of quantification (LOQ) was calculated as the mean value +10 standard deviations. The limit of LOD and LOQ values were estimated to be 0.021 and 0.069 $\mu\text{g kg}^{-1}$, respectively. Concentrations of both the TAF and AFB₁ in the samples were measured by interpolation of the standard curve by constructing a dose-response curve using the average of OD values expressed as a percentage (%B/B₀) of the OD of the zero (0.0) standard against the aflatoxin content of the standard.

The unknown TAF and AFB₁ concentrations were measured by interpolation from the standard curve. The concentrations of the standards were labeled on the vial (0.0, 0.02, 0.05, 0.1, 0.2, and 0.4). However, the sample was diluted at a 5:1 ratio by extraction solvent as instructed in the extraction procedure. The level of aflatoxin shown by the standard was multiplied by 5 to indicate the ng per gram (ppb) of the samples. Linear regression and coefficient of determination (R^2) were calculated to assess the linearity of the calibration curve using six points of the standard curve. The R^2 values for all the curves (R^2 0.989 - 0.996) were within acceptable values.

5.2.5 Estimation of TAF and AFB₁ dietary intake

In this study, deterministic methods were performed by combining normalized MMSSC daily intake per body weight data. Whereby, the MMSSC intake data was collected using a modified food frequency questionnaire, and 24-hour recall for three consecutive days. The average was

used as earlier done by Huong *et al.* (2019). Children's weights were measured using a precise weighing balance. Exposure of individual children to TAF and AFB₁ was assessed as Estimated Daily Intake, EDI ($\text{ng kg}^{-1}\text{bwday}^{-1}$) = (TAF or AFB₁ concentration x consumption/day/body weight) (Huong *et al.*, 2019).

5.2.6 Statistical analysis

Using SPSS version 21, data were analyzed using descriptive statistics and inferential statistics. Specifically, means, standard deviation, standard error, and median were analyzed for the dependent variables. The significance of the means was tested using a one-way analysis of variance (ANOVA) and Duncan post-ANOVA test for mean comparison in the case of TAF and AFB₁ concentrations across the five processing points. The t-test was used to assess differences in mean for EDI TAF and EDI AFB₁ between stored and processed products. A pairwise correlation analysis was also performed to test for the significance of the correlation between aflatoxin exposure indicators; age and weight.

5.3. Results

5.3.1 TAF concentrations at processing points

Overall, TAF concentration was highest in soybean ($1.17 \pm 0.99 \mu\text{gkg}^{-1}$) and least in millet ($0.71 \pm 0.26 \mu\text{gkg}^{-1}$), was least contaminated. The concentration of TAF significantly differed at processing points in the wet season. In the wet season, highest concentration was observed in sesame ($1.19 \pm 1.12 \mu\text{gkg}^{-1}$), and least in the millet ($0.75 \pm 0.31 \mu\text{gkg}^{-1}$), (Table 7). However, there was no significant difference in TAF concentration between the soybean, freshly processed MMSSC, and processed stored products

In the dry season, TAF was highest in stored processed products ($0.85 \pm 0.11 \mu\text{gkg}^{-1}$), and least in the millet ($0.665 \pm 0.183 \mu\text{gkg}^{-1}$). There was no significant difference in TAF concentration between the millet and freshly processed. However, TAF was significantly higher in the stored products in the dry season. In the dry season, Soybean was not included in the analysis because there was only one sample. Overall, there was no significant difference between freshly processed MMSSC and stored MMSSC.

Table 7: TAF concentrations at process points of locally made malted millet sesame soy composite (MMSSC) across the wet and dry seasons

Process points	N (%)	Mean $\pm$ SD (μgkg^{-1})	Median	LL	UL
Wet season					
Soybean (raw material)	45 (21.6)	1.18 $\pm$ 1.00 ^{ab}	0.89	0.59	4.94
Sesame (raw material)	41 (19.7)	1.19 $\pm$ 1.12 ^a	0.86	0.43	4.87
Millet (raw material)	46 (22.1)	0.75 $\pm$ 0.31 ^b	0.73	0.08	1.35
FP-MMSSC	32 (15.4)	0.91 $\pm$ 0.26 ^{ab}	0.91	0.54	1.53
PS-MMSSC	44 (21.2)	0.86 $\pm$ 0.22 ^{ab}	0.83	0.49	1.49
Dry season					
Soybean (raw material)	1* (0.6)				
Sesame (raw material)	35 (22.6)	0.82 $\pm$ 0.16 ^{ab}	0.90	0.55	0.98
Millet (raw material)	46 (29.7)	0.66 $\pm$ 0.18 ^a	0.63	0.08	1.35
FP-MMSSC	30 (19.4)	0.69 $\pm$ 0.53 ^a	0.59	0.09	3.43
PS-MMSSC	43 (27.7)	0.85 $\pm$ 0.11 ^b	0.83	0.61	1.27
Overall	TAF				
concentrations					
Soybean (raw material)	46 (12.7)	1.18 $\pm$ 1.00 ^a	0.90	0.59	4.94
Sesame (raw material)	76 (20.9)	1.03 $\pm$ 0.85 ^{ab}	0.87	0.43	4.87
Millet (raw material)	92 (25.4)	0.71 $\pm$ 0.26 ^c	0.69	0.08	1.35
FP-MMSSC	62 (17.1)	0.81 $\pm$ 0.42 ^{bc}	0.68	0.09	3.43
PS-MMSSC	87 (23.9)	0.81 $\pm$ 0.17 ^{bc}	0.83	0.49	1.49

The values reported in the tables are mean $\pm$ standard deviation, median, and upper and lower limits. Means with a different subscript letter(s), a, ab, b, bc, and c indicate significant differences from each other (Duncan's test, $P \leq 0.05$). LL = lower limit, UL = upper limit, * Sample not included in the analysis. The proportion of the composite was: malted millet (76%), malted sesame (3.8%), and soybean (20.2%).

5.3.2 AFB₁ concentrations at process points

Table 8: AFB₁ concentrations at process points of locally made malted millet sesame soy composite (MMSSC) across the wet and dry seasons

Process points	N (%)	Mean $\pm$ SD (μgkg^{-1})	Median	LL	UL
Wet season					
Soybean (raw material)	45 (21.6)	0.65 $\pm$ 0.26 ^a	0.66	0.06	0.12
Sesame (raw material)	41 (19.7)	0.26 $\pm$ 0.31 ^b	0.09	0.00	0.1.1
Millet (raw material)	46 (22.1)	0.22 $\pm$ 0.27 ^b	0.05	0.01	1.04
FP-MMSSC	32 (15.4)	0.40 $\pm$ 0.27 ^c	0.51	0.01	0.81
PS-MMSSC	44 (21.2)	0.51 $\pm$ 0.24 ^c	0.52	0.06	0.86
Dry season					
Soybean (raw material)	1*(0.6)				
Sesame (raw material)	35 (22.6)	0. 48 $\pm$ 0.18 ^a	0.55	0.09	0.87
Millet (raw material)	46 (29.7)	0.43 $\pm$ 0.26 ^a	0.52	0.01	1.60
FP-MMSSC	30 (19.4)	0.35 $\pm$ 0. 21 ^a	0.34	0.03	0.68
PS-MMSSC	43 (27.7)	0.41 $\pm$ 0.28 ^a	0.44	0.01	0.83
Overall AFB₁ concentrations					
Soybean (raw material)	46 (12.7)	0.65 $\pm$ 0.26 ^a	0.66	0.06	1.22
Sesame (raw material)	76 (20.9)	0.38 $\pm$ 0.28 ^{bc}	0.38	0.00	1.05
Millet (raw material)	92 (25.4)	0.35 $\pm$ 0.33 ^b	0.46	0.01	2.00
FP-MMSSC	62 (17.1)	0.37 $\pm$ 0.24 ^{bc}	0.44	0.01	0.81
PS-MMSSC	87 (23.9)	0.46 $\pm$ 0.26 ^c	0.50	0.01	0.86

The values reported in the tables are mean $\pm$ standard deviation, median, and upper and lower limits. Means with a different subscript letter(s), a, ab, b, bc, and c indicate significant differences from each other (Duncan's test, $P \leq 0.05$). LL = lower limit, UL = upper limit. * Sample not included in the analysis. The proportion of the composite was: malted millet (76%), malted sesame (3.8%), and malted soybean (20.2%).

Generally, concentration was highest in soybean (0.65 $\pm$ 0.26 μgkg^{-1}) and least in millet (0.35 $\pm$ 0.33 μgkg^{-1}). However, there was no significant difference in AFB₁ concentration between freshly processed MMSSC and sesame.

In the wet season, AFB₁ concentration was highest in soybean (0.649 $\pm$ 0.038 μgkg^{-1}) and least in millet (0.22 $\pm$ 0.04 μgkg^{-1}) (Table 8). However, there was no significant difference in AFB₁

concentrations between sesame and millet, freshly processed products, and stored processed products. On the other hand, in the dry season AFB₁ contamination was highest in sesame ($0.48 \pm 0.03 \mu\text{gkg}^{-1}$), and least in the freshly processed product ($0.37 \pm 0.04 \mu\text{gkg}^{-1}$). Despite this, there was no significant difference in AFB₁ contamination in processed and stored products across the seasons, but overall, stored products had significantly high contaminations.

5.3.3 Exposure to TAF and AFB₁

As indicated in Figure 7, on average the most exposed age to both TAF and AFB₁ across wet and dry season was 5 months ($37.51 \text{ ng kg}^{-1} \text{ bw day}^{-1}$). The mean age for the children was 15.69 ± 6.92 months and the typical weight was $11.2 \pm 3.24 \text{ kg}$.

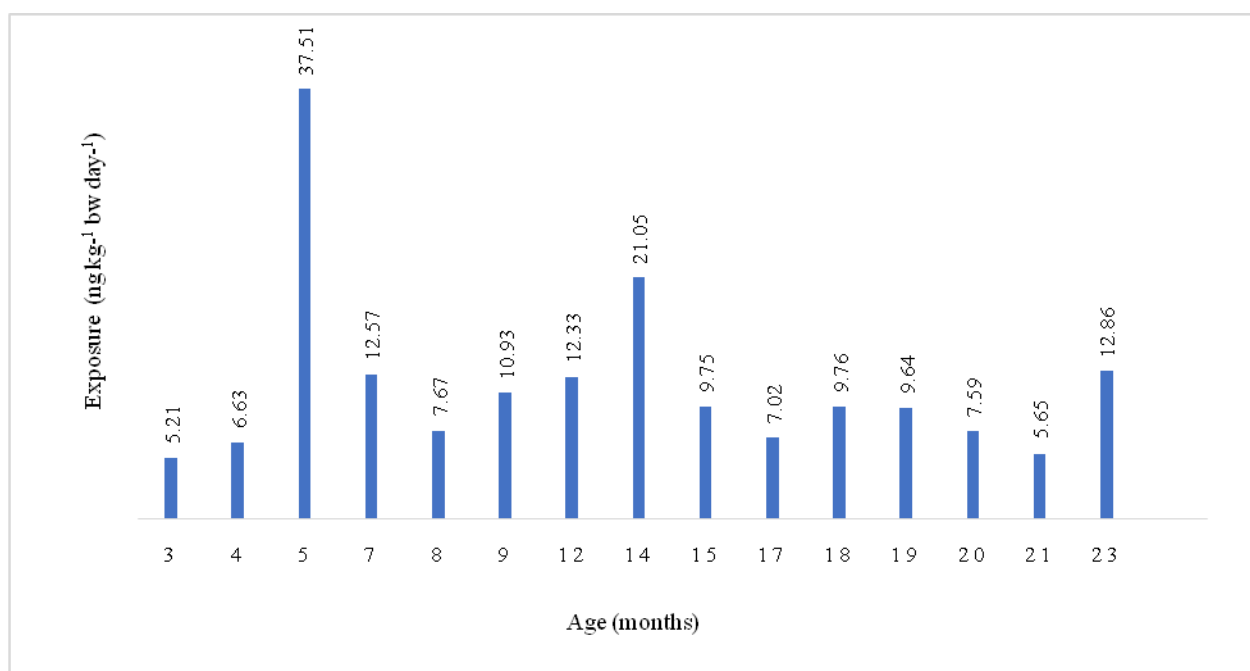


Figure 5: Average exposure ($\text{ng kg}^{-1} \text{ bw day}^{-1}$) of children with age (months)

As illustrated in Table 9, the mean EDI for TAF in the freshly processed products was higher in the wet season than in the dry season ($14.65 \pm 2.11 \text{ ngkg}^{-1} \text{ bw day}^{-1}$ and $13.58 \pm 2.21 \text{ ngkg}^{-1} \text{ bw day}^{-1}$) respectively. A similar scenario was observed in the stored processed MMSSC where the mean EDI for TAF in the wet season was higher than in the dry season ($18.08 \pm 2.15 \text{ ngkg}^{-1} \text{ bw day}^{-1}$ and $16.8 \pm 1.90 \text{ ngkg}^{-1} \text{ bw day}^{-1}$) respectively. Equally, the mean EDI for AFB₁ in freshly processed MMSSC was higher in the dry season than in the wet season (8.14 ± 1.58

ngkg⁻¹bwday⁻¹ 6.66 ± 1.38 ng kg⁻¹bwday⁻¹). The mean EDI for AFB₁ in the wet season was in stored processed product than in the dry season at 10.66 ± 1.69 ngkg⁻¹bwday⁻¹ and 7.81 ± 1.29 ngkg⁻¹bw day⁻¹ respectively. However, there was no significant difference in the TAF and AFB₁ EDI resulting from consumption of the freshly processed and stored processed MMSSC, $P \leq 0.05$ as indicated in Table 10.

Table 9: Mean values of TAF and AFB₁EDI resulting from consuming freshly processed and stored processed MMSSC

Parameters	Freshly processed products	Stored processed products
	Mean ± SD	Mean ± SD
Age (months)	15.95± 6.89	15.43 ± 7.03
Weight (kg)	11.24 ± 3.26	11.15 ± 3.26
EDI TAF S-1 (ng kg ⁻¹ bw day ⁻¹)	14.65 ± 13.01	18.08 ± 12.71
EDI AFB ₁ S-1(ng kg ⁻¹ bw day ⁻¹)	6.66 ± 8.52	10.66 ± 10.05
EDI TAF S-2 (ng kg ⁻¹ bw day ⁻¹)	13.58 ± 13.59	16.86 ± 11.24
EDI AFB ₁ S-2 (ng kg ⁻¹ bw day ⁻¹)	8.14 ± 9.73	7.81 ± 7.76

The daily intake was calculated based on the mean concentration reported with consumption data of processed products. EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively), EDI AFB₁ S-1 and EDI AFB₁ S-2 (Estimated Daily Intake for AFB₁ in the wet season and dry season respectively).

Table 10: Mean values of TAF and AFB₁ EDI resulting from the consumption of freshly processed food and the stored processed MMSSC

EDI	Stored product		Processed Product		Mean difference (μgkg^{-1})	t-stat	p-value
	Mean $\pm$ SD	Mean $\pm$ SE	Mean $\pm$ SD	Mean $\pm$ SE			
	(μgkg^{-1})	(μgkg^{-1})	(μgkg^{-1})	(μgkg^{-1})			
TAF L S-1	18.08 $\pm$ 12.70	18.08 $\pm$ 12.70	14.65 $\pm$ 13.01	14.65 $\pm$ 2.11	3.43	1.14	0.26
AFB ₁ S-1	10.66 $\pm$ 10.05	10.66 $\pm$ 10.05	6.66 $\pm$ 8.52	6.66 $\pm$ 1.38	4.01	1.84	0.07
TAF L S-2	16.86 $\pm$ 11.24	16.86 $\pm$ 11.24	13.58 $\pm$ 13.59	13.58 $\pm$ 2.21	3.27	1.12	0.27
AFB ₁ S-2	7.81 $\pm$ 7.76	7.81 $\pm$ 7.76	8.14 $\pm$ 9.73	8.14 $\pm$ 1.58	-0.33	0.16	0.87

EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively),
EDI AFB₁ S-1 and EDI AFB₁ S-2 (Estimated Daily Intake for AFB₁ in the wet season and dry season respectively).

5.3.4. Correlation between EDI for total and AFB₁ across the season, processing points, and age and weight of children

As indicated in Table 11, in fresh processed and stored MMSSC, there was a weak negative correlation between EDI for total and AFB₁ and age. However, these were not significant ($P \leq 0.05$) level of significance in both seasons. Yet, the weight of the children significantly ($P = 0.024$) correlated negatively to EDI for TAF in the wet season in the stored processed products.

Table 11: Correlation between EDI for total and AFB₁ and process levels, age, and weight of children across the wet and dry seasons

EDI of TAF and AFB ₁ in wet and dry seasons	Age		Processed		Weight		Processed	
	Stored		Product		Stored		Product	
	Correlation	P-value	Correlation	P-value	Correlation	P-value	Correlation	P-value
EDI TAF S-1	-0.075	0.667	-0.091	0.586	-0.382	0.024*	-0.179	0.281
EDI AFB ₁ S-1	-0.009	0.960	-0.041	0.807	-0.212	0.222	-0.132	0.429
EDI TAF S-2	0.089	0.611	-0.115	0.492	-0.258	0.135	-0.133	0.425
EDI AFB ₁ S-2	0.107	0.535	-0.106	0.527	-0.085	0.624	-0.231	0.163

EDI TAF S-1 and EDI TAF S-2 (Estimated Daily Intake for TAF in the wet season and dry season respectively),
EDI AFB₁ S-1 and EDI AFB₁ S-2 (Estimated Daily Intake for AFB₁ in the wet season and dry season respectively).

5.4 Discussions

Total aflatoxin and AFB₁ contamination were observed throughout the processing points. The highest contamination was in the raw materials (sesame and soybean). The higher TAF and AFB₁ contamination of sesame and soybean than millet or processed MMSSC could be differences in crop structure and or pre and postharvest management according to the respective food types (Matumba *et al.*, 2021; Ortega-Beltran & Cotty, 2020; Seetha *et al.*, 2017). Similarly, other studies reported differential contamination by aflatoxins due to differences in postharvest practices of many staple crops including oil seeds (Goncalves *et al.*, 2019). According to Marshall *et al.* (2020), contamination along processing points could be reduced by using innovative technologies such as sorting produce using fluorescence devices and secondary processing (Özer *et al.*, 2018). Equally, Özer *et al.* argued that ensuring good agricultural practices and proper storage may not guarantee the reduction of mycotoxin (Özer *et al.*, 2018). However, Boudergue *et al.* (2017) reported that decontamination approaches such as sorting, sieving, washing, and heat treatment may ensure reduction. Additionally, Özer *et al.* (2018) noted that chemical treatment using acids, alkalis, oxidizing agents, and microbial agents such as enzymes may also be employed to minimize contamination. Thus, it may not be reasonable to conclude that once the raw materials are free from contaminants then the processed products are

safe. Contamination can occur at any stage along the processing chain including at storage. However, combinations of decontamination methods may be appropriately applied to guarantee minimal contamination to acceptable levels.

The TAF contamination was most common in the raw materials and freshly processed products was the least contaminated in the wet season. Overall, the mean TAF concentration was highest in soybean and least in millet. The report showed that depending on the availability of favorable conditions, fungal growth, and toxin production may occur at any stage of the food chain (Perrone *et al.*, 2020). The low prevalence of the total and AFB₁ in millet is comparable to a study by Akello *et al.*, (2021) who noted that small cereals were less prone to aflatoxin contamination. The literature further explained that measures to control/prevent fungal infection at pre-harvest are critical for sustainably minimizing aflatoxin contamination at the post-harvest level (Mahuku *et al.*, 2019a). This could be the reason for the high contamination of the raw materials than the processed and stored products since contamination of the product could have most likely happened in the field. Literature also indicated that high aflatoxin contamination of the product may occur due to late detection and removal of contaminated ones in the processing continuum, and their spread along the chain can be exacerbated and promoted by poor storage conditions (Ahlberg *et al.*, 2019). This is supported by Casquete *et al.* whose findings showed that the proliferation of *A. flavus* and AFB₁ production significantly resulted from optimum temperature, pH, and water activity of the substrate during storage (Casquete *et al.*, 2017). Furthermore, they observed aflatoxin contamination level was highest in the stored processed product. This could be due to poor storage conditions that might have exacerbated and promoted contamination (Casquete *et al.*, 2017). Therefore, to minimize contamination, there is an urgent need to sort raw material before storage of grains.

The most contaminated raw materials were soybean and sesame which could be explained by the fact that oil seed plants promote the proliferation of *A. flavus* and subsequent toxin production. This was evidenced by several studies, for instance, Rajasekaran *et al.* observed that total lipid content significantly enhances AFB₁ production in cotton while in the field (Rajasekaran *et al.*, 2017). Comparably, (Liu *et al.*, 2016) revealed that growth and toxin production were significantly negatively affected when oily grains and cereals such as soybean and peanuts were defatted and vice versa when corn oil was reintroduced. Additionally, the same study showed a positive correlation between AFB₁ concentrations and an increase in substrate-soluble sugars such as glucose, maltose, fructose, sucrose raffinose, and stachyose (Liu *et al.*, 2016). Similarly,

Singh and Sinha's report showed that the total starch and amylopectin content of the substrate were positively correlated with AFB₁ levels (Jallow *et al.*, 2021). However, on the contrary, other substrate components such as Tocopherols have been shown to negatively influence the production of aflatoxins (Jallow *et al.*, 2021). But soybean that was heavily contaminated compared to millet has been shown to have higher tocopherol content compared to millet (Himanshu *et al.*, 2018). Therefore, susceptibility to fungal infestation and subsequent production of aflatoxins may vary among crop produce depending on their nutrient content, crop structure, and harvest management (Ortega-Beltran & Cotty, 2020; Shanakhath *et al.*, 2018; Los *et al.*, 2018).

Generally, the low contamination observed in freshly processed products could be explained by the fact that processing reduces fungal contaminants (Los *et al.*, 2018; Juodeikiene *et al.*, 2018; Taheri *et al.*, 2012). For instance, previous studies indicated a reduction of fungal load in processed cereals. Equally, malting, a process that was employed in this study has been sought to reduce mycotoxin concentration (Taheri *et al.*, 2012), which could be due to the low release of the toxins into the food but remain in the mycelium during processing (Jallow *et al.*, 2021).

Based on the season, the total and the mean TAF concentrations were higher in the wet season (wet) than in the dry season (dry). Samples collected in the wet season had high contaminations of both TAF and AFB₁ in the dry season. This is analogous to a study by Taheri *et al.* who registered higher concentrations of TAF in winter than in summer (1.99 ng g⁻¹ and 0.82 ng g⁻¹), respectively (Bashiry *et al.*, 2021). That study also reported a high level of AFB₁ during winter to be above the globally acceptable limit than during summer (7.4% and 3.4%, respectively). Likewise, Bashiry *et al.* (2021) noted that AFB₁ occurrence was higher (60%) in cold seasons than in warm seasons. According to Atongbiik *et al.* (2017), high concentrations observed in the wet season may indicate the availability of favorable environmental conditions (water activity of between 0.93 to 0.99 and temperature of 0 to 33°C) for the proliferation of aflatoxin-producing *Aspergillus* species. Contrarily, Damianidis *et al.* (2018) findings showed significantly high aflatoxin contamination in the dry seasons due to night heat stress.

Conversely, AFB₁ concentration in the wet season was highest in the soybean and least in millet. In the dry season, the mean concentration of AFB₁ was highest in the sesame and least in freshly processed MMSSC. Generally, the mean concentration varied, with concentrations of both TAF and AFB₁ being higher in the wet season than in the dry season. This could have been due moist

environment during the wet season that favored fungal growth (Makori *et al.*, 2018; Perrone *et al.*, 2020). In agreement with the current finding, Negash reported that contamination may vary from year to year (Negash, 2018). These may result from the availability of favorable factors such as optimal growth conditions, pest invasion, and poor pre- and post-harvest management (Lukwago *et al.*, 2019). Specifically, the production of aflatoxin by *A. flavus* varies depending on growth conditions and the availability of another microorganism that degrades the produced toxins (Jallow *et al.*, 2021).

The current study demonstrated that 100% of the locally made malted millet-sesame-soy composite had detectable levels of TAF and AFB₁. Additionally, 100% of the composite analyzed seems to be contaminated by TAF and AFB₁ above the EU maximum allowable limits of 0.10 µgkg⁻¹ and 0.40 µgkg⁻¹ for TAF and AFB₁ respectively, especially for processed baby foods made from cereals (Atongbiik *et al.*, 2017). Although, contamination levels for both TAF and AFB₁ of the raw materials were within the EU acceptable levels of 2 µgkg⁻¹ for AFB₁ and 4 µgkg⁻¹ for TAF for processed foods for other human consumption and 8 µgkg⁻¹ unprocessed foods (Atongbiik *et al.*, 2017). In terms of compliance with the EAC and USFDA standards, 5 µgkg⁻¹ and 10 µgkg⁻¹ for AFB₁ and TAF and 20 µgkg⁻¹ for TAF in food and feeds respectively (Ali *et al.*, 2020), the studied product met the standard. Nevertheless, due to frequent daily consumption, children consuming this product could be exposed to a high load of aflatoxins.

Globally, vulnerability to health risks of exposure to aflatoxins is registered more in weaning children than adults because of their increased special food intake mainly cereals for each kg/body weight (Adetunji & Atanda, 2017). The present study showed that exposure to both TAF and AFB₁ was higher when stored products were consumed than freshly processed products in both seasons. However, there was no significant difference in both the TAF and AFB₁ EDI resulting from the consumption of freshly processed food and stored processed food in both seasons ($P = 0.05$). This could be due to the short storage period (one month used in the current study) that limits the proliferation of toxigenic fungi and results in toxin production.

The study also demonstrated that on average when both the processed and stored products are consumed across wet and dry seasons, the most exposed age was 5 months followed by 14 months. The least exposed group was 3 months. This could be because this age group complementary composite is introduced and frequently taken, compared to the younger (3 months) and older ones who may depend on breast milk and family food respectively. Contrarily,

Bashiry *et al.* (2021) found the most exposed age to be 6-12 months with an average EDI of $153 \pm 0.13 \mu\text{gkg}^{-1}\text{bwday}^{-1}$. This was higher than the overall level of exposure in the current study. Eshete *et al.* (2021) on the other hand found that in Nigeria, children below 12 months were less exposed in comparison with older children. This could have been due to a myriad of factors such as the quantity and frequency of consumption of contaminated weaning foods given to this age group in addition to proper hygiene care rendered to this age group to avoid cross-contamination and consequent exposure. Several other studies have also reported levels higher than PMTDI. For instance, an average exposure level of $68 \text{ ngkg}^{-1}\text{bwday}^{-1}$ due to the consumption of cereal was reported (Sirma *et al.*, 2018). Another finding also indicates higher aflatoxin exposure levels (763.6 to $1901.1 \text{ ng kg}^{-1}\text{bwday}^{-1}$) in children who consumed stored maize grains (Zhang *et al.*, 2020). Similarly, in Tanzania, a very high aflatoxin exposure level of $1,337 \pm 392.5 \text{ ngkg}^{-1}\text{bwday}^{-1}$ was registered (Herrera *et al.*, 2019).

In general, exposure levels in these studies were above the aflatoxin PMTDI of $1.0 \text{ ngkg}^{-1}\text{bwday}^{-1}$ and $0.4 \text{ ngkg}^{-1}\text{bwday}^{-1}$ for children who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively (Boni *et al.*, 2021). Aflatoxin exposure levels in other continents apart from Africa have also been explored and found on average relatively lower levels such as 0.93 to $2.45 \text{ ng kg}^{-1}\text{bw day}^{-1}$ in Europe, and $2.7 \text{ ngkg}^{-1}\text{bwday}^{-1}$ in the USA (Zhang *et al.*, 2020). This variation could be because countries with high levels of exposure may rely much on local production of complementary food compared to those with low levels of exposure who could mainly depend on industrialized exported products whose production and processing strictly adhere to recommended safety practices. Literature affirms that locally-produced foods had reasonably high levels of aflatoxin compared to the imported ones possibly due to contaminants in the raw materials and along the chain due to negligence for safety among households (Alamu *et al.*, 2018; Echodu *et al.*, 2019). This calls for urgent action to ensure the safety of locally made complementary food to minimize exposure of weaning children because of health implications resulting from exposure to aflatoxin.

Exposure to both TAF and AFB₁ was higher in the stored products than in freshly processed products in both seasons. Although no significant difference was observed in both the TAF and AFB₁ EDI resulting from the consumption of the freshly processed food and the stored processed food, in both seasons ($P \leq 0.05$). There was a weak negative correlation between EDI for total and AFB₁ and age in both the freshly processed and stored products. However, these were not

significant in both seasons. This finding suggests that exposure of children to total and AFB₁ is independent of the season.

5.5 Study Limitations

The study was conducted in a rural domestic environment because it is vital for the public health of rural communities that cannot access and afford standardized complementary foods made under controlled safety conditions. Thus, the study contributed to the information on the safety of complementary food produced in a rural setting with minimal controlled conditions. However, the study had the following methodological limitations: The ELISA method used for determining aflatoxins in the current study is a routine test with a detection limit higher than other methods such, as HPLC. Therefore, the accuracy may be lower. However, in resource-limited settings, ELISA is used and, it provides reliable results.

The study also only examined aflatoxins contamination of samples obtained from the trained group so our findings may not be generalized to the entire community. Further study is thus needed to compare the aflatoxin contamination of samples collected from the trained and untrained groups. Also, the number of soybean samples in season 2 was limited due to the dry season when the food grains produced in the households were inadequate, so it could not be statistically analyzed.

5.6 Conclusion and recommendation

This study has demonstrated that TAF and AFB₁ contamination in MMSSC varied along processing points from raw materials, stored, and freshly prepared composite in descending order. The variation in TAF and AFB₁ along the processing points was higher in the wet than dry season except in the stored composite. Estimated Daily intake for both total and AFB₁ exceeded the Codex PMTDI with no variation in the wet and dry seasons. Based on food safety standards, all the raw materials, processed, and stored products met the EU, US-FDA, and EAC standards limit for general consumption but were below EU standards for processed cereal baby food. Therefore, a concerted effort is needed to reduce contamination and exposure of the children consuming locally made MMSSC to aflatoxin and AFB₁ taking sesame and soybean as priority ingredients for formulating composite based on the season.

CHAPTER SIX: Status of food safety knowledge, attitude, and practices of caregivers of children in Amuru and Nwoya districts of Uganda

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6.1 Introduction

Food safety is a universal precondition necessary for achieving good complementary feeding outcomes among children 6-23 months of age (Issaka *et al.*, 2014). Particularly, because complementary foods are introduced at 6 months, a point in time at which breast milk solely cannot adequately support a child's nutrient needs for optimal growth despite the child having an underdeveloped immune system (Agostoni *et al.*, 2009; Du Plessis *et al.*, 2013). In many low- and middle-income countries, contamination of complementary foods is one of the factors that compromise the nutritional outcome due to the occurrence of diarrheagenic food-borne diseases caused by nontyphoidal *Salmonella* and *Escherichia coli* (Scallan *et al.*, 2015). As such, complementary feeding of children with safe food becomes a prerequisite for reducing diarrheagenic food-borne diseases and consequently attaining optimal growth among children (Sihag *et al.*, 2015). Evidence in the literature shows that local production of safe complementary foods and their utilization necessitates that caregivers practice hygienic food behavior (Kunadu *et al.*, 2016). Therefore, efforts to address food safety challenges, especially among caregivers of children 6–23 months from a behavioral viewpoint are necessary for realizing good nutrition outcomes and attaining sustainable development goal 3, good health and well-being (Nabyonga-Orem, 2017).

Universally, food-borne illnesses (FBI) threaten public health and socioeconomic progression due to the associated morbidity and mortality (Devleesschauwer *et al.*, 2018). FBI are caused by microbiological, chemical, and physical hazards causing health challenges. Among the food-borne hazards, microbiological hazards have been regarded as a threat in recent years (Gizaw, 2019) and are caused by pathogenic viruses, bacteria, molds, yeast, protozoa, and their associated toxins (Lyashchuk *et al.*, 2021; Vandeweyer *et al.*, 2021). These micro-biological agents in food beyond acceptable limits render the food unsafe for human consumption (Bersisa *et al.*, 2019;

Negash, 2018; Saipullizan *et al.*, 2018). Recent studies show that the burden of FBI is attributable to pathogenic bacteria such as *E. coli*, *campylobacter*, norovirus, and nontyphoidal *Salmonella enterica* that cause diarrhea (Aworh, 2021; Chau *et al.*, 2019; Lund, 2015). Globally, 40% of the burden of all FBI is on children under 5 years due to their underdeveloped immune systems (Havelaar *et al.*, 2015; Kloc *et al.*, 2020).

Although data on food-borne diseases are often scanty in the case of low-middle-income countries, data are often available in the form of food-borne disease-associated manifestations such as diarrhea prevalence. For instance, a recent study in Uganda reported that 41% of hospitalized children under 5 years had diarrhea caused by *Salmonella* infection (Appiah *et al.*, 2021). Particularly in northern Uganda, over the last 10 years, there has been a high prevalence of diarrheal diseases in children under (24.4% in 2016 and 22.2% in 2022), (Uganda Bureau of Statistics, UBOS, 2019; Uganda Bureau of Statistics, UBOS, 2023). The prevalence of diarrheal disease among children suggests the existence of non-adherence to good food hygiene practices among caregivers. Indeed, studies on complementary feeding have shown that diarrheal disease incidence among children becomes more pronounced within half a year when complementary foods are introduced (Ogbo *et al.*, 2018; Rao *et al.*, 2011).

Owing to their low purchasing power of standardized hygienically produced complementary foods, households in rural areas of several low- and middle-income countries largely rely on locally produced (homemade) complementary foods (Pelto *et al.*, 2013). Even though locally produced complementary foods are widely acceptable, used, and nutritious (Alowo *et al.*, 2018; Nassanga *et al.*, 2018; Theurich *et al.*, 2019), their microbiological safety is of major public health concern. However, it is important to note that the safety of such locally produced complementary food is largely dependent on the food-handling practices of caregivers involved in their preparation and utilization (Wodnik *et al.*, 2018). Thus, sanitary practices during the preparation of such products have to be strictly followed to minimize the occurrence of FBI among children fed on locally made complementary foods (Mshida *et al.*, 2020; Teshome *et al.*, 2022).

Additionally, because most rural communities including those in northern Uganda produce complementary foods that are largely plant-based mainly cereals such as maize (*Zea mays*), millet (*Eleusinecoracana*), and legumes such as soybeans (*Glycinemax*) (Alowo *et al.*, 2018; Lukwago *et al.*, 2019), aflatoxin contamination may be another threat. A typical example is the

malted millet–sesame–soy composite (MMSSC) that was recently formulated and promoted among caregivers of children aged 6–23 months in Acholi, a sub-region of northern Uganda (Alowo *et al.*, 2018). Locally produced complementary foods are potentially prone to microbial contamination if FHP is not stringently adhered to. Therefore, to ensure that children 6–23 months are fed microbiologically safe, locally produced complementary foods, it is vital that caregivers have sufficient food safety knowledge and a positive attitude associated with food-handling practices from the production to the consumption stage.

Though there is a high reported occurrence of food-borne diseases among children under 5 years (WHO, 2015), the caregivers' knowledge, attitude, and practices regarding food safety of locally produced complementary foods in the context of the rural environment have remained under investigation. Studies on the significance of knowledge, attitude, and practices on the outcome of complementary feeding have focused on aspects associated with nutrient quality, quantity, and frequency (Aguayo *et al.*, 2018; Pratik *et al.*, 2017) and the elements of primary health-care (Assefa *et al.*, 2021; Chidziwisano *et al.*, 2019; Gebresilasie *et al.*, 2018; Hassan Khatib & Joho, 2022; Yimenu *et al.*, 2022). This leaves a lacuna in the knowledge of whether caregivers who produce local complementary foods have sufficient food safety knowledge, positive attitudes, and follow good FHP; which is essential in reducing the vulnerability of children to food-borne diseases (Vasco & Alvito, 2011). Therefore, the knowledge, attitude, and practices of the caregivers towards safety of complementary food formulae produced in rural domestic environments should be adequately investigated. Hence, the objective of this study was to determine the status of food safety knowledge, attitude, and food hygiene practices of the caregivers of children aged 6–23 months utilizing MMSSC as complementary food and their predictors as well as barriers to FHP.

6.2 Materials and Methods

6.2.1 Study design

This study adopted a mixed cross-sectional design involving a survey using in-depth household interviews and focus group discussions (FGDs). The study was conducted in the Acholi sub-region in Amuru (02°50'N 33°05'E) and Nwoya (02°38'N 32°00'E) districts, Northern Uganda, between March and June 2019. The two districts were selected to follow up on a study done by Alowo *et al.* (2018). The study population consisted of caregivers (mother/father of the child or

legal guardian who takes care of the child in the absence of the mother/father) of children (6–23 months).

6.2.2. Sample and sampling procedure

A total of 180 participants in the previous study conducted by Alowo *et al.* (2018) participated. They included all caregivers trained on local production of MMSSC from two sub-counties per district in Nwoya and Amuru districts of northern Uganda. From the two purposively selected sub-counties per district, one parish was purposively selected. In each parish, one village was then purposively selected. The inclusion criteria were a caregiver who received training on local production of MMSSC and was willing to participate in the study. The training of the caregivers lasted for 82 days and aimed at equipping them with good hygiene and good manufacturing practices to produce safe complimentary food composite in a domestic environment. The training content included using safe water and raw materials for making the composite, hand washing the caregiver and the child, keeping the utensils and environment clean, thorough cooking, and keeping cooked food covered in a safe place. Training of participants was done in Local dialect (Acoli). Figure 8 shows the detailed porridge production process using three ingredients, millet (*Eleusine coracana*), sesame (*Sesamum indicum*), and soybeans (*Glycine max*).

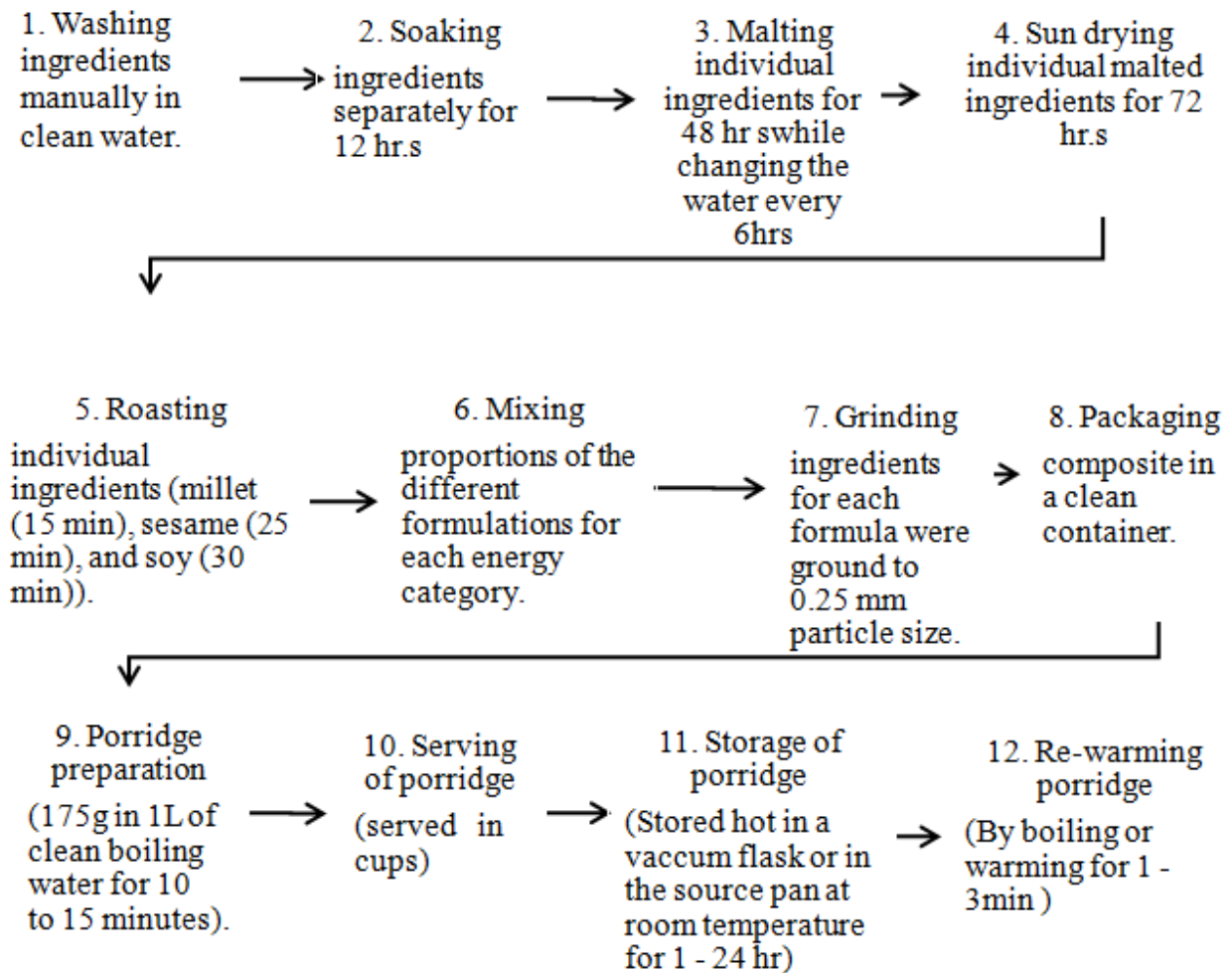


Figure 6: The detailed porridge production process

6.2.3 Data collection

A modified pretested questionnaire with provision for socio-demographic characteristics of the household adopted from FAO (2014) and Marías and Glasauer (2014) was used for assessing the knowledge, attitude, and practices (KAP) of the caregivers. To attenuate the general answer bias ten food safety knowledge questions positively and negatively were framed (Dunsch *et al.*, 2018). Also, six both positively and negatively framed questions were used for measuring attitude on a 3-point Likert scale (0=disagree, 1=neither agree nor disagree, and 2=agree) (Anand & Puri, 2013).

Caregivers' self-reported practices were assessed by asking caregivers to articulately express how they handle food, especially food for complementary feeding from postharvest to consumption.

To supplement the information obtained from household interviews, the community's perspective on the safety of MMSSC and food safety, in general, was obtained using the pretested FGDs guide based on health belief model constructs (Pourtaheri *et al.*, 2018; Rosenstock, 1974; Rosenstock *et al.*, 1988). Before household interviews and FGDs, modified semi-structured questionnaires and FGD guide adopted from WHO (2010) and Marías and Glasauer (2014) were pretested among 30 caregivers of children aged 6–23 months attending postnatal care at Laroo Health Center III in Gulu district to ensure accuracy, clarity, and consistency in interpretation of questions. The responses were analyzed to check for validity and ambiguity. Results of the first pretest showed that respondents found difficulties interpreting questions such as, “What is the source of birth?” This question was revised to “What is the place of birth?”

Additionally, participants did not respond to the question, “What is the estimated household income allocated to food?” This question was later split into two questions: “What is the estimated household income in a month?” and “What is the estimated amount spent on food?” The checked and validated pretested tools were then re-pretested among 30 caregivers of children aged 6–23 months in Unyama Village, Gulu District (a similar distant community), within 1 week. From the second pretest, respondents did not have much difficulty, and the responses were in agreement with the study objective. However, results from the second pretest showed that most (97%) respondents failed to estimate the money spent on food in a month. Therefore, this question was removed from the data collection instrument.

Trained research assistants with experience in nutrition surveys and fluent in English and the local dialect (Acholi) administered the study questionnaires and conducted FGDs in the local dialect (Acholi). The study instruments were written in English and translated into the local dialect (Acholi). To guarantee confidentiality and prevent any interference, other members of the household and members of the neighboring household were not allowed to participate or be present during the interview and FGDs. To ensure the originality of information, FGD consisted of 6 to 10 participants (caregivers of children aged 6–23 months) who had not participated in the household interview. As affirmed by Guest *et al.* (2006) and McCafferty (2004), a total of eight FGDs were conducted, this is beyond the requirement (six focus group discussions) for data saturation.

6.2.4 Data analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 21. For the case of knowledge questions, correct answers scored 1, wrong answers, and in scenarios where the respondent did not know, awarded a score of 0. An attitude score of 0, 1, and 2 was given for disagreeing, neither agree nor disagree, and agree, respectively. Conversely, the reverse scores were given to negatively framed questions. This implied that 0, 1, and 2 were given for agreeing, neither agree nor disagree, and disagree, respectively (Anand & Puri, 2013). Knowledge and attitude of each caregiver were calculated by summing up scores on each question. The overall score ranked as sufficient or insufficient knowledge and positive or negative attitude, respectively. The cutoff for insufficient knowledge and a positive attitude was 70% based on previously published studies (Tamang *et al.*, 2020).

Descriptive statistics (frequency, percentages, mean, and standard deviation) where applicable, were used for summarizing the socioeconomic demographic characteristics and knowledge, attitude, and practices (KAP) data. To establish the socioeconomic and demographic predictors of sufficient knowledge and positive attitude, Pearson correlation analysis was run before regression analysis to eliminate highly correlated variables ($r > .7$) (Dormann *et al.*, 2013). Meanwhile, multivariate binary logistic regression was performed using Equation (i):

$$Y_i = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \dots \dots \dots \beta_{17} X_{17} + \mu \dots \dots \dots (i).$$

Y_i is the binary dependent variable representing the status of knowledge and attitude; α is the regression constant; β is the regression coefficient; X_1 to X_{17} are the independent variables; and μ is the error term.

The independent variables selected to run the multivariate binary logistic regression are shown in Table 12. Binary multivariate logistic regression analysis was performed to establish the socio-demographic predictors of sufficient knowledge and positive attitude. Significant statistical differences were considered at a 95% confidence level ($P \leq .05$). FGD data were transcribed, coded, and organized into common themes based on the health belief model constructs (Pourtaheri *et al.*, 2018) and then analyzed using qualitative content analysis (Cho & Lee, 2014).

Table 12: Independent variables selected to run the multivariate binary logistic regression

S/N	Social demographic variables	Responses
1	District/location	Nwoya = 1, Amuru = 2
2	Age of the caregiver	Years
3	Gender of the caregiver	1 = male, 0 = female
4	Education level of household head	1 = formal, 0 = non-formal
5	Occupation of household head	1 = farmer, 0 = others
6	Marital status	1 = married, 0 = others
7	The main source of income	1 = sale of agricultural produce, 0 = others,
8	Frequency of food safety training	1= two or more times, 0 = once.
9	Food safety training	1= yes, 0 = no
10	Household size	Number of people,
11	Households with children.	1= 1-17 years, 0 = children aged ≤ 5 .
12	Gender of the child	1= male, 0 = female
13	Whether a child has ever suffered from symptoms of food-borne illness.	1= yes, 0 = no
14	Where the child was treated.	1 = medical unit, 0 = others.
15	Decision on family income.	1 = male, 0 = others
16	Decision on food to cook.	1 = male, 0 = female,
17	Responsibility for provision for the household.	1 = male, 0 = female

6.3 Results

6.3.1 Socio-demography of the caregivers

Table 13 shows that the female caregivers were three times more than the male caregivers. The majority (91.3%) of the caregivers were within the age bracket of 18 - 45 years. The most predominant age group of the caregivers was between 18 and 25 years and three-quarters of the caregivers were married. Three-quarters of both the household heads and caregivers were subsistence farmers. Others were formally employed or casual laborers, but more caregivers (12%) were involved in casual work than household heads (9.5%). In terms of the level of

education attained by the caregivers, a greater percentage attained primary level of education (57%), followed by secondary (31.1%), no formal education (11.2%), and lastly tertiary education (1.7%). On average, the household size in this community was seven people. Families with household sizes less than seven were more than those with household sizes greater than seven by 13%.

The respondents reported varied ages when complementary feeding was introduced. More than half (54.3%) of the caregivers did not adhere to the WHO and MOH of Uganda guidelines of introducing complementary feeding at 6 months while less than half (45.7%) adhered to the guideline. Additionally, the study found that an equal proportion of the children (50:50) were still breastfeeding and had been weaned. Own-grown foods were 22 times more consumed in the studied households than the purchased foods and the sale of agricultural produce contributed three-quarters of the main source of income in the studied households compared to other sources of income such as formal employment and casual labor that altogether contributed to only one-quarter of the total household income.

Concerning gender roles and responsibilities, the male family head was 13 times more responsible for household needs than the female caregiver and had 33 times more than the shared responsibility between the wife and the husband. Further, the male family head was two times more responsible for the decision-making on family income usage than the female caregivers and had six times more than the shared responsibility between the wife and the husband. Feeding and child care were women largely (94%) done by women. The current study revealed that more than 42% of the caregivers gave birth in the medical facility than at home. Birth records indicated that more than three-quarters (77.6%) of children had normal birth weights. Among the studied children, there were an equal proportion of boys to girls.

Table 13: Socio-demographic characteristics of the caregivers

Variables	n	%	Variables	n	%
Sex of the caregiver			How Food is Obtained		
Male	29	25	Farming	111	95.7
Female	87	75	Purchase	5	4.3
Age of the caregiver (Years)			Who is responsible for providing for the household		
<18	2	1.7	Family head	55	47.4
18- 25	41	35.3	Wife/mother	40	34.5
26-35	36	31	Both husband and wife	17	14.7
36-45	29	25	Grandparent	4	3.4
46 +	8	7			
Marital status			Decides family income use		
Single	7	6	Family head	66	
Married	88	75.9	Wife/mother	36	31
Separated	12	10.3	Both husband and wife	11	9.5
Widowed	9	7.8			
Occupation			Who is responsible for cooking and home/child care		
Household head			Grandparent	3	2.6
Formal employment	8	6.9			
Causal laborer	11	9.5	Who is responsible for cooking and home/child care		
			Family head	1	0.9
Farmer	87	75	Wife/mother	109	94
Others	10	8.6	Both husband and wife	4	3.4
Occupation caregiver			Grandparent	2	1.7
Formal employment	3	2.7	The main source of family income		
Causal laborer	14	12	Sale of agricultural produce	87	75
			Others	12	10.3
Farmer	87	75	Where the caregiver gave birth		
Others	12	10.3	Hospital	82	70.7

Education level of the caregiver			Home	33	28.4
No formal education	13	11.2	Prison	1	0.9
Primary	66	57	Sex of the child		
Secondary	35	30.1	Female	58	50
Tertiary	2	1.7	Male	58	50
Household size			Childbirth weight		
> 7 people	54	46.5	< 2.5	9	7.8
< 7 people	62	53.5	≥ 2.5	90	77.6
Age of the children			Do not know	17	14.6
Children aged 6 months to 23 months	38	32.8	Child breastfeeding		
Children aged 24 months -59 months	49	42.2	Yes	58	50
Children age > 59 months	29	25	No	58	50
Age porridge introduced					
< 6 months	28	24.1			
6 months	53	45.7			
> 6 months	35	30.2			

Note: Data were based on a sample size of 116 caregivers. A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father.

6.3.2 Food safety knowledge of the caregivers

Figure 9 shows the overall distribution scores of food safety knowledge, attitude, and level of adherence to food hygiene practices of the caregivers. There were high proportion of caregivers with sufficient food safety knowledge (74.1%) and positive attitude (68.1%) although caregivers' adherence to food hygiene practices was low by factors of 4 and 3.8, respectively.

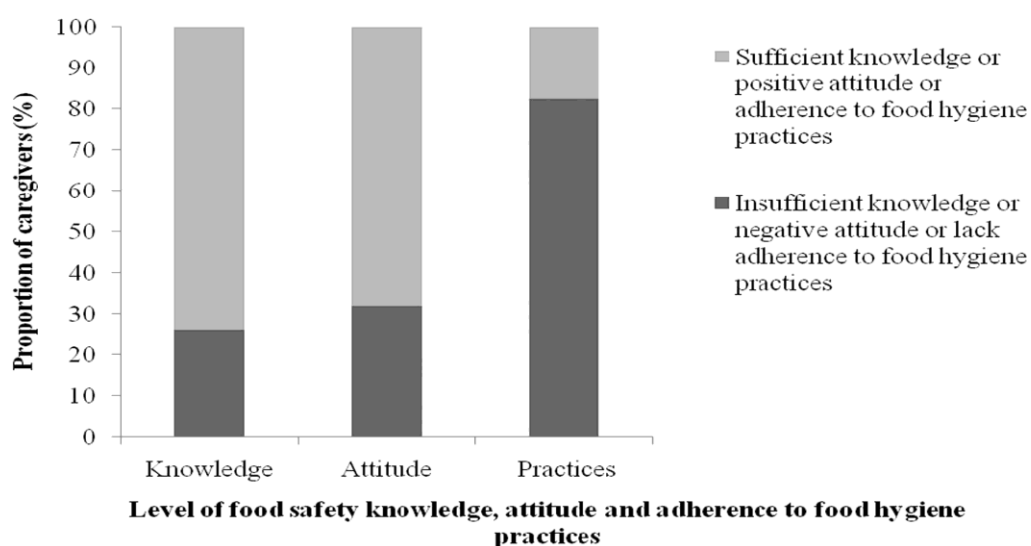


Figure 7: Distribution of food safety knowledge, attitude, and level of adherence to food hygiene practices among caregivers based on a sample size of 116 caregivers. A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father.

Table 14 indicates that the largest proportion (93.1%) of the caregivers was aware of the importance of ensuring food safety, and above average (60.3%) of them could correctly define safe food. This perceived benefit is vital in ensuring food safety at the household level. Majority (81.9%) of the caregivers perceived agrochemical residues and microorganisms as the greatest threat to food safety. All (100%) of the caregivers were unable to recognize mycotoxin, allergens, veterinary drug residues, heavy metals, unsafe packaging materials, food chemical issues (e.g., hormones and food additives), health issues (e.g., food nutrient imbalance), and food regulatory issues (e.g., food inspection and labels) as a threat to food safety. Therefore, food safety training should be focused on threats that are less known to rural communities. There are many ways by which one can acquire food safety knowledge. In this study, the largest proportion (38%) of the caregivers acquired food safety knowledge through formal food safety training in health facilities, especially during ante-natal

care. Nearly equal proportions (20%, 22%, and 20% respectively) of the caregivers acquired food safety from schools where caregivers attended formal education, from friends and relatives, and radio programs. These means of knowledge acquisition mode should be sustainably strengthened. Eight of ten (80%) respondents stated stomach upset and diarrhea as symptoms of food-borne illness. None of the caregivers mentioned paralysis, fatigue, and dizziness as symptoms of food-borne illness. In addition, although more than half (53.5%) of the caregivers were aware of the food safety authority, more than 17% did not know their roles in ensuring food safety.

Table 14: Distribution of food safety knowledge, attitude, and food hygiene practices among caregivers based on a sample size of 116 caregivers

Food safety knowledge questions	Responses	
	(n)	(%)
Never Participated in a food safety training		
Yes	51	44
No	65	56
Source of information regarding food safety		
Doctor/health work	19	38
Friends and relatives	11	22
Radio	10	20
School	10	20
Basic food hygiene practices		
Washing hands with soap	4	3.4
Wash baby's hands	1	0.9
cover food well	7	6
Clean utensils	4	3.4
Keep flour in a clean dry place	3	2.6
Ensure a clean working environment	1	2.6
Don't know	96	82.8
Importance of preparing a safe food		
Protect children from food-borne diseases,	108	93.1
Don't Know	8	6.9
Definition of safe food		
Nutritious food	33	28.4

Food free from contaminants	70	60.3
Well Covered food	4	3.4
Well cooked food	5	4.3
Hot food	3	2.6
No response	1	0.9
The perceived greatest threat to food safety		
Chemical and chemical residues	58	50
Microorganisms and their metabolites	37	31.9
Physical elements e.g., sand, metal pieces	17	14.7
Don't know	4	3.4
Symptoms of food-borne illnesses		
Vomiting	12	10.3
Stomach upset	50	43.1
Diarrhea	54	46.6
Suffer Food borne illness after taking porridge?		
Yes	72	62.1
No	44	37.9
Things that are done to make food unsafe		
Not washing hands with soap	4	4.3
Not covering food well	7	6
Un clean utensils	4	3.4
Keep flour in an unclean damp place	3	2.6
Unclean working environment	1	0.9
Nothing	96	82.8
Not aware of food safety authority		
Yes	54	46.5
No	62	53.5
Do not know the roles of food safety authority		
Yes	68	58.6
No	48	41.4

Note: Data were based on a sample size of 116 caregivers. A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father.

6.3.3 Caregivers food safety attitude

The food safety attitude of the caregivers is summarized in Table 15. The result indicates that nearly all (97.4%) caregivers have positive attitude concerning food safety. For instance, half (51%) caregivers had a positive attitude toward the safety of purchased raw materials used for making malted millet–sesame–soy composite. And, nearly three-quarters (70.7%) were willing to pay the extra price for safe raw materials for making malted millet–sesame–soy composite.

Table 15: Attitude toward food safety

Questions for food safety attitude	Households proportion	
	(n)	(%)
1. Food safety is not important		
a) Disagree	113	97.4
b) Neither agree nor disagree	1	0.9
c) Agree	2	1.7
2. Have confidence in the food safety authority		
a) Disagree	18	15.5
b) Neither agree nor disagree	27	23.3
c) Agree	71	61.2
3.1 know the roles of food safety authority		
a) Disagree	95	81.9
b) Neither agree nor disagree	9	7.8
c) Agree	12	10.3
4. Home grown raw materials are safe		
a) Disagree	7	6
b) Neither agree nor disagree	8	6.9
c) Agree	101	87.1
5. Not willing to pay the extra price for safe raw materials		
a) Disagree	82	70.7
b) Neither agree nor disagree	10	8.6
c) Agree	24	20.7

6. Purchased raw materials are safe

a) Disagree	45	38.8
b) Neither agree nor disagree	11	9.5
c) Agree	60	51.7

6.3.4 Food safety self-reported practices

Food safety practices of caregivers of children aged 6 to 23 months are shown in Table 16. Generally, most caregivers embraced general hygiene practices such as the use of clean water sources (borehole and protected well) (74.1%), use of pit latrines (98.3%), and washing hands with water and soap after visiting the pit latrine (84.5%). However, 7–9 in 10 households did not adhere to specific areas of food hygiene such as hand washing before preparing food and feeding the child.

For storage of the locally made complementary food composite, the majority (93.1%) of caregivers stored processed composite flour for 2 to 14 days, and only 1.7% of the caregivers stored for more than 30 days. Nearly half (47%) of the households cook the composite for an estimated 15 to 30 minutes. Only 13% of the caregivers could not estimate the time taken to cook the composite. Figure:8 indicate a detailed porridge production process. Three-quarters of the respondents stored the cooked porridge at ambient temperature (typically 25 to 30°C) and used it within 8 hours. In utilization of the stored porridge, the majority (76.7%) did not warm, and largely (61.2%) were stored in vacuum flasks. Those who do not use vacuum flasks reheated before boiling for 10 to 15 minutes and reheated without reaching boiling temperature for 2 to 3 min.

Table 16: Self-reported food safety practices of the caregivers

Questions	Responses	
	(n)	(%)
Source of clean water		
1. Borehole	58	50
2. Protected well	28	24.1
3. Well	30	25.9
Do you have a pit latrine?		
1. Yes	114	98.3
2. No	2	1.7
What is done after visiting the pit latrine?		
1. Hand washing	98	84.5
2. Nothing	18	15.5
What is done before preparing food?		
1. Hand washing	8	6.9
2. Nothing	108	93.1
What is done before feeding a child?		
1. Hand washing	33	28.4
2. Nothing (“Just get food and feed the child”)	83	71.6
Household members treated when suffering from food-borne illness		
Yes	32	72.7
No	12	27.3
If yes, treated from where?		
Medical unit	22	52.4
Home (self-medication both conventional and traditional)	20	47.6
How household produce is dried		
Spreading on the ground	18	15.5
Spreading on the mat/tarpaulin	91	78.4
Spreading on Polythene/ 'Kavera '	4	3.4
Spreading in the Tray	2	1.7
No response	1	0.9

How raw materials stored

Sack	47	40.5
Sack and raise the ground	12	10.3
Polythene bag (kaveera)	8	6.9
Polythene bag (kaveera) and raised ground	2	1.7
Closed container /Bucket	16	13.8
In a dry place	4	3.4
In the bags	1	0.9
N/A	1	0.9
No response	8	6.9
Don't know	17	14.7

Where raw materials stored

House/grass thatched	107	92.2
Store	3	2.6
Granary	6	5.2
Days the MMSSC are stored		
2-7 days	73	62.9
8-14 days	35	30.2
15- 30 days	5	4.3
>30 days	3	2.6

How long the porridge is cooked

10min -15 min	22	18.9
>15 min-30 min	54	46.6
>30min - 60 min	20	17.2
>60min-90 min	1	0.9
>90 min- 120 minin	4	3.4
Don't Know	15	12.9

Stores cooked porridge

Yes	86	74.1
No	30	25.9

Hours cooked porridge is stored

1 - 4 hours	36	42.4
5 - 8 hours	29	34.1

9 - 12 hours	20	23.5
How did the respondents store the cooked porridge?		
Covered cooking pan	7	6
Vacuum Flask	71	61.2
Covered in a jug	9	7.8
N/A	29	25
How the respondent warms the cooked porridge		
Boil	6	5.2
Warm	14	12.1
Do not warm it	89	76.7
N/A	7	6

Note: Data were based on a sample size of 116 caregivers. A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father.

6.3.5 Association of caregivers' competency and sociodemographic factors

Multivariate binary logistic regression analysis in Table 17 indicated that gender role in decision-making on the household income ($P = 0.034$), frequency of food safety training ($P = 0.041$), households with children who had ever suffered from food-borne illness ($P = 0.001$), and household with children aged 1 to 17 years ($P = 0.002$) were significantly associated with food safety knowledge of the caregivers. Households with the male gender taking the lead in decision-making on household income were associated with nearly two (1.831) fold increases in food safety knowledge. Similarly, caregivers who frequently attend food safety training were more than half (0.602) better in food safety knowledge than their counterparts who only attended once. Furthermore, households with children who have ever suffered from food-borne illness were nearly 1–fold (0.852) better in food safety knowledge than those who have never. Households with older children aged 1 to 17 years have almost more than half (0.58) fold increase in food safety knowledge compared to those who have only younger children aged under 5 years.

Regarding attitude, binary multivariate logistic regression analysis indicated that both genders taking the lead in decision-making on household income and households with children aged 1 to 17 years were significantly associated with a positive attitude toward food safety ($P = 0.006$ and $P = 0.041$), respectively. Households with the male gender taking the lead in decision-making on household income are associated with a virtually two-fold (1.925) decrease in a positive attitude

toward food safety. Households with children aged 1 to 17 years were associated with nearly a quarter-fold (0.254) increase in a positive attitude toward food safety. Generally, decision-making on household income and households with children aged 1 to 17 years significantly predicted FSK and FSA, which showed a significant positive correlation ($r = 0.406$, $P=.000$).

Table 17: Association of caregivers' characteristics with food safety knowledge and attitude

Sociodemographic variables	Knowledge			Attitude		
	β	SE	p-Value	β	SE	p-Value
Residence of caregiver	1.049	.610	.086			
Sex of the caregiver	.691	.893	.439	-.251	.642	.695
Age of the caregiver	-.036	.033	.274	-.037	.026	.153
Occupation of household head	-	.960	.094			
	1.609					
Occupation of the caregiver	1.001	.900	.266	-.767	.653	.240
Marital status	1.251	1.108	.259	.010	.749	.989
The main source of income	-.904	.901	.316	.889	.610	.145
Decision on household income	1.831	.865	.034*	1.925	.706	.006*
Decision on food to cook	-	1.622	.130			
	2.453					
Responsibility for the household provision	-.999	.755	.186	-	.724	.119
				1.128		
Trained in food safety	-.318	.990	.748	-.578	.797	.468
Frequency of food safety training	.602	.295	.041*	.356	.202	.079
Children in the household ever suffered from food-borne illness	.852	.266	.001*	-.111	.166	.504
Where the child was treated	-.168	.902	.852			
Children in the household aged 1-17 years	.583	.192	.002*	.254	.124	.041*
Children in the household aged ≤ 5 years	.342	.379	.368			
Children in the household aged ≤ 2 years	.029	.419	.946	-.148	.348	.672
Constant	1.173	2.759	.671	1.048	1.586	.509

Note: A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother/father. Abbreviations: β , regression coefficients; SE, standard error.

* Indicates the variables that significantly predicted food safety knowledge and food safety attitude.

6.3.6 Common themes and quotes generated from FGD based on Health Belief Model

The summary of common themes generated from FGD and caregivers' quotes is shown in Table 18. Common themes are reported below: -

6.3.6.1 Perceived susceptibility and severity

The verbatim from caregivers include: “children were more susceptible to food-borne illness than other household members”. “Household members suffered from symptoms of food-borne illness such as vomiting, stomach ache, and diarrhea resulting from consuming contaminated food” These conditions lasted from 1 day to more than 14 days among children with the above symptoms”. “The majority of the cases were treated and others got healed without treatment. Commonly, treatment was from medical facilities, but others did self-medication from home using both conventional and traditional medicine”.

6.3.6.2 Perceived barriers

Inadequate sanitation facilities such as pit latrines and drying equipment, especially tarpaulin/polyethylene bags, were reported as barriers to food safety. The community believed that more than half of the households share pit latrines, and some dry their agricultural produce on bare ground. Additionally, women's workload was considered a big challenge. Caregivers reported they get exhausted and take shortcuts to prepare food and feed the hungry crying children. Furthermore, caregivers reported adequate knowledge of preparing food for the children. For instance, the time needed to cook porridge was unknown. They prepare according to convenience. The caregivers stated, “When the bubbles in the porridge clear and a good aroma starts, I will know the porridge is ready. But when the child starts crying, nobody can wait for the above features”.

6.3.6.3 Self-efficacy

High self-efficacy in food safety practices was observed. Group members reported that most caregivers have safe water sources or share a pit latrine. They also believed that only a few households do not use a pit latrine. Community discussions indicated that virtually all the

caretakers wash their hands with soap and water after visiting the pit latrine and only a small portion do not.

Additionally, the community perceived majority of the caregivers were willing to pay an extra price for safer materials for making malted millet–sesame–soy composite. They believed that food safety is vital at home. However, some group members did not know basic food hygiene. Although the FGD members knew the roles of food safety authority, they had no confidence in food safety authority, especially in regulating the safety of cereals safety.

6.3.6.4 Cues to action

The FGDs revealed that important messages concerning food safety knowledge and practices can be passed from generation to generation from parents/caregivers to the children in addition to acquiring it formally right from schools and medical facilities where the caregivers attend antenatal care. Posters in hospitals and clinics showing a person(s) with diarrhea and vomiting as a result of consuming unsafe food or not following proper food hygiene practices need to be put appropriately in medical centers where community members usually go to seek medical attention. Poster pictures should be brightly colored and information translated into the local language. Additionally, scary media reports on television and radio on the health and economic impact of food contamination and experts' talk shows on the dangers of consuming contaminated foods were perceived by the FGDs members to trigger recommended food safety actions.

Table 18: Quotes from FGD based on Health belief constructs

Common themes	Quotes from focus group discussions
Perceived susceptibility The susceptible group in the household.	"Anybody in the community is susceptible to food-borne illness. But children especially those from six months (on the introduction of complementary foods) to 23 months."
Perceived severity Gastrointestinal disturbances.	"Adults mostly suffer stomach ache and diarrhea. But children suffer from vomiting, loss of appetite, and fever in addition to stomach ache and diarrhea."
How long the condition lasted?	"The time lag varies, from 1 day to 14 days but rare cases last for more than 14 days."

Medical treatment	"Many of those who get sick seek medical attention due to the severity of the illness. Children especially were taken to hospitals or medical clinics. Adults and children with the less severe case do self-medication from home or get healed without medication."
Perceived benefits	
Importance of preparing safe food.	"Prevent children from being infected with diseases"
Identify safe food practices that prevent food-borne illness	Hand-washing with clean water and soap after visiting the pit latrine, before preparing food, and before feeding the child. Know the time taken to cook porridge, Best way to re-warm porridge
Perceived barrier	
Hygiene practice (Inadequate pit latrine)	"More than half of the community share pit latrine. This makes cleaning and provision of water and soap for washing hands after use very challenging."
Challenge of drying agricultural produce	"Most of us dry on the mat, polyethylene bags ('Kavera') and tarpaulin because those who don't have them can borrow them. But those who don't have and can't borrow do dry their agricultural produce on bare ground."
Time and caregiver's workload	"We (women) are the ones responsible for household care mostly obtaining and preparing food, household hygiene in addition to children's care. This work is overwhelming and makes us take shortcuts, especially in food preparation and child feeding."
Self - efficacy	"We always get food safety knowledge when we go for antenatal visits in hospitals and health centers"
Confidence	"Locally made malted-millet-sesame-soy composite porridge is a safe product for our children aged 6 to 23 months". "Both home grown and purchased raw materials used for

	making malted millet sesame soy composite are safe"
	"Most of us are willing to pay the extra price for safe raw materials for making malted-millet-sesame-soy composite. I rather spend money on a good thing that will prevent diseases."
	"I have confidence in food safety authority, especially in meat inspection but not other produce".
Food handling control	"Only what you have produced is under your control but one has to decide to either purchase expensive good quality produce or cheap poor-quality produce". I go for cheap products because high-quality products are very expensive, some of us cannot afford them."
Food safety handling of left-over	"Most of us prepare baby's porridge once a day and it is consumed for the whole day. The leftover is either put in a well-covered flask, Jug, or saucepan. The one left in the Jug or saucepan will then be re-warmed, boiled, or given to the child cold depending on the time available
False confidence	Nutritious food is a safe food
Cues to action	"Posters in hospitals and clinics showing a person with diarrhea, vomiting resulting from consumption of unsafe food or not following proper food hygiene practices"
Audio-visual catching message	"Scary media report on television and radios highlighting socio-economic losses resulting from unsafe foods".
Shocking message	"Experts talk shows on radios and televisions educating the public on the dangers of consuming contaminated foods".

The information was generated from a total of 8 focus groups consisting of 6 to 10 caregivers. A caregiver is the mother/father of the child or legal guardian who takes care of the child in the absence of the mother.

6.4 Discussions

6.4.1 Socio-demographic characteristics of the caregivers

In terms of complementary feeding, the study indicated that less than half (Table 13) of the caregivers introduce complementary feeding at 6 months as per the World Health Organization (WHO, 2003) and the Ministry of Health (2009) recommendation. However, the remaining proportion of caregivers introduced complementary foods either before or after 6 months. Similar to the finding of this study, Aber *et al.* (2018) noted low adherence to complementary feeding guidelines in a rural setting in Uganda. As a result, there is a need to discourage practices that hinder adherence to recommended practices because, before 6 months of age, the child's digestive and immune systems are still underdeveloped to digest complimentary food and overcome any food-related illnesses (Rao *et al.*, 2011).

Additionally, the late introduction of complementary foods after 6 months is equally inappropriate because of the energy gap since breast milk alone from a well-nourished mother cannot support a child's optimal growth. This is because the metabolizing energy intake of breastmilk is 2.2–2.4MJ/day, and the mean energy requirement for babies at 6 months is approximately 2.6–2.7MJ/day (632–649Kcal/ day) (Reilly & Wells, 2005). Prescott *et al.* (2008) argued that there is a growing need to revise the age at which weaning foods are introduced to 4 months due to various needs while maintaining breastfeeding to a minimum of 6 months. There is a conflict between age complementary feeding being introduced from 4 to 6 months by some allergy prevention guidelines practiced in Australia and the WHO recommendation of complementary feeding at 6 months (Prescott and Tang, 2005). These conflicting ideas need to be resolved through extensive research to harmonize the age of introduction of complementary feeding for realizing good nutritional outcomes from complementary feeding.

The current study showed that nearly all (95.7%) of the consumed food was own-grown; only a small portion (4.3%) was purchased. The finding suggests that the foods consumed by this community are relatively safe since this study further revealed that the studied community perceived that their grown produce is relatively safe compared to what is purchased. This applies to poor households who are economically constrained and may not buy relatively safe quality agricultural products sold in the market. The implication is that, in case of crop failure, this community may suffer food insecurity coupled with the utilization of unsafe food.

In agreement with the findings, other studies revealed that rural areas in low- and middle-income countries extensively depend on their grown produce for household consumption (Adeyeye *et al.*, 2022; Ejeromedoghene *et al.*, 2020; Gebre & Rahut, 2021). This is a positive scenario because the current study revealed that poor households can only be sure of the food quality and safety when it is grown. Therefore, the government should strengthen the capacity of households to grow their food and diversify their livelihood activities to enhance resilience in case of crop failure.

Concerning gender roles and responsibilities, caregivers reported that in nearly half (47.4%) of the male-headed households, decision-making on income usage and responsibility for household needs was met by the male gender. This could have been because the female gender earns low income due to their unpaid care and domestic work (Gulobaet *et al.*, 2018). Largely women (94%) only decided on food acquisition and preparation. This was typical of rural African households where women chiefly decide on food since they are gatekeepers of food and nutrition security. And, mainly men decide on household income. Gender roles and responsibilities have changed over time. In an African setting in northern Uganda, the husband/family head was responsible for all the household decision-making and standard of living. Similarly, in Tanzania, Anderson *et al.* (2017) observed that men held more power in terms of household decision-making and provision except in specialized areas. This may have poor implications on household food safety and food and nutrition security in case of crop failure since the gender that makes the critical decision on household food consumption may not be fully involved in household income usage. Thus, it is necessary to make collective decisions on household income to ensure food safety and food and nutrition security.

6.4.2 Food safety knowledge of the caregivers

In terms of knowledge, all caregivers knew the importance of preparing safe food to prevent food-borne illnesses. However, the majority (82.4%) did adhere to food hygiene practices such as handwashing with detergent and clean water before food preparation, feeding the child, ensuring a clean working environment, cleaning utensils, covering food well to avoid cross-contamination, and keeping food in a dry place. The explanation could be the lower educational stages attained by the caregivers (Table 13). A similar scenario reported by Webb and Morancie (2015) indicate that most (81%) knew the importance of hygiene practices such as proper hand washing, but only 2% practiced. This reflected weakness could be due to time constraints and

inadequate resources needed to perform the task (Brannon *et al.*, 2009). However, washing hands with antibacterial soap eliminates >95% of total coliforms even for a shorter exposure time (Toshima *et al.*, 2001). The barriers to good food hygiene practices thus need to be addressed by enhancing social-demographic factors such as frequent food safety training to improve knowledge. However, Ncube *et al.* (2020) acknowledged that hygiene practices such as hand washing need validation by observation because of significant deviation in the observation checklist from the reported practice Da Cunha *et al.* (2019) affirmed that influenced by knowledge and attitude and is closer to actual practice.

Kunaduet *al.* (2016) observed that most of the participants in their study reported washing their hands with antibacterial soap after visiting a toilet and before food preparation to prevent food-borne illnesses. This deviation could be due to the formal environment (restaurant) where they researched not being similar to the typically rural households considered in this study. Also, this study showed that although over half of the caregivers were aware of the authority responsible for safe foods, the same proportion of the caregivers did not know their roles the authority in ensuring food safety.

Policymakers should reinforce functional food safety departments at all levels.

6.4.3 Caregivers' food safety attitude

Most (97.4%) caregivers had a positive attitude toward food safety as an important concern at home with more than half (61%) of them having confidence in the food safety authority. Nonetheless, the caregivers had a negative attitude toward the safety of both the homegrown and purchased raw materials used for making malted millet–sesame–soy composite (87% and 51.7%, respectively). Although the FGDs pointed out that “as long as you have money, you can always buy a good quality safe food, some times even better than own grown.” This statement was attested by Safariet *al.*, (2017) who reported that cultural, social, and economic status influences food safety attitudes.

The current study found that nearly three-quarters (70.7%) of the caregivers were willing to pay the extra money for safe raw materials for making malted millet–sesame–soy composite. Less than a quarter of the caregivers were unwilling because of financial constraints. More than a third of the caregivers had a negative attitude toward the safety of purchased raw materials for making

malted millet–sesame–soy composite because quality raw materials are usually highly priced compared to poor-quality ones. The willingness to pay the extra price depends on the economic status. Therefore, households need to be economically empowered to utilize safe foods. The finding from this study replicated Meysenburg et al. 2014) which shows that “the only thing you can control is what you have in your house.” The positive attitude of the caregiver should be translated into good food hygiene practices. This should be achieved by addressing barriers such as economic constraints caregiver's workload, and social-economic factors like the decision on household income that influence attitude. The decision of the male gender on household income positively influences attitude because they own more resources and earn more money. The female gender thus needs to be empowered since they are door keepers of food safety as revealed by this study.

6.4.4 Food Safety self-reported practices

The majority of caregivers (83.6%) did not dry their agricultural produce on the bare ground since they had personal or shared/borrowed tarpaulin for drying produce. Tarpaulin ownership was limited in rural setting because they are expensive. After drying agricultural products, most (89.7%) households did not store their produce on raised ground. Poor practices such as not storing produce on raised ground encourage mold growth, rodents, and insect infestations that compromise food quality and safety. Only 10.3% of households stored the produce on raised ground. Thus, there is a need to sensitize the community about the danger of improper postharvest management and training on best practices. Almost all (92.2%) of the agricultural produce was stored by stockpiling on polypropylene sacks and baskets in grass-thatched houses that were used for sleeping by the household members due to fear of thieves as reported by the community. This practice should be discouraged because it predisposes the produce to cross-contamination, so the local authority in rural communities should ensure social security. Our finding is comparable to that of Viola (2017), who found that three-quarters of the products are stored in rooms at home after harvesting for fear of thieves.

The caregivers further revealed that only 2.6% of the households stored their agricultural produce in the local community store. Community storage of the product should be encouraged because the facility offers better services that reduce contamination, thus ensuring food quality and safety. Further, more than half (62.9%) of the caregivers reported storing processed composite flour for feeding weaning children for 2 to 7 days. The caregivers reported that they

were trained to keep the composite for a short duration to prevent the proliferation of spoilage agents. The rest of the caregivers (34.5%) stored for 8 to 30 days, and only very few (2.6%) of caregivers stored for more than 30 days. These varying maximum storage days, however, are challenging in case they need to access the market. Therefore, irrespective of the source of raw materials, shelf-life should be determined.

Concerning time and temperature control, nearly half (46.6%) of the caregivers took an estimated 15 to 30 min to cook MMSSC, and only 12.6% did not know the time taken to cook the composite. In this rural setting, arbitrary cooking is practiced. The cooked porridge is stored for a range of 1 to 24 hours. The stored porridge is usually rewarmed by boiling (5.2%) and warming (12.1%) for a very few (2–3) minutes before taking it. Warming by boiling should be encouraged because, in case of any germination of spores, the vegetative form can be destroyed by boiling. Although in case of toxin production resulting from contamination at pre-harvest and postharvest, mostly during improper storage of raw materials and flour, simple boiling may not eliminate hazards. Therefore, proper pre- and postharvest management should be encouraged. Additionally, porridge should be properly stored to eliminate hazards. Warming for very few (2–3) minutes, however, should be discouraged as it does not destroy the vegetative form of the microorganism but provides optimum conditions for their proliferation.

Additionally, a greater percentage of caregivers (76.7%) do not warm the leftover porridge because they store it in a vacuum flask to keep it hot until use. Several studies have reported an enormous challenge of temperature and time control to many food handlers (Webb & Morancie, 2015; Kamboj *et al.*, 2020; Kwol *et al.*, 2020; Ncube *et al.*, 2020).

6.4.5 Association of caregivers' characteristics with food safety knowledge and attitude

Results from binary multivariate logistic regression models substantiated that; several socio-demographic characteristics could not significantly be associated with food safety knowledge and attitude. However, gender roles in decision-making on household income significantly predicted knowledge and attitude ($P = 0.006$). The model further revealed that households with the male gender taking the lead in decision-making on household income were associated with a two-fold increase in the level of food safety knowledge, which could have been because men earn more income than women enabling men to support food safety programs thus the household's food safety knowledge (Guloba *et al.*, 2018). The study revealed that women make a

decision on food acquisition and preparation, thus they need to be empowered and participate equally in household income decision-making. Conversely, households with the male gender taking the lead in decision-making on household income are associated with a virtually twofold decrease in a positive attitude toward food safety. The decreased positive attitude could have been because most caregivers play a lesser role in household income so they cannot decide on appropriate food safety measures that require finances.

Furthermore, the frequency of food safety training and households with children who have ever suffered from food-borne illness significantly predicted sufficient food safety knowledge. Caregivers who frequently attended food safety training had more than half an increase in food safety knowledge than their counterparts who only attended once ($P = 0.041$). Also, households with children who had ever suffered from food-borne illness had a nearly one-fold increase in food safety knowledge than those who had never ($P = 0.001$). Equally, households with children aged 1 to 17 years had significantly sufficient food safety knowledge and positive attitudes with a more than half-fold (0.58) increase in food safety knowledge compared to those with children under 5 years. Also, a quarter-fold (0.254) increase in a positive attitude toward food safety was observed in a household with children aged 1 to 17 years. The above predictors could plausibly be explained by the experience of food safety that a caregiver gained during training and over the years of childbearing which builds their self-efficacy and positive attitude (Meysenburg *et al.*, 2014). Nee and Sani (2011) and Abdul-Mutalib *et al.* (2012), however, found deviant results, indicating an insignificant correlation between experience in food-handling operation and increased food safety knowledge and attitude.

In this study, however, a significant positive correlation between food safety knowledge and attitude was observed ($r = 0.406$, $P = 0.000$). Several studies have revealed that good predictors of safe food-handling practices are sufficient food safety knowledge and a positive attitude (Kunadu *et al.*, 2016; Al-Kandari *et al.*, 2019). However, Nassanga *et al.* (2018) and Ncube *et al.* (2020) argued that knowledge may not necessarily be translated into appropriate practices, and self-reported practices may not be the actual practice. Therefore, self-reported practices need to be verified with an observation checklist.

6.4.6 Themes generated from FDG based on the health belief model

6.4.6.1 Perceived susceptibility and severity

FGDs based on health belief model constructs shown in Table 18, The caregivers, verbatim is quoted as “children are more susceptible to foodborne illness than other age groups in the household, they frequently fall ill”. According to literature, this is due to their underdeveloped immune systems (Agostoniet *al.*, 2009; Du Plessis *et al.*, 2013). FGDs verbatim further quoted “Adults generally suffer from stomach aches and diarrhea, and children suffer from vomiting, loss of appetite, and fever in addition to stomach aches and diarrhea”. These findings are strongly supported by a study by Meysenburg *et al.* (2014), indicating that children and the elderly were more susceptible to food-borne illness due to their weaker immune systems. The caregivers believed that their household members have ever suffered from symptoms such as vomiting, stomach ache, and diarrhea resulting from consuming contaminated food. These conditions were reported to last from 1day to more than 14 days in some cases. This implied that the caregivers knew some of the consequences of eating contaminated foods. However, the study findings presented in Table 18 indicate that the caregivers had a low perception of susceptibility and severity to food-borne illnesses as quoted “Adults and children with the less severe case do self-medication from home or get healed without medication.”. This could have resulted from inadequate and late diagnosis of food-borne diseases and lack of experience with food-borne illnesses. Another reason includes ignorance of the serious health consequences of food contamination such as stunting in children and a more life-threatening condition such as liver cancer and hemolytic uremic syndrome (Buzby, 2001; Meysenburg *et al.*, 2014).

Additionally, this could be due to respondents' failure to recognize that they are susceptible to foodborne illness and the high degree of severity of infection. Caregivers' low perceptions of their susceptibility to FBI and its severity could have resulted from not acknowledging the reality due to fear of undesired outcomes. Thus, this opportunistic bias may lead to invalid conclusions. This can be overcome by rigorous methods of data collection that may be costly (DeCosteret *al.*, 2015). Among the children with the above symptoms, the majority (72.7%) was treated, and 27.3% healed without treatment. Treatment was obtained from the medical unit (52.4%) and others (47.6%) used self-medication.

In this study, caregivers did not understand the risk of food contamination caused by unhealthy food handlers. Similar findings by Tuglo *et al.* (2021) showed that food handlers lacked adequate knowledge of the dangers caused by unhealthy primary food preparers. However, factors such as food contamination due to poor agricultural practices and cross-food contamination from the food handlers suffering from diseases such as diarrhea, cough, and flu increase the risk of foodborne illnesses (Al-Kandari *et al.*, 2019; Ncube *et al.*, 2020). Training emphasis concerning safe foods ought to focus on threats that are unknown to caregivers.

6.4.6.2 Perceived benefits

Caregivers acknowledged that the goal of safety practices is to prevent children and other household members from getting food-borne illnesses as quoted, "Prevent children from being infected with diseases" The food safety practices pointed out by the members included:- hand washing after visiting the latrine, before preparing food, and before feeding the child; drying agricultural produce on tarpaulin; cleaning utensils before preparing food, covering leftover foods, and re-heating leftover foods. Although these food hygiene practices were well known, they were not practiced. These include hand washing with soap and clean water, washing hands before preparing food and feeding the child, not drying agricultural produce on the bare ground, storing the produce on raised ground, and reheating leftover food. These conflicting food safety practices are expected because the health belief model points out that the lower the perceived susceptibility and severity, the lesser the perceived benefits of taking action (food safety practices) and vice versa (Pourtaheri *et al.*, 2018).

6.4.6.3 Perceived barriers

As indicated in table 18, verbatim concerning verbatins include: "More than half of the community share pit latrine, this makes cleaning and provision of water and soap for washing hands after use very challenging", "Most of us dry on the mat, polyethylene bags ('Kavera') and tarpaulin because those who don't have them can borrow them. But those who don't have and can't borrow do dry their agricultural produce on bare ground", "We (women) are the ones responsible for household care mostly obtaining and preparing food, household hygiene in addition to children's care. This work is overwhelming and makes us take shortcuts, especially in food preparation and child feeding".

Caregivers believed barriers to practicing food safety include inadequate sanitation facilities such as pit latrines and drying equipment, especially Turpin/polyethylene bags. Concerning the identified barrier, more than half of the households share a pit latrine, and some caregivers dry their agricultural produce on the bare ground. Furthermore, the workload of women was considered a big challenge. Caregivers reported that the women get exhausted and take shortcuts to prepare food and feed the hungry and crying children. This finding is comparable to other studies where employers in a formal setting (restaurants) fail to adhere to set food safety practices due to time constraints and limited resources (Meysenburg *et al.*, 2014; Webb & Morancie, 2015).

Another barrier stated by the caregivers was inadequate knowledge of preparing food for the children. For example, the time needed to cook a safe porridge, whereby they prepare according to convenience, “When the bubbles in the porridge clear and the good aroma starts, I will know the porridge is ready. But sometimes when the child starts crying, nobody can wait for the above features.” Most participants in various studies did not know the critical temperatures and time requirement in the control of microorganisms (Webb & Morancie, 2015; Kwol *et al.*, 2020; Kamboj *et al.*, 2020; Webb & Morancie, 2015). In agreement to the study, Kwol *et al.* (2020) reported that lack of adherence to food safety guidelines could be due to time constraints and limited resources. Anon (2003) emphasizes that improper time and temperature control is one of the causes of food-borne diseases. There is a need for intervention to address the highlighted barriers to food hygiene practices and promote food safety within rural communities.

6.4.6.4 Self-efficacy

High self-efficacy refers to the ability of a person to competently execute the action to avert health threats such as food-borne illness (Rosenstock *et al.*, 1988). Verbatims quoted include: “We always get food safety knowledge when we go for antenatal visits in hospitals and health centers”, “Locally made malted-millet-sesame-soy composite porridge is a safe product for our children aged 6 to 23 months”, “Both home grown and purchased raw materials used for making malted millet sesame soy composite are safe”, “Most of us are willing to pay the extra price for safe raw materials for making malted-millet-sesame-soy composite. I rather spend money on a good thing that will prevent diseases.”, “I have confidence in food safety authority, especially in meat inspection but not other produce”, “Only what you have produced is under your control but one has to decide to either purchase expensive good quality produce or cheap poor-quality

produce", I go for cheap products because high-quality products are very expensive, some of us cannot afford them", "Most of us prepare baby's porridge once a day and it is consumed for the whole day" and the leftover is either put in a well-covered flask, Jug, or saucepan. The one left in the Jug or saucepan will then be re-warmed, boiled, or given to the child cold depending on the time available.

Comparable to the quantitative data, current study revealed caregivers' high self-efficacy in food safety practices. For instance, most caregivers had a safe water source (74.1%) and have or share a pit latrine (98.3%). Virtually all (84.5%) of the caretakers voiced washing their hands with detergent after visiting the pit latrine. Additionally, almost three-quarters (70.7%) were willing to pay an extra price for safe raw materials for locally making malted millet–sesame–soy composite. This finding indicates that as long as a household is economically stable, it acquires safe food. But those who cannot afford costly safe food will always go for cheap unsafe food. This phenomenon was explained by Safari *et al.* (2017) as food safety knowledge, attitudes, and practices can be influenced culturally, socially, and economically, such as food prices. This deviates from the finding by Meysenburget *al.* (2014), who indicated that "the only thing you can control is what you have in your house."

6.4.6.5 Cues to action

Some cues to action identified in this study included:-"putting posters in hospitals and clinics showing a person with diarrhea, vomiting resulting from consumption of unsafe food or not following proper food hygiene practices", "scary media report on television and radios highlighting socio-economic losses resulting from unsafe foods", "experts talk shows on radios and televisions educating the public on the dangers of consuming contaminated foods" and "passing important messages concerning food safety knowledge and practices from generation to generation (parents/caregivers to the children) and acquiring formal rights from schools, and medical units where we attend antenatal care". All these cues to action were the verbatims from FGD that was may enhance knowledge. However, knowledge may be insufficient for behavior change; therefore, food safety programs should not only be designed to increase awareness about food safety but also to emphasize the benefits of adopting proper food-handling practices. For these reasons, in our study, caregivers pointed out the following as perceived triggers for the recommended food safety actions: posters in hospitals and clinics showing a person with diarrhea and vomiting resulting from consumption of contaminated food or not following proper

food hygiene practices needs. The posters should be put appropriately in medical units where expectant mothers and community members usually go to seek medical attention and the pictures should be brightly colored and the information translated into the local languages. Additionally, a social marketing communication strategy with frightening media information about the dangers of consuming unsafe food should be aired. This can be passed on to radios and television engaging experts to teach about the risks associated with producing and consuming contaminated foods.

The current study showed that more than an average (60.3%) of the caregivers had sufficient knowledge of safe food. This can be explained by the fact that apart from formal training, caregivers acquire knowledge informally. Caregivers reported acquiring knowledge from family and friends, radio programs, schools they attended, and health workers during antenatal care. However, nearly a third (28.4%) had false confidence that safe food is the same as nutritious food. Similar spurious confidence was reported by Meysenburg *et al.* (2014), whereby study respondents reported that safe food is determined by appearance or smell. However many (60.3%) participants in this study affirmed that even if food is nutritious and looks and smells good, it may contain small organisms that cannot be seen macroscopically.

The study observed an equal proportion (50%) of the caregivers perceived chemical and agrochemical residues as the greatest threat to food safety, followed by microorganisms (31.9%), and finally, extraneous physical elements (14.7%) such as sand and metal pieces. This finding deviates from those of Kunadu *et al.* (2016) who reported that nearly all participants in their study reported microorganisms as the greatest threat to food safety. However, in both studies, participants did not recognize allergens, veterinary drug residues, heavy metals, unsafe packaging materials, food chemical issues (e.g., hormones and food additives), health issues (e.g., food nutrient imbalance), food regulatory issues (e.g., food inspection and labels), and microbial metabolites such as mycotoxin as the greatest threat to food safety. The authors in the current study argue that the divergent views could have resulted from participants' characteristics, the prior being from an informal rural household setting and the latter from a formal food industrial setting. There are many ways by which one can acquire food safety knowledge. In this study, more than half (56%) of the caregivers reported that they acquired knowledge from food safety training through non-governmental organizations (NGOs). The rest of the caregivers who had not attended training reported having acquired food safety knowledge from health workers, especially during antenatal care, friends, relatives, radio program, and

schools where they attended classes. The findings from the study agrees with those of Meysenburg *et al.* (2014) whose findings revealed that gaining experience in food-related jobs, and food safety knowledge can be acquired formally from school and informally from family. Thus, informal and formal knowledge acquisition should be strengthened to ensure community health and well-being.

6.5 Study strengths and limitations

The study tool was pretested and validated. Additionally, data were collected by trained research assistants. Furthermore, the study involved caregivers of children (3–23 months of age) in a rural setting. This contributed to the body of knowledge that was lacking in the area of food safety KAP in an informal rural domestic environment.

The present study, however, had the following methodological constraints: The study not examine the KAP of the untrained group; only the caregivers who were trained on the use of locally made food formulae were recruited into the study; and since training has been associated with good knowledge and attitude, our findings may not be generalized to the entire community. Therefore, further research should be carried out to compare the KAP of trained and untrained caregivers. The study design was cross-sectional; thus, it could not demonstrate a cause-effect relationship. Also, the purposive sampling method used is susceptible to sampling bias. Finally, the self-reported practice technique of reporting food safety practices may result in inconsistency with the actual practice. Future research should use an observation checklist to validate caregivers' food safety practices.

6.6 Conclusion

This study established that caregivers in the Acholi sub-region of Uganda trained in the local production of malted millet–sesame–soy composite have sufficient food safety knowledge and a positive attitude. However, most caregivers did not adhere to food hygiene practices. The identified socio-demographic factors that hinder adherence to food safety practices included inadequate sanitation facilities such as pit latrines and drying equipment, especially Turpin/polyethylene bags. Furthermore, women's workload was considered a huge hindrance. Another barrier stated by the caregivers was inadequate knowledge of preparing children's food.

6.7 Recommendation

To address food safety issues at home, where food is grown for consumption and sale, food safety knowledge and attitude should be strengthened by delivering food safety education based on the health belief model. This will increase the perceived susceptibility and severity to food-borne illness while increasing perceived benefits, thus reducing barriers to safe food-handling practices.

The study highlighted the urgent need to strengthen medical centers to include food safety training during nutrition education for mothers/caregivers attending antenatal care. The target participants in this setting are the doorkeepers of food safety based on findings from this study. They will thus acquire food safety knowledge on proper household sanitation and hygiene, elimination of food contamination from ‘garden to fork’ through good agronomic practices, good pre- and postharvest management, and recommended food preparation while considering time and temperature control.

The government of Uganda through the Uganda Nutrition Action Plan and Ministry of Agriculture Animal Industry and Fisheries (MAAIF) should enforce by-laws on postharvest handling including abolishing drying produce on the bare ground. Domestic and field workloads of women should be addressed by providing appropriate agricultural technologies that save time.

Finally, laboratory analyses should be done on selected food commodities to ascertain the safety of agricultural produce and determine whether food safety management systems are being practiced at household levels. This should be done alongside shelf-life determination of the locally made complementary food to empower the rural to access local and international markets.

CHAPTER SEVEN: The influence of caregivers' competency on aflatoxin contamination and exposure of the children through consumption of locally made complementary food formulae

7.1 Introduction

Proper complementary feeding is vital for ensuring good nutrition outcomes in children (Auria *et al.*, 2020). This is critical because poor quality and unsafe complementary foods may lead to malnutrition and its complications during this vital stage of life (Pietravalle *et al.*, 2023; Asmawati & Patrina, 2024). Due to this crucial need, WHO and MOH of different countries recommend introducing quality and safe complementary foods at 6 months (WHO, 2023). For this reason, several certified companies produce relatively safe and nutritious complementary foods. However, in resource-poor communities, these industrially manufactured complementary foods are expensive thus, not readily available because of their high cost (Theurich *et al.*, 2019; Theurich *et al.*, 2020; Baker *et al.*, 2021; Nejad *et al.*, 2023). Therefore, they produce home-made complementary foods from locally available raw materials, and the ingredients may be susceptible to aflatoxin contamination (Echodu *et al.*, 2019b; Lukwago *et al.*, 2019; Wokorach, *et al.*, 2021; Mshida *et al.*, 2020; Teshome *et al.*, 2022). In this respect, WHO reported that only 25% of the children are fed based on the recommended guidelines, and the remaining higher percentage of non-adherence is observed in rural communities in low and middle-income countries (White *et al.*, 2017).

Furthermore, it is evident that development in complementary feeding has mainly focused on nutritional quality, and for many years, food safety has not been prioritized in low and middle-income countries where complementary foods are produced from locally available materials (Du Plessis *et al.*, 2013; Alowo *et al.*, 2018; Lukwago *et al.*, 2019; Nordhagen *et al.*, 2022; Harrison *et al.*, 2023; Grace, 2023). Moreover, several studies have shown that in those countries, aflatoxin contamination levels in foods are above recommended threshold levels (Seetha *et al.*, 2017; Echodu *et al.*, 2019; Grosshagauer *et al.*, 2020; Wokorach *et al.*, 2021; Achiro *et al.*, 2023). Yet, exposure of children to aflatoxin is of great concern due to its impact ranging from mild (diarrhea) to chronic (cancer) illnesses (Kimanya *et al.*, 2021). Additionally, exposure to aflatoxin in children may be associated with growth retardation and disability-adjusted life years (DALYs) (Havelaar *et al.*, 2015; Fung *et al.*, 2018; Kimanya *et al.*, 2021; Nejad *et al.*, 2023). The impact of aflatoxin contamination of complementary food formulae and exposure in children

has been observed in low- and middle-income countries (Owaga *et al.*, 2011; Asiki *et al.*, 2014; Aron *et al.*, 2017; Achiro *et al.*, 2023). In those countries, exposure to aflatoxin is attributable to poor sanitary practices typical of poor pre and post-harvest management practices and insufficient food safety education (Baluka *et al.*, 2015; Chidziwisano *et al.*, 2019; Teshome *et al.*, 2022).

The challenge of children's exposure to aflatoxin requires a holistic approach involving all the stakeholders, government and non-government agencies, policymakers, funding bodies, and all the localized communities such as food handlers, consumers, and researchers. In this regard, emphasis needs to be put on responsive food safety education to reduce exposure to the risk of foodborne illness at home in unindustrialized communities.

Several factors play a role in regulating the rate of foodborne diseases, including competency in food safety knowledge, attitudes, and practices of food handlers (Ali *et al.*, 2023; Ahmed *et al.*, 2024). Authors emphasized that food safety knowledge, attitude, and food hygiene practices during food handling and preparation play a role in the outbreak of foodborne illnesses including illnesses due to aflatoxin exposure (Cissé, 2019; Abdulrahman *et al.*, 2020; Namubiru *et al.*, 2022; Abdeta & Milki, 2023). Nevertheless, the risk of aflatoxin contamination and related exposure is manageable. As the case in sub-Saharan Africa, technologies that employ good agricultural practices right from primary production at pre- and post-harvest levels where contamination can occur are implemented (Udomkun *et al.*, 2018; Gonçalves *et al.*, 2019; Awuchi *et al.*, 2020; Jallow *et al.*, 2021; Abdeta & Milki, 2023). At pre-harvest, such technologies may include the following: - good agronomic practices, for example, planting crops in time, appropriate application of fertilizers and manure, supplementing rain-fed agriculture with irrigation, rotational cropping, and application of non-toxigenic species of *Aspergillus* such as toxigenic *A. flavus* and *A. Niger* (Njoroge, 2018; Bandyopadhyay *et al.*, 2016). Likewise, at the post-harvest level, measures to manage aflatoxin control may include ensuring proper moisture content of cereals through adequate drying, and segregation of damaged and good grains before appropriately storing them in a properly aerated storage facility (Waliyar *et al.*, 2015; Mannaa & Kim, 2017).

Addressing the challenge of aflatoxin contamination and its impact on health in rural communities requires implementing control technologies both at pre- and post-harvest levels.

Additionally, several studies have linked positive attitude, sufficient knowledge, and appropriate practices to safe food (Cissé, 2019; Abdulrahman *et al.*, 2020; Namubiru *et al.*, 2022; Abdeta & Milki, 2023). These studies have been carried out in formal settings, not informal rural settings where production is done without recognized food safety guidelines. Moreover, these studies have not explicitly examined the influence of the competency of the caregivers in terms of KAP on aflatoxin contamination levels and related exposure levels, especially of weaning children in rural settings. Although competency factors are essential in assuring the safety of foods, limited studies done in this area do not indicate the influence of the competency of the caregivers of children weaning on aflatoxin contamination along the processing points and related exposure of the children. Thus, to bridge the information gap, examining the influence of food safety KAP on aflatoxin contamination and related exposure to aflatoxin in such settings remained a critical need. This study, therefore, assessed the effect of the competency of the caregivers in terms of the status of KAP on the level of total aflatoxin (TAF) and aflatoxin B₁ (AFB₁) contamination of home-made malted millet-sesame-soy composite (MMSSC) along the processing point across the wet and dry seasons of agricultural year and resultant exposure as a mitigation measure to reduce exposure of weaning children to aflatoxins.

7.2 Materials and Methods

7.2.1 Aflatoxin extraction and analysis

The levels of aflatoxin concentrations were determined by extracting and analyzing the samples along the processing points across the wet and dry seasons. Toxin extraction and analysis were done following the manufacturer's instructions (TAF Assay- low matrix and AFB₁ Assay - low matrix HELICA Biosystems, Inc. 2019). The detailed description of the procedures is indicated in chapter five, subsections 5.2.4 and 5.2.5 of this dissertation.

7.2.2 Determination of exposure

Based on Estimated Daily Intake, EDI (ng kg⁻¹bwday⁻¹), assessment of exposure of individual children to TAF and AFB₁ was done by multiplying TAF or AFB₁ concentration by consumption/day/body weight. The assessment procedure is detailed in chapter five, sub-section 5.2.5 of this dissertation.

7.2.3 Status of food safety knowledge, attitude and practices

In order to ascertain caregivers' competency in terms of food safety knowledge, attitudes, and practices, a cross-sectional design involving a survey using in-depth household interviews and focus group discussions (FGDs) was carried out. Details of the sample size, sampling procedure, and data collection are described in chapter four, sub-section 6.2 of this dissertation.

7. 2.4 Data analysis

Statistical Package for Social Sciences (SPSS) version 21 was used to perform statistical analyses whereby, levels of KAP were dichotomized, level of aflatoxin concentrations, exposure of the children to aflatoxin, and status of KAP were compared. This was achieved by using the t-test. The use of the student's t-test allowed for pairwise comparison of the different levels of aflatoxin as well as exposure by the KAP categories. Specifically, the t-test was used to test whether the mean level of aflatoxin and mean exposure at a given point/product is significantly different for households with sufficient knowledge and those with insufficient knowledge, those with a positive attitude, and those with a negative attitude and those that practice food hygiene and those that did not.

7.3. Results

7.3.1 The influence of the status of knowledge on total aflatoxin contamination total aflatoxin and aflatoxin B₁ concentrations along the processing points across the wet and dry seasons

As indicated in Table 19, in the wet season, the highest total aflatoxin contamination was observed in households with insufficient food safety knowledge. In contrast, in households with sufficient knowledge, the highest total aflatoxin contamination was observed in sesame. However, households with insufficient food safety knowledge significantly influenced total aflatoxin contamination in the processed stored MMSSC ($P = 0.027$). Whereas along the processing points, total aflatoxin concentration was highest in one of the raw materials, soybean ($1.52 \pm 1.51 \mu\text{gkg}^{-1}$), and processed stored products ($0.99 \pm 0.31 \mu\text{gkg}^{-1}$). During the dry season, the highest total aflatoxin contamination was observed in households with insufficient food safety knowledge. Along the processing points, total aflatoxin contamination in sesame ($1.48 \pm$

1.60 μgkg^{-1}) nearly doubled all others and it was the only variable that was significantly linked to knowledge ($P = 0.03$).

Table 19: The influence of the status of knowledge on mean total aflatoxin concentration along the processing points across the wet and dry seasons

Processing points	Level of total Aflatoxin ($\mu\text{g kg}^{-1}$)			MD	SED	t-stat	P-value
		Insufficient Knowledge	Sufficient Knowledge				
	N (%)	Mean $\pm$ SD	Mean $\pm$ SD				
Wet season							
Millet	46 (22.1)	0.84 $\pm$ 0.24	0.81 $\pm$ 0.23	0.02	0.09	0.252	0.80
Sesame	41 (19.7)	0.92 $\pm$ 0.25	1.18 $\pm$ 1.16	-0.26	0.37	-0.71	0.48
Soybeans	45 (21.6)	1.52 $\pm$ 1.51	1.04 $\pm$ 0.72	0.48	0.34	1.44	0.16
FP-MMSSC	32 (15.4)	0.84 $\pm$ 0.40	0.78 $\pm$ 0.37	0.06	0.14	0.42	0.67
PS-MMSSC	44 (21.2)	0.99 $\pm$ 0.31	0.82 $\pm$ 0.17	0.17	0.07	2.30	0.03*
Dry season							
Millet	46 (29.7)	0.77 $\pm$ 0.17	0.63 $\pm$ 0.21	0.14	0.07	1.86	0.07
Sesame	35 (22.6)	1.48 $\pm$ 1.60	0.78 $\pm$ 0.22	0.70	0.31	2.26	0.03*
Soybeans	1 (0.6)	0.62 $\pm$ 0.05	0.29 $\pm$ 0.41	0.33	0.29	1.12	0.38
FP-MMSSC	30 (19.4)	0.97 $\pm$ 1.01	0.59 $\pm$ 0.16	0.38	0.20	1.87	0.07
PS-MMSSC	43 (27.7)	0.82 $\pm$ 0.19	0.84 $\pm$ 0.06	-0.03	0.04	-0.73	0.47

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.2 The influence of the status of knowledge on aflatoxin B₁ contamination along the processing points across the wet and dry seasons

Table 20 shows that in both the wet and dry seasons, households with insufficient food safety knowledge had higher aflatoxin B₁ contamination than those with sufficient food safety knowledge. Conversely, in households with sufficient knowledge, the highest total aflatoxin

contamination was observed in sesame in the wet season, sesame had higher aflatoxin B₁ contamination and in the dry season, freshly processed MMSSC and stored processed MMSSC had higher aflatoxin B₁ contamination in households with sufficient food safety knowledge. Nonetheless, food safety knowledge did not significantly link to aflatoxin B₁ contamination along the processing points across the wet and dry seasons.

Table 20: The influence of the status of knowledge on mean aflatoxin B₁ concentration along the processing points across the wet and dry seasons

Processing points	Level of Aflatoxin B ₁ (µgkg ⁻¹)		MD	SED	t-stat	P-value	
	Insufficient Knowledge	Sufficient Knowledge					
	N (%)	Mean ± SD					
Wet season							
Millet	46 (22.1)	0.29± 0.36	0.18± 0.25	0.11	0.09	1.15	0.26
Sesame	41 (19.7)	0.17 ± 0.16	0.26± 0.34	-0.09	0.11	-0.85	0.40
Soybeans	45 (21.6)	0.66± 0.29	0.63± 0.24	0.03	0.08	0.34	0.73
FP-MMSSC	32 (15.4)	0.23± 0.26	0.40± 0.28	-0.17	0.09	-1.69	0.09
PS-MMSSC	44 (21.2)	0.38± 0.25	0.54± 0.25	-0.16	0.08	-1.92	0.06
Dry season							
Millet	46 (29.7)	0.49 ± 0.28	0.36 ± 0.26	0.13	0.09	1.36	0.18
Sesame	35 (22.6)	0.48 ± 0.23	0.39± 0.26	0.09	0.11	0.81	0.43
Soybeans	1 (0.6)	0.34± 0.37	0.04± 0.05	0.30	0.26	1.12	0.38
FP-MMSSC	30 (19.4)	0.37± 0.24	0.35± 0.22	0.02	0.08	0.26	0.79
PS-MMSSC	43 (27.7)	0.37± 0.28	0.41± 0.28	-0.03	0.10	-0.34	0.74

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.3 The influence of the food safety attitude on total aflatoxin contamination along the processing points across the wet and dry seasons

Regarding attitude as (Table 21), the contamination levels of total aflatoxin indicated a variation along the processing points across the wet and dry seasons. Total aflatoxin contamination was generally higher in households with negative attitudes, especially in the raw materials. Contrarily in the dry season, only sesame and the processed stored products had higher total aflatoxin contamination. However, attitude did not influence total aflatoxin contamination along the processing points across the wet and dry seasons.

Table 21: The influence of the food safety attitude on mean total aflatoxin concentration along the processing points across the wet and dry seasons

Processing points		Level of total Aflatoxin (µg kg ⁻¹)		MD	SED	t-stat	p-value
		Negative	Positive				
		Attitude	Attitude				
		N (%)	Mean ± SD				
Wet season							
Millet	46 (22.1)	0.81± 0.21	0.82± 0.24	-0.01	0.08	-0.09	0.93
Sesame	41 (19.7)	1.08± 1.03	1.14± 1.02	-0.05	0.34	-0.16	0.87
Soybeans	45 (21.6)	1.32± 1.36	1.10± 0.78	0.21	0.33	0.65	0.52
FP-MMSSC	32 (15.4)	0.88± 0.33	0.76± 0.40	0.12	0.13	0.92	0.36
PS-MMSSC	44 (21.2)	0.91± 0.26	0.84± 0.19	0.07	0.07	0.94	0.35
Dry season							
Millet	46 (29.7)	0.709 ± 0.218	0.644 ± 0.197	0.065	0.070	0.924	0.362
Sesame	35 (22.6)	1.112 ± 1.099	0.775 ± 0.245	0.338	0.259	1.301	0.203
Soybeans	1 (0.6)	0.654	0.385 ± 0.334	0.269	0.385	0.697	0.558
FP-MMSSC	30 (19.4)	0.624 ± 0.125	0.719 ± 0.644	-0.095	0.189	-0.502	0.620
PS-MMSSC	43 (27.7)	0.816 ± 0.169	0.834 ± 0.053	-0.087	0.078	-1.126	0.268

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means,

Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.4 The influence of the food safety attitude on aflatoxin B₁ contamination along the processing points across the wet and dry seasons

As indicated in Table 22, in both the wet and dry seasons, households with negative food safety attitudes mainly had higher aflatoxin B₁ contamination than those with positive food safety attitudes. Except for soybeans in the wet season and freshly processed and stored processed MMSSC in the dry season respectively. However, aflatoxin B₁ contamination was not significantly related to attitude, ($P \leq 0.05$).

Table 22: The influence of the food safety attitude on mean aflatoxin B₁ concentration along the processing points across the wet and dry seasons

Processing points		Level of Aflatoxin B ₁ (µg kg ⁻¹)		MD	SED	t-stat	p-value
		Negative Attitude	Positive Attitude				
N (%)		Mean ± SD	Mean ± SD				
Wet season							
Millet	46 (22.1)	0.28± 0.33	0.17± 0.24	0.11	0.08	1.23	0.23
Sesame	41 (19.7)	0.21± 0.26	0.26± 0.33	-0.05	0.10	-0.53	0.60
Soybeans	45 (21.6)	0.57± 0.23	0.68± 0.27	-0.11	0.08	-1.37	0.18
FP-MMSSC	32 (15.4)	0.35± 0.26	0.36± 0.30	-0.01	0.09	-0.11	0.92
PS-MMSSC	44 (21.2)	0.55± 0.26	0.47 ± 0.26	0.07	0.08	0.93	0.36
Dry season							
Millet	46 (29.7)	0.39± 0.28	0.40± 0.26	-0.01	0.09	-0.08	0.94
Sesame	35 (22.6)	0.41± 0.24	0.41± 0.27	0.00	0.08	0.02	0.98
Soybeans	1 (0.6)	0.59			0.55	0.05	11.6
FP-MMSSC	30 (19.4)	0.29± 0.22	0.38± 0.22	-0.09	0.15	-0.65	0.52
PS-MMSSC	43 (27.7)	0.35± 0.31	0.43± 0.26	-0.08	0.08	-1.13	0.27

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.5 The influence of the food hygiene practices on total aflatoxin contamination along the processing points across the wet and dry seasons

Generally, in the wet season, as shown in Table 23, high total aflatoxin contamination was registered in households that did not practice food hygiene. In contrary, in the dry season, higher concentrations of total aflatoxin were observed in raw materials in households that adhered to food hygiene practices. However, food hygiene practices did not significantly relate to the level of total aflatoxin contamination along the processing points across the wet and dry seasons.

Table 23: The influence of the food hygiene practices on mean total aflatoxin concentration along the processing points across the wet and dry seasons

Processing points		Level of total Aflatoxin (µg kg ⁻¹)	MD	SED	t-stat	p-value	
		Don't practice food hygiene	Practice food hygiene				
N (%)		Mean ± SD	Mean ± SD				
Wet season							
Millet	46 (22.1)	0.84± 0.23	0.77± 0.24	0.07	0.08	0.84	0.41
Sesame	41 (19.7)	1.19 ± 1.14	0.93± 0.58	0.25	0.36	0.70	0.48
Soybeans	45 (21.6)	1.30± 1.18	0.87 ± 0.18	0.44	0.35	1.26	0.21
FP-MMSSC	32 (15.4)	0.78± 0.43	0.84± 0.28	-0.06	0.13	-0.45	0.66
PS-MMSSC	44 (21.2)	0.89± 0.23	0.78± 0.18	0.11	0.07	1.44	0.16
Dry season							
Millet	46 (29.7)	0.65 ± 0.22	0.69± 0.17	-0.04	0.07	-0.57	0.57
Sesame	35 (22.6)	0.73± 0.24	1.18±1.07	-0.45	0.25	-1.78	0.08
Soybeans	1 (0.6)	0.38± 0.33	0.65		-0.27	0.38	-0.69
FP-MMSSC	30 (19.4)	0.70± 0.64	0.65± 0.19	0.05	0.19	0.25	0.81
PS-MMSSC	43 (27.7)	0.83± 0.10	0.81 ± 0.14	0.02	0.04	0.46	0.65

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.6 The influence of the food hygiene practices on aflatoxin B₁ contamination along the processing points across the wet and dry seasons

The influence of food hygiene practices on aflatoxins B₁ contamination along the processing points across the wet and dry seasons is shown in Table 24. In both the wet and dry seasons, there was no significant difference in aflatoxins B₁ contamination along the processing points across all the households that did not practice food hygiene those that practice food hygiene. Except in the dry season, food hygiene practices significantly influenced the contamination level of aflatoxin B₁ in soybean ($P = 0.007$).

Table 24: The influence of the food hygiene practices on mean aflatoxin concentration along the processing points across the wet and dry seasons

Processing points		Level of Aflatoxin ($\mu\text{g kg}^{-1}$)		MD	SED	t-stat	p-value
		Practice food hygiene	Do not practice food hygiene				
N (%)							
Wet season							
Millet	46(22.1)	0.24 $\pm$ 0.30	0.16 $\pm$ 0.23	0.08	0.08	0.94	0.35
Sesame	41(19.7)	0.24 $\pm$ 0.30	0.23 $\pm$ 0.31	0.02	0.12	0.16	0.87
Soybeans	45(21.6)	0.65 $\pm$ 0.26	0.60 $\pm$ 0.26	0.05	0.08	0.59	0.55
FP-MMSSC	32(15.4)	0.38 $\pm$ 0.29	0.29 $\pm$ 0.28	0.09	0.09	0.93	0.36
PS-MMSSC	44(21.2)	0.53 $\pm$ 0.25	0.39 $\pm$ 0.26	0.14	0.09	1.58	0.12
Dry season							
Millet	46(29.7)	0.42 $\pm$ 0.29	0.36 $\pm$ 0.22	0.05	0.09	0.60	0.550
Sesame	35(22.6)	0.39 $\pm$ 0.22	0.44 $\pm$ 0.29	-0.05	0.08	-0.60	0.552
Soybeans	1(0.6)	0.05 $\pm$ 0.04	0.59	0.55	0.05	11.65	0.007*
FP-MMSSC	30(19.4)	0.32 $\pm$ 0.24	0.43 $\pm$ 0.18	-0.11	0.08	-1.43	0.163
PS-MMSSC	43(27.7)	0.40 $\pm$ 0.28	0.38 $\pm$ 0.27	0.03	0.12	0.23	0.819

MD, SD, SED, t-stat, P -value, FP – MMSSC, PS – MMSSC denotes, mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, probability value, freshly processed malted millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite and * indicate a significant difference.

7.3.7 The influence of the status of food safety knowledge on exposure to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

The influence of the status of food safety knowledge on exposure to total aflatoxin and aflatoxin B₁ in the wet and dry seasons is shown in Table 25 below. In the wet and dry seasons, the highest exposure to total aflatoxin is in households with insufficient food safety knowledge. In the wet season, households that consumed processed stored MMSSC had higher exposure to total aflatoxin ($26.9 \pm 14.3 \text{ ngkg}^{-1}\text{bwday}^{-1}$) than those that consumed freshly processed MMSSC ($22.0 \pm 19.53\text{ngkg}^{-1}\text{bwday}^{-1}$). Exposure to total aflatoxin in the wet season was significantly related to knowledge in households that consumed freshly processed products and processed stored products ($P = 0.026$ and $P = 0.005$).

Contrarily, during the dry season, equal exposure to total aflatoxin was observed in households that consumed freshly processed products and processed stored products ($25.0 \pm 21.6 \text{ 3ng kg}^{-1}\text{bwday}^{-1}$ and $25.3 \pm 15 \text{ 3ngkg}^{-1}\text{bwday}^{-1}$) respectively. Exposure to total aflatoxin in households that consumed freshly processed products and processed stored products was related to food safety knowledge ($P = 0.001$ and $P = 0.003$). In contrast, the status of food safety knowledge and exposure to aflatoxin B₁ in both the wet and dry seasons indicates that in both the wet and dry seasons, households with insufficient food safety knowledge were more exposed to aflatoxin B₁ than those with sufficient food safety knowledge. However, knowledge significantly influenced exposure to aflatoxin B₁ in households that consumed freshly processed products in the dry season ($P = 0.003$).

Table 25: The influence of the status of food safety knowledge on mean estimated dietary intake (EDI) to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

Processing points across wet and dry seasons	Level of Exposure (ng kg ⁻¹ bw day ⁻¹)		MD	SED	t-stat	p-value
	Insufficient Knowledge	Sufficient Knowledge				
	Mean ± SD	Mean ± SD				
Wet season						
TAFL for wet season for FP-MMSSC	22.0 ± 19.5	11.5 ± 7.3	10.5	4.5	2.339	0.026*
TAFL for wet season for PS-MMSSC	26.9 ± 14.3	14.1 ± 9.9	12.8	4.2	3.032	0.005*
AFB ₁ for wet season for FP-MMSSC	10.5 ± 13.7	5.5 ± 5.6	5.0	3.2	1.542	0.133
AFB ₁ for wet season for PS-MMSSC	14.1 ± 15.1	9.2 ± 7.1	4.9	3.7	1.307	0.200
Dry season						
TAFL for dry season for FS-MMSSC	25.0 ± 21.6	9.2 ± 5.7	15.8	4.5	3.507	0.001*
TAFL for dry season for PS-MMSSC	25.3± 15.0	13.3 ± 6.9	11.9	3.7	3.245	0.003*
AFB ₁ for dry season for FS-MMSSC	15.6 ± 15.1	5.2 ± 4.9	10.4	3.3	3.160	0.003*
AFB ₁ for dry season for PS-MMSSC	11.6 ± 10.2	7. 0 ± 6.5	4.6	2.9	1.578	0.124

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC, TAFL, AFB₁ (Mean difference, Standard Deviation, Standard Error Difference, Statistical test used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite, Total aflatoxin, Aflatoxin B₁) respectively and * indicate a significant difference of a P-value.

7.3.8 The influence of food safety attitude on exposure to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

As indicated in Table 26, in the wet and dry seasons, households with negative food safety attitudes were more exposed to total aflatoxin and aflatoxin B₁. Households that consumed the freshly processed and processed stored MMSSC were more exposed to total aflatoxin in the wet season than dry the season, (19.1 ± 18.1 and 20.3 ± 16.7 ngkg⁻¹bwday⁻¹). Those who consumed processed stored products were more exposed to aflatoxin B₁ (13.7 ± 14.3 ng kg⁻¹bw day⁻¹) in the wet season. However, in both seasons, exposure to total aflatoxin and aflatoxin B₁ did not significantly relate to attitude towards food safety.

Table 26: The influence of food safety attitude on mean estimated dietary intake (EDI) to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

Estimated daily intake	Level of exposure (ngkg ⁻¹ bwday ⁻¹)		MD	SED	t-stat	P-value
	Negative Attitude	Positive Attitude				
	Mean SD	Mean SD				
TAFL for wet season for FP-MMSSC	19.1 ± 18.1	11.8 ± 7.3	7.3	4.3	1.683	0.102
TAFL for wet season for PS-MMSSC	20.3 ± 16.7	16.2 ± 9.5	4.0	4.4	0.918	0.365
AFB ₁ for wet season for FP-MMSSC	9.4 ± 12.0	5.5 ± 6.1	3.9	3.0	1.272	0.212
AFB ₁ for wet season for PS-MMSSC	13.7 ± 14.3	8.8 ± 6.1	4.9	3.5	1.406	0.169
Dry season						
TAFL for dry season for FP-MMSSC	15.0 ± 15.1	12.8 ± 13.5	2.2	4.9	0.442	0.662
TAFL for dry season for PS-MMSSC	18.4 ± 16.5	15.7 ± 6.4	2.7	3.9	0.693	0.493
AFB ₁ for dry season for FP-MMSSC	11.1 ± 14.9	6.4 ± 5.1	4.7	3.4	1.392	0.173
AFB ₁ for dry season for PS-MMSSC	8.8 ± 10.9	8.1 ± 5.8	0.7	2.8	0.262	0.795

MD, SD, SED, t-stat, P-value, FP – MMSSC, PS – MMSSC, TAFL, AFB₁ denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite, Total aflatoxin, Aflatoxin B₁ and * indicate a significant difference.

7.3.9 The influence of food hygiene practices on exposure to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

As indicated in Table 27, in the wet and dry seasons, households with poor food hygiene practices were more exposed to total aflatoxin and aflatoxin B₁. In the wet season, children who consumed freshly processed MMSSC were significantly exposed to Aflatoxin B₁ ($P = 0.029$) and In the dry season, children who consumed processed stored MMSSC were significantly exposed to Aflatoxin B₁ ($P = 0.041$)

Table 27: The influence of food hygiene practices on mean estimated dietary intake (EDI) to total aflatoxin and aflatoxin B₁ in the freshly processed and processed stored MMSSC across the wet and dry seasons

Estimated daily Intake	Level of exposure (ng kg ⁻¹ bw day ⁻¹)		MD	SED	t-stat	p-value
	Do not practice food hygiene	Practice food hygiene				
	Mean ± SD	Mean ± SD				
Wet season						
TAFL for wet season for FP- MMSSC	16.2 ± 14.0	9.6 ± 5.8	6.6	4.9	1.370	0.180
TAFL for wet season for PS- MMSSC	19.9 ± 13.4	11.6 ± 7.4	8.3	4.7	1.747	0.090
AFB ₁ for wet season wet for FP- MMSSC	8.8 ± 9.5	1.5 ± 1.1	7.3	3.2	2.289	0.029*
TAFL for wet season for PS- MMSSC	19.9 ± 13.4	11.6 ± 7.4	8.3	4.7	1.747	0.090
AFB ₁ for wet season for PS-MMSSC	12.0 ± 10.9	6.6 ± 5.8	5.4	3.8	1.409	0.168
Dry season						
TAFL for dry season for FP-MMSSC	15.4 ± 15.6	8.3 ± 4.0	7.1	5.3	1.334	0.191
TAFL for dry season for PS- MMSSC	18.6 ± 12.0	11.4 ± 5.6	7.3	4.2	1.738	0.092
AFB ₁ for dry season for FP- MMSSC	9.2 ± 11.1	4.8 ± 3.8	4.4	3.8	1.169	0.250
AFB ₁ for dry season for PS- MMSSC	9.9 ± 8.4	3.8 ± 3.8	6.2	2.9	2.123	0.041*

MD, SD, SED, t-stat, P- value, FP – MMSSC, PS – MMSSC , TAFL, AFB₁ denotes, Mean difference, Standard Deviation, Standard Error Difference, test statistical value used to compare means, Probability value, Freshly processed Malted Millet Sesame Soy Composite, Processed Stored Malted Millet Sesame Soy Composite, Total aflatoxin, Aflatoxin B₁ and * indicate a significant difference.

7.4 Discussions

Overall, the competency of the caregivers of children variedly influenced the level of total aflatoxin and aflatoxin B1 contaminations and related exposures. Total aflatoxin contaminations varied along the processing points across the two seasons with the highest total aflatoxin contamination observed in households with insufficient food safety knowledge (Table 19). This is because knowledge influences practice which might have led to high contamination and increased aflatoxin levels in households with insufficient food safety knowledge. In support of these findings, da Cunha *et al.* (2019) affirmed that knowledge influences observed practice. However, literature shows that knowledge may not be translated into good practice (Nassanga *et al.*, 2018; Ncube *et al.*, 2020). Precisely, the highest total aflatoxin concentration was in one of the raw materials, soybean ($1.519 \pm 1.505 \mu\text{gkg}^{-1}$ and processed stored MMSSC ($0.994 \pm 0.307 \mu\text{gkg}^{-1}$) in households with insufficient food safety knowledge. Literature reflects that soybean is an oil seed and, stored processed MMSSC are prone to aflatoxin contamination (Rajasekaran *et al.*, 2017).

Sufficient knowledge in handling during processing and storage is thus required to control aflatoxin contamination. The high total aflatoxin contamination within the processed stored MMSSC may also indicate the importance of sufficient knowledge to improve storage conditions in reducing the proliferation of aflatoxigenic fungi and related toxins. These findings agree with other authors who showed that awareness of storage conditions play a role in the contamination of stored products (Mannaa & Kim, 2017; Udomkun *et al.*, 2018; Omotayo *et al.*, 2019; Ojuri *et al.*, 2019; Ncube *et al.*, 2020; Hoffmans *et al.*, 2022). Contrarily, in households with sufficient knowledge, the highest concentration of total aflatoxin was observed in sesame. This observation may show that control of aflatoxin in sesame may require specific knowledge when handling it.

The literature points out that sufficient knowledge may not reflect actual practice, and therefore, the status knowledge should be verified with actual practice through observation, not self-reported practice (Ncube *et al.*, 2020). However, knowledge was only significantly correlated with the concentration of total aflatoxin in the processed stored MMSSC ($P = 0.027$). This may indicate that to control aflatoxin contamination in the wet season, food handlers should have sufficient food safety knowledge regarding the storage of products. In agreement with this, studies have shown that during storage in the wet season, aflatoxigenic fungi are likely to proliferate and produce aflatoxin due to favorable conditions during storage such as optimum

humidity, temperature and water activity, and insect infestation (Casquete *et al.*, 2017; Udomkun *et al.*, 2018; Massomo, 2020; Adeyeye *et al.*, 2022).

During the dry season, the highest total aflatoxin contamination was observed in households with insufficient food safety knowledge. Whereas, along the processing points, the total aflatoxin contamination in sesame nearly doubled all others and was the only variable that significantly related to knowledge (Table 19). The high total aflatoxin contamination of sesame may be due to the oil in sesame and other factors such as moisture content that promotes the proliferation of aflatoxigenic fungi and related toxins. Literature showed that lipid content positively enhances the growth of aflatoxigenic fungi and toxins. Interplay of factors may influence the proliferation of aflatoxigenic fungi (Liu *et al.*, 2016, Rajasekaran *et al.*, 2017). This is affirmed by other studies indicating that factors such as environmental conditions and grain/ cereal structure may also play a role in the colonization and eventual proliferation, (Seetha *et al.*, 2017; Norlia *et al.*, 2019; Ayofemi Olalekan Adeyeye, 2020; Akello *et al.*, 2021). Therefore, in the dry season food safety knowledge in handling sesame should be emphasized to control contamination of oily cereals like sesame by aflatoxigenic fungi and related toxins, (Liu *et al.*, 2016).

The influence of the status of food safety knowledge on the level of aflatoxin B1 concentration along the processing points across the wet and dry seasons is shown in Table 20. In both the wet and dry seasons, households with insufficient food safety knowledge had higher contamination with aflatoxin B1 than those with sufficient food safety knowledge except for sesame and freshly processed and stored MMSSC in the wet and dry seasons respectively. This finding suggests that the interaction of factors such as favorable environmental conditions, and grain/cereal structure may contribute to aflatoxin contamination in both the wet and dry seasons. Therefore, specific awareness should be paid to the raw materials such as sesame in the wet season and freshly processed MMSSC and stored MMSSC in the dry season respectively. This is attested by Achiro *et al.* who reported that high contamination occurred in sesame in the wet season (Achiro *et al.*; 2023). In agreement with this finding, other scholars observed that oily seeds/grains are more prone to aflatoxin contamination, especially in the wet season due to availability of optimal proliferation conditions (Casquete *et al.*, 2017; Udomkun *et al.*, 2018; Massomo, 2020; Adeyeye *et al.*, 2022). Akello *et al.* argued that small gains and cereals are less prone to aflatoxin contamination due to the decreased surface for microbial colonization, (Akello *et al.* 2021). In the dry season, contamination of stored processed MMSSC by aflatoxin was more pronounced. This could have resulted from poor storage conditions (Casquete *et al.*, 2017). Nonetheless, food

safety knowledge did not significantly influence aflatoxin B₁ contamination levels along the processing points across the wet and dry seasons.

Regarding attitude (Table 21), the pairwise comparison of the status of attitude and the contamination levels of total aflatoxin specified that in the wet season, households with positive attitude had nearly ¾ of the raw materials having higher total aflatoxin contamination levels compared to households with negative food safety knowledge. This may indicate that contamination by aflatoxin may not be influenced by attitude toward safe food although literature showed that attitude may directly influence practices that influence food contamination (da Cunha *et al.*, 2019; Kunaduet *et al.*, 2016; Ncube *et al.*, 2020). Contrarily in the dry season, all the raw materials and freshly processed MMSSC had higher total aflatoxin levels in households with negative attitudes. Stored MMSSC had nearly equal levels of total aflatoxin contamination in both households with positive and negative attitudes. The results revealed that attitude had no significant linkage between attitude and levels of total aflatoxin concentrations along the processing points across the wet and dry seasons.

Aflatoxin B₁ in both the wet and dry seasons (Table 22), households with negative food safety attitudes mainly had higher contamination with aflatoxin B₁ than those with positive food safety attitudes. This finding reveals that attitude may influence aflatoxin B₁ contamination except for soybeans and freshly processed MMSSC and stored products that may not be influenced by attitude. Nonetheless, attitude towards food safety was not significantly associated with aflatoxin B₁ levels along the processing points in the wet season. In the dry season, attitude significantly influenced the contamination levels of aflatoxin B₁ in soybeans ($P = 0.007$). This may be explained by the fact that an array of factors have an influence on aflatoxin B₁ contamination not necessarily attitude (Liu *et al.*, 2016; Mannaa & Kim, 2017; Aron *et al.*, 2017; Bhardwaj *et al.*, 2022). The significant linkage between aflatoxin contamination levels in soybeans and attitude may reveal that during the dry season, specific attention should be given to soybeans to control contamination.

In the wet season, higher levels of total aflatoxin contaminations were registered in households that did not practice food hygiene compared to those that practiced food hygiene except for freshly processed MMSSC that had higher levels of total aflatoxin concentration observed in households who practiced to food hygiene practices (Table 23). This finding is in agreement with other scholars indicating that practicing food hygiene has an influence on contamination levels of

total aflatoxins in the wet season due to favorable climatic conditions (Mannaa & Kim, 2017; Jallow *et al.*, 2021; Lee *et al.*, 2022; Bhardwaj *et al.*, 2022).

During the dry season, higher concentrations of total aflatoxin were observed in raw materials in households that practiced food hygiene. This could be because of the inaccuracy of reporting by respondents whereby self-reported practice may not be the actual practice as affirmed by authors (Nassanga *et al.*, 2018; Ncube *et al.*, 2020). And so, self-reported practices need to be verified by observation. Contrarily, in the processed MMSSC, higher levels of total aflatoxin contamination were observed in households that did not practice food hygiene. However, food hygiene practices did not significantly relate to the total aflatoxin contamination along the processing points across the wet and dry seasons. In the wet season, all the households that did not practice food hygiene had higher contamination with aflatoxin B₁. According to this finding, adherence to food hygiene practices and good postharvest practices may be the control strategies of total aflatoxin control. In line with this finding, authors have pointed out that food hygiene practices and postharvest practices have a linkage to food safety and control of food contamination in particular (Aron *et al.*, 2017; Echodu *et al.*, 2019; Ojuri *et al.*, 2019; Mollay *et al.*, 2021; Hoffmans *et al.*, 2022; Namubiru *et al.*, 2022). In the dry season, most households who did not practice food hygiene had higher contamination with aflatoxin B₁ than those who practiced food hygiene. Nonetheless, in the wet season, food hygiene practices did not significantly relate to the contamination levels of aflatoxin B₁ along the processing points. In the dry season, this lack of relatedness may indicate that the reported practice may not be the actual practice reported by Ncube *et al.*, 2020; Mshida *et al.*, 2020). Therefore, such self-reported practices should be verified with observation to make concrete conclusions. Conversely, the concentration levels of aflatoxin B₁ in soybeans were significantly related to food hygiene practices in the dry season ($P = 0.007$). This may point out that during the dry season, attention should be paid to soybeans to avoid contamination with aflatoxin B₁.

As shown in Table 25, the influence of the status of food safety knowledge on exposure to total aflatoxin and aflatoxin B₁ in the freshly processed and stored MMSSC across the wet and dry seasons shows that in both the wet and dry seasons, households with sufficient food safety knowledge have low exposure levels to total aflatoxin. This could be explained by the fact that knowledge is closely associated with the practice (Namubiru *et al.*, 2022). Accordingly, authors have linked good food hygiene practices to low aflatoxin contamination (Udomkun *et al.*, 2018). Other authors argued that knowledge is not necessarily translated into good practice (Nassanga *et*

al., 2018, Ncube *et al* 2020; Achiro *et al* 2023). Thus the gap that leads to the non-translation of knowledge into practice should be bridged, (Yimenu *et al.*, 2022).

In the wet season, households that consumed processed stored MMSSC had higher exposure to total aflatoxin than those that consumed freshly processed MMSSC (Table 25). This could be explained by the fact that contamination can occur during storage due to factors such as cross-contamination and poor storage conditions. Exposure to total aflatoxin in the wet season was significantly associated with knowledge in households that consumed freshly processed and processed stored MMSSC (Table 25). This means that there may be a need for sufficient food safety knowledge during processing and storage to address the challenge of exposure to aflatoxin in the dry season. Inversely in the dry season, equal exposure to total aflatoxin was observed in households that consumed freshly processed products and processed stored products.

Exposure to total aflatoxin in households that consumed freshly processed and processed stored MMSSC were both significantly influenced by food safety knowledge (Table 25). In contrast, in both the wet and the dry seasons households with insufficient food safety knowledge were more exposed to aflatoxin B₁ than those with sufficient food safety knowledge. However, knowledge only influenced exposure to aflatoxin B₁ in households that consumed freshly processed MMSSC in the dry season (Table 25). Therefore, addressing exposure may require sufficient knowledge both during processing and storage. Specific knowledge may thus be needed to control aflatoxin B₁. According to scholars, knowledge may not be translated into practice leading to non-food hygiene practices and proper postharvest practices. However, knowledge was only related to exposure to aflatoxin B₁ in households that consumed freshly processed MMSSC in the dry season (Table 25). Thus, to control exposure to aflatoxin B₁ resulting from the consumption of freshly processed MMSSC in the dry season, food hygiene practices during processing need to be emphasized. Literature attests to this that contamination can occur during processing due to several factors including cross-contamination and the use of contaminated raw materials (Mannaa & Kim, 2017; Miklós *et al.*, 2020; Acur *et al.*, 2020).

Regarding attitude as indicated in Table 26, in both the wet and dry seasons, households with negative food safety attitudes were more exposed to total aflatoxin and aflatoxin B₁. Households that consumed the freshly processed and processed stored MMSSC were more exposed to total aflatoxin in the wet season. Those who consumed processed stored MMSSC in the wet season were more exposed to aflatoxin B₁. However, in both seasons, exposure to total aflatoxin and

aflatoxin B₁ was not influenced significantly by attitude towards food safety. This finding points out that, in both the wet and dry seasons, exposure to the total aflatoxin and aflatoxin B₁ may not be influenced by attitude. Many scholars found that attitude may not influence practice although practice is closely linked to food contamination and resultant exposure (Neme & Mohammed, 2017; Mahuku *et al.*, 2019).

Furthermore, the linkage between food hygiene practices and exposure to total aflatoxin and aflatoxin B₁ in Table 27 showed that in both the wet and dry seasons, higher exposure levels to total aflatoxin and aflatoxin B₁ were in households that did not practice food hygiene compared to those who practice food hygiene. This means that exposure to total aflatoxin and aflatoxin B₁ may be directly influenced by food hygiene practices as attested by scholars who found that food hygiene practices may influence food contamination and subsequent exposure to the related hazards (Seetha *et al.*, 2017; Aron *et al.*, 2017; Echodu *et al.*, 2019; Mollay *et al.*, 2021). Also, this study indicated that food hygiene practice only significantly influenced exposure to total aflatoxin and aflatoxin B₁ in households that consumed freshly processed products in the wet season and those that consumed processed stored products in the dry season. Strategies to reduce and control exposure to total aflatoxin and aflatoxin B₁ may require specific practices along the processing points during the wet and dry seasons. This may be due to the interplay of different factors (Lahouar *et al.*, 2016; Rajasekaran *et al.*, 2017).

7.5 Study strengths and limitations

Data used in this study was collected by competent research assistants with adequate knowledge of agriculture, food science, and human nutrition. Additionally, the data collection tools were pretested and validated. Further, the study participants were caregivers of children that were producing complementary food across the wet and dry seasons at the domestic level without recognized validated standard processing procedures. The study highlighted the influence of the status of KAP on the caregivers and the contamination levels of total and aflatoxin B₁ along the processing points across the wet and dry seasons and subsequent exposure of the children. This information has contributed to the body of knowledge as one of the strategies for controlling aflatoxin contamination and exposure in homemade products produced under uncontrolled conditions. The study, however, had the following methodological constraints; the study examined the KAP of the only caregivers trained in preparing locally made food formulae. Therefore, our findings do not generalize to the entire community. Moreover, the study employed

a cross-sectional design; thus, the cause-effect relationship may not be demonstrated.

Additionally, the selection of the respondents was purposive and prone to sampling bias. Also, the self-reported practice technique may need validation due to inconsistency with the actual practice. Furthermore, although the Enzyme-linked immunosorbent Assay (ELISA) method used for determining aflatoxin is reliable, the accuracy may be limited compared to methods such as High-performance Liquid Chromatography (HPLC). Other methodological limitations included inadequate soybean samples for the dry season that could not be statistically analyzed satisfactorily.

7.6 Conclusion

Food safety knowledge, attitude, and food hygiene practices influence aflatoxin contamination and related exposure of the children. The influence was dependent on processing points and seasonality. The highest aflatoxin contamination was in households with insufficient food safety knowledge. In the wet season, food safety knowledge significantly influenced total aflatoxin in the processed stored MMSSC. In the dry season, food safety knowledge had a significant influence on total aflatoxin contamination in sesame.

In the wet and dry seasons, knowledge influenced the children's exposure to total aflatoxin and aflatoxin B₁. The highest exposure to total aflatoxin and aflatoxin B₁ was in households with insufficient food safety knowledge.

Attitude did not influence contamination levels of total aflatoxin and aflatoxin B₁ along the processing points across the wet and dry seasons.

Food hygiene practices had influence on exposure to total aflatoxin and aflatoxin B₁ in households that consumed freshly processed MMSSC in the wet season and those that consumed processed stored MMSSC in the dry season.

7.7 Recommendation

Inevitable control of aflatoxin contamination and related exposure at household levels is required. As evidenced by the study findings, the strong influence of competency factors on

aflatoxin contamination and related exposure calls for food safety education to enhance knowledge of the community that produces homemade complementary food formulae. Specifically, providing awareness of the levels of aflatoxin contamination and exposures of the vulnerable populace as weaning children through consumption is vital. Sufficient knowledge and good hygiene practices should be prioritized when handling oil seeds such as sesame and soybeans during the wet and dry seasons.

Sufficient knowledge, a positive attitude, and good hygiene practices are needed along the processing points across the wet and dry seasons to control aflatoxin contamination and related exposure. However, alongside controlling cross-contamination along the processing points, attention should be paid to the raw materials, processing conditions during the wet season, and storage conditions during the dry season. Policies should be put in place highlighting specific conditions along the processing points based on seasons.

Thus, controlling and reducing aflatoxin contamination and related exposure requires a multi-sectoral approach. The producers, processors, and consumers need to be knowledgeable on food safety issues, have a positive food safety attitude, and practice good food hygiene and pre and postharvest management. Additionally, regulating bodies and policymakers need to put policies and guidelines in place regarding local processing in a domestic environment. This should highlight specific conditions along the processing points based on seasons. Researchers need to conduct a study while taking into consideration the association of food safety knowledge, attitude food hygiene practices, and exposure to aflatoxin while considering both the trained and untrained of the respondents. Additionally, while conducting any KAP study reported practice needs to be validated by observation checklist.

CHAPTER EIGHT: General discussions, conclusion and recommendations

8.0 General discussions

Although complementary foods are usually cooked, heat-resistant contaminants remain a safety threat. This study investigated the prevalence of *Aspergillus* species (heat resistant), the total aflatoxin, and aflatoxin B₁ contamination levels along the processing points locally made MMSSC across the wet and dry seasons. Subsequently, exposure of the children to both the total and aflatoxin B₁ was determined. Additionally, the study assessed the status of KAP of caregivers of children aged 6-23 months regarding the microbial safety of locally formulated complementary food composite. The study further evaluated the influence of the status of KAP on the level of total and aflatoxin B₁ contamination and related exposure. The results of this study are below.

8.1. Contamination of MMSSC with *Aspergillus* species along the processing points across the wet and dry season

Throughout the processing points, less than a quarter of the total samples were contaminated with *Aspergillus* species across the wet and dry seasons. Millet was the most contaminated and sesame was the least contaminated. This could have resulted from how different raw materials were managed at the pre-and postharvest levels (Gonçalves *et al.*, 2019).

The structural characteristic of small grains could have contributed to the high contamination in millet since sorting as a strategy to control contamination may not be adequately effected in small grains (Boudergue *et al.*, 2017; Marshall *et al.*, 2020). The presence of soluble sugars such as glucose, maltose, fructose, sucrose raffinose, and stachyose favors the biosynthesis of aflatoxin B₁ (Singh and Sinha 2013; Liu *et al.* 2016). However, because of the co-occurrence of both aflatoxigenic and non-aflatoxigenic *Aspergillus* species, biosynthesis of aflatoxins may be altered hence less aflatoxin contamination of millet as observed in chapter 5, sub-section 5.3 of this dissertation.

Contrarily, literature reports lower contamination of small grains than large grains due to their structure, which does not support colonization and subsequent aflatoxin production by the fungal species (Akello *et al.*, 2021; Achiro *et al.*, 2023). Though seasonal variation and processing point

had no significant statistical influence on *Aspergillus* species contamination, the current study noticed soybean having the highest contamination in the wet season followed by freshly processed products, millet, and least in processed stored products and sesame. The high prevalence of *Aspergillus spp* in the soybean could have resulted from favorable environmental conditions and structural and chemical properties that favor the colonization of *Aspergillus* species (Khan *et al.*, 2021; Ajmal *et al.*, 2022).

Aflatoxigenic fungal contamination during the dry season was due to poor storage conditions that favor their growth and aflatoxin production (Ajmal *et al.*, 2022; Achiro *et al.*, 2023). Ensuring the safety of stored products requires controlling the biotic (insect invasions and microorganisms) and abiotic factors such as temperature, carbondioxide, oxygen, and humidity) appropriately. For instance, water content should be 94 g/kg, temperature 30 - 40°C if possible, and relative humidity should not exceed 70%. Soil cadmium and iron play a role in controlling the proliferation of fungi, and subsequent toxin production should be increased to optimum levels as part of good agronomic practices (Khan *et al.*, 2021). The finding agrees with a study by Achiro *et al.*, 2023a that showed households store their produce in the same house where they dwell. Such practice makes controlling the biotic and abiotic factors that promote the proliferation of these fungal species difficult. Therefore, it is advisable to keep produce in distinct stores to control these factors appropriately.

The main aflatoxin-producing and non-aflatoxigenic species; *A. flavus*, *A. parasiticus*, *A. niger*, and *A. fumigatus* respectively were recovered in relatively low frequencies from raw materials (Millet, sesame, and soybean), freshly processed products and stored processed products cross the wet and dry season. Studies showed that millet especially is less contaminated with aflatoxigenic fungi (Njoroge 2018; Akello *et al.*, 2021; Gichohi-Wainaina *et al.*, 2022). This disagrees with the current finding which indicated that millet was the most contaminated. However when the same samples were analyzed for aflatoxin contamination, millet and processed products had the least aflatoxin concentrations (Achiro *et al.*, 2023b). The interaction between *A. flavus* and *A. niger* that were more prevalent in millet could have resulted in this observation since the literature points out that the interaction between the two organisms has been known to reduce levels of aflatoxin (Xing *et al.*, 2017; Ting *et al.*, 2020). The present study attests to the reported interaction whereby, *A. flavus* and *A. niger* were more in millet.

The current study revealed variation in the mycobiome along processing points across the wet and dry seasons. Generally, *A. parasiticus* was more detected followed by *A. flavus*, *A. niger*, and *A. fumigatus* was the least detected across the two seasons. Similarly, other studies have observed a high prevalence of *A. flavus* and *A. parasiticus* in cereals compared to other *Aspergillus* species (Dangwa and Dhlamini, 2014; Alkuwari *et al.*, 2022). The result indicates aflatoxin contamination since these microorganisms are the major producers of aflatoxin (Ting *et al.*, 2020). This scenario threatens food safety and food and nutrition security of communities in rural settings that depend on cereals as staple foods, but also negatively affects trade both at the local and international levels. The contamination spread along the processing points with *A. flavus* and *A. parasiticus* more frequently detected in millet and soybean. *A. niger* on the other hand was more frequent in millet and stored processed products. The presence of *A. niger* in millet samples could have contributed to the counteraction of aflatoxin production by *A. flavus* thus leading to the observed low concentrations of aflatoxin in the millet samples (Achiro *et al.*, 2023b). And, *A. fumigatus* was only detected in millet and sesame. Though cross-contamination has frequently been reported during processing due to poor hygiene and manufacturing practices, food handlers are usually trained on proper food handling practices but the knowledge gained is usually not translated into good practices (Nassanga *et al.*, 2018; Achiro *et al.*, 2023a).

About season, there was a seasonal variation of specific *aspergillus* species contamination whereby, in the wet season soybean had the highest number of *A. flavus* contamination followed by millet and freshly processed products and the least number of *A. flavus* contaminations was observed in sesame and stored products. Comparably, sesame has been recorded to be contaminated with potential aflatoxigenic *A. flavus* (Ajmal *et al.*, 2022). Although sesame like other oily seeds has an oily component that supports the growth of aflatoxigenic *Aspergillus* (Liu *et al.*, 2016; Rajasekaran *et al.*, 2017), it was among one of the least contaminated. This could be explained by the fact that an array of intrinsic and extrinsic factors contributes to contamination by *Aspergillus* species (Jallow & Li, 2021). Millet and processed stored products on the other hand had the highest frequency of *A. niger*. As pointed out earlier, the high prevalence of *A. niger* has got a comparative advantage of positively competing with *A. flavus* hence minimizing aflatoxin production (Xing *et al.*, 2017). This positive impact on food safety calls for further investigations to determine the safe concentrations of the inhibitors for maximum reduction of aflatoxin occurrence. A high prevalence of *A. fumigatus* was in sesame and millet in the dry season. This aligns with other studies indicating the occurrence of *A. fumigatus* more than other mycobiota in millet (Bamkefa *et al.*, 2020).

8.2 Contamination of MMSSC with TAF and AFB₁ along the processing points across the wet and dry season

The AF and AFB₁ contamination were detected throughout the processing points. The highest contamination was in the raw materials (sesame and soybean). The higher TAF and AFB₁ contamination of sesame and soybean than millet or processed products could be differences in crop structure and or pre and post-harvest management according to the respective food types (Matumba *et al.*, 2021; Ortega-Beltran & Cotty, 2020; Seetha *et al.*, 2017). Another study reported differential contamination by aflatoxins due to differences in post-harvest practices of many oily staple crops (Goncalves *et al.*, 2019). According to Marshall *et al.* (2020), contamination could be reduced by using innovative technologies such as sorting produce using fluorescence devices and secondary processing (Özer *et al.*, 2018). Equally, Özer *et al.* argued that ensuring good agricultural practices and proper storage may not guarantee the reduction of mycotoxin (Özer *et al.*, 2018). Boudergue *et al.* (2017) showed that decontamination approaches such as sorting, sieving, washing, and heat treatment may ensure reduction.

Özer *et al.* (2018) noted that chemical treatment using acids, alkalis, oxidizing agents, and microbial agents such as enzymes may also be employed to minimize contamination. Thus, it may not be reasonable to conclude that once the raw materials are free from contaminants then, the processed products are safe. Contamination can occur at any stage along the production-consumption continuum. However, combinations of decontamination methods may be appropriately applied to guarantee minimal contamination to acceptable levels.

The TAF contamination was most common in the raw materials followed by the stored products and freshly prepared products in the wet season. Overall, the mean TAF concentration was highest in soybean followed by sesame, stored processed products, freshly processed products, and millet. The report showed that depending on the availability of favorable conditions, fungal growth, and toxin production may occur at any stage along the production-consumption continuum (Perrone *et al.*, 2020). The low prevalence of the total and AFB₁ in millet is comparable to a study by (Akello *et al.*, 2021), who found that small cereals were less prone to aflatoxin contamination. The literature further explained that measures to control/prevent fungal infection at pre-harvest are critical for sustainably minimizing aflatoxin contamination at the post-harvest level (Mahuku *et al.*, 2019a). This could be the reason for the high contamination of the raw materials than the freshly processed and processed stored products since contamination

of the product could have most likely happened in the field. A previous study also indicated that high aflatoxin contamination of the product may occur due to late detection and removal of contaminated ones in the processing continuum, and their spread along the chain can be exacerbated and promoted by poor storage conditions (Ahlberg *et al.*, 2019). Casquete *et al.* (2017) affirmation showed that the proliferation of *A. flavus* and AFB₁ production results from optimum temperature, pH, and water activity of the substrate during storage (Casquete *et al.*, 2017). Furthermore, aflatoxin contamination was high in the processed stored MMSSC. This could be due to poor storage conditions that might have exacerbated and promoted contamination (Casquete *et al.*, 2017). Therefore, to minimize contamination, raw materials should be sorted before storage.

The highly contaminated raw materials were soybean and sesame. This could be because oil seed plants promote the proliferation of *A. flavus* and subsequent toxin production. Several studies affirmed the findings. For instance, Rajasekaran *et al.* (2017) found that total lipid content significantly enhances AFB₁ production in cotton while in the field. Comparably (Liu *et al.* (2016) revealed that growth and toxin production were significantly negatively affected when oily grains and cereals such as soybean and peanuts were defatted and vice versa when corn oil was reintroduced. The same study showed a positive correlation between AFB₁ concentrations and substrate-soluble sugars such as glucose, maltose, fructose, sucrose raffinose, and stachyose (Liu *et al.*, 2016). Similarly, Singh and Sinha's report showed that the total starch and amylopectin content of the substrate were positively correlated with AFB₁ levels (Singh & Sinha, 2013). However, on the contrary, other substrate components such as tocopherols have been shown to negatively influence the production of aflatoxins (Jallow *et al.*, 2021). However, soybean was heavily contaminated compared to millet though it has higher tocopherols than millet (Himanshu *et al.*, 2018). Therefore, susceptibility to fungal infestation and subsequent production of aflatoxins may vary among crop produce depending on their nutrient content, crop structure, and harvest management (Ortega-Beltran & Cotty, 2020; Shanakhat *et al.*, 2018; Los *et al.*, 2018).

Overall, the low contamination observed in freshly processed products could be because processing reduces fungal contaminants (Taheri *et al.*, 2012; Los *et al.*, 2018; Juodeikiene *et al.*, 2018). For instance, previous studies indicated a reduction of fungal load in processed cereals. Equally malting, a process that was employed in this study has been sought to reduce mycotoxin

concentration (Taheri *et al.*, 2012), which could be due to the low release of the toxins into the food but remain in the mycelium during processing (Jallow *et al.*, 2021).

Based on the season, the total and the mean TAF concentration was higher in the wet season (wet) than in the dry season (dry). High concentrations of both TAF and AFB₁ observed in the wet than in the dry season is analogous to a study by Taheri *et al.*, 2012 who registered higher concentrations of TAF in winter than in summer (1.99 ng g⁻¹ and 0.82 ng g⁻¹) respectively (Bashiry *et al.*, 2021). The authors reported a higher level of AFB₁ during winter to be above the globally acceptable limit than during summer (7.4% and 3.4%, respectively). Likewise, Bashiry *et al.* (2021) noted that AFB₁ occurrence was higher (60%) in the cold than in the warm season. According to Atongbiik *et al.* (2017), high concentrations observed in the wet season may indicate the availability of favorable environmental conditions (water activity of between 0.93 to 0.99 and temperature of 0 to 33°C) for the proliferation of aflatoxin-producing *Aspergillus* species. Contrarily, Damianidis *et al.* (2018) showed significantly high aflatoxin contamination in the dry seasons due to night heat stress. Conversely, AFB₁ concentration in the wet season was highest in soybean followed by stored products, freshly processed products, sesame, and millet. In the dry season, the mean concentration of AFB₁ was highest in sesame followed by stored product, millet, and processed product (not determined for soybean).

The mean concentration varied. The concentrations of both TAFs and AFB₁ were higher in the wet than in the dry season. This was due to a damp environment during the wet season that favored fungal growth (Makori *et al.*, 2018; Perrone *et al.*, 2020). In agreement with the current finding, Negash (2018) reported that contamination may vary across season. These may result from the availability of favorable factors such as optimal growth conditions, pest invasion, and poor pre- and post-harvest management (Lukwago *et al.*, 2019). Specifically, the production of aflatoxin by *A. flavus* varies depending on growth conditions and the availability of another microorganism that degrades the produced toxins (Jallow & Li, 2021)

The present findings demonstrated that 100% of the locally made malted millet-sesame-soy composite had detectable levels of TAF and AFB₁. Additionally, 100% of the composite analyzed was contaminated by TAF and AFB₁ above the EU maximum allowable limits of 0.10 µgkg⁻¹ and 0.40 µgkg⁻¹ for TAF and AFB₁, especially for processed baby foods made from cereals (Atongbiik *et al.*, 2017). Although, contamination levels for both TAF and AFB₁ of the raw materials were within the EU acceptable levels of 2 µgkg⁻¹ for AFB₁ and 4 µgkg⁻¹ for TAF for

processed foods for other human consumption and $8 \mu\text{g kg}^{-1}$ unprocessed foods (Atongbiik *et al.*, 2017). In terms of compliance with the EAC and USFDA standards, $5 \mu\text{g kg}^{-1}$ and $10 \mu\text{g kg}^{-1}$ for AFB₁ and TAF and $20 \mu\text{g kg}^{-1}$ for TAF in food and feeds (Ali *et al.*, 2020), the studied product met the standard. Nevertheless, due to frequent consumption, children consuming this product could be exposed to a high load of aflatoxin.

8.3 Exposure of weaning children resulting from consuming freshly processed and processed stored MMSSC across the wet and dry seasons

Globally, the most exposed population to the danger of aflatoxin contamination is children because of their increased special cereal intake for each kg/body weight (Adetunji & Atanda, 2017). The present study showed that exposure to both TAF and AFB₁ was higher when stored products were consumed than freshly processed products in both seasons. However, there was no difference in the TAF and AFB₁ EDI resulting from consuming freshly processed and stored processed MMSSC in both seasons (Table 9). This could be due to the short storage period (one month used in the current study) that limits the proliferation of toxigenic fungi and results in toxin production.

The study also demonstrated that when the processed and stored products were consumed across wet and dry seasons, the most exposed age was 5 months followed by 14 months. The least exposed group was 3 months. This is because at this age group, complementary composite is introduced and frequently taken, compared to the younger (3 months) and older ones who may depend on breast milk and family food. Contrarily, Bashiry *et al.* (2021) found the most exposed age to be 6-12 months with an average EDI of $153 \pm 0.13 \mu\text{g kg}^{-1}\text{bw day}^{-1}$. This was higher than the overall level of exposure in the current study. Eshete *et al.* (2021) reported that in Nigeria, children below 12 months were less exposed than older children. This could have been due to a myriad of factors like the quantity and frequency of consumption of contaminated weaning foods given to this age group in addition to proper hygiene care rendered to this age group to avoid cross-contamination and consequent exposure. Several other studies have also reported levels higher than PMTDI. For instance, an average exposure level of $68 \text{ ng kg}^{-1}\text{bw day}^{-1}$ due to consumption was reported (Sirma *et al.*, 2018). Another finding also indicates higher aflatoxin exposure levels (763.6 to $1901.1 \text{ ng kg}^{-1}\text{bw day}^{-1}$) of children who consumed stored maize grains (Zhang *et al.*, 2020). Similarly, in Tanzania, a high aflatoxin exposure level of $1,337 \pm 392.5 \text{ ng kg}^{-1}\text{bw day}^{-1}$ was registered (Herrera *et al.*, 2019).

Exposure levels in these studies were above the aflatoxin PMTDI of $1.0 \text{ ng kg}^{-1}\text{bw day}^{-1}$ and $0.4 \text{ ng kg}^{-1} \text{ bwday}^{-1}$ for children not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV (Boni *et al.*, 2021). Aflatoxin exposure levels in other continents apart from Africa have also been explored and reported low contamination (0.93 to $2.45 \text{ ngkg}^{-1}\text{bwday}^{-1}$) in Europe and $2.7 \text{ ng kg}^{-1}\text{bwday}^{-1}$ in the USA (Zhang *et al.*, 2020). This variation could be because countries with high levels of exposure may rely much on local production of complementary food compared to those with low levels of exposure who depend on industrialized exported products whose production and processing strictly adhere to recommended safety practices. Literature affirms that locally-produced foods had reasonably high levels of aflatoxin compared to the imported ones possibly due to contaminants in the raw materials and along the chain due to negligence for safety among households (Alamu *et al.*, 2018; Echodu *et al.*, 2019). This call for urgent action to ensure that locally made complementary foods are free from aflatoxin contamination hence minimizing exposing children to aflatoxins, owing to its health implications.

Exposure to both TAF and AFB₁ was higher in the stored products than in freshly processed products in both seasons. No significant difference was observed in both the TAF and AFB₁ EDI resulting from the consumption of the freshly processed and the stored processed MMSSC, in both seasons ($P > 0.05$). There was a weak negative correlation between EDI for total and AFB₁ and age in both the freshly processed and stored products. However, these were not significant in both seasons. This finding suggests that Children's exposure to total and AFB₁ are independent of the season.

8.4 Socio-demographic characteristics of the caregivers

Generally, the results indicated that less than half (45.7%) of the caregivers introduce complementary feeding at 6 months as recommended by the World Health Organization (WHO, 2003) and the Ministry of Health (2009). However, the remaining proportion of caregivers introduced complementary foods either before or after 6 months. Similar to the finding of this study, Aber *et al.* (2018) noted low adherence to complementary feeding guidelines in a rural setting in Uganda. As a result, there is a need to discourage practices that hinder adherence to recommended practices because, before 6 months of age, the child's digestive and immune systems are still underdeveloped to digest complimentary food and overcome any food-related

illnesses (Rao *et al.*, 2011). Also, the late introduction of complementary foods after 6 months is equally inappropriate because of the energy gap since breast milk alone even from a well-nourished mother cannot support a child's optimal growth. This is because the metabolizing energy intake of breastmilk is 2.2–2.4MJ/day and, the mean energy requirement for babies at 6 months is approximately 2.6–2.7MJ/day (632–649Kcal/ day) (Reilly & Wells, 2005). However, Prescott *et al.* (2008) argued that there is a growing need to revise when weaning foods need to be introduced. According to this argument, complementary food should be introduced at 4 months due to various needs while maintaining breastfeeding for a minimum of 6 months. However, as pointed out by Prescott and Tang (2005), there is a conflict between introducing complementary feeding from 4 to 6 months by some allergy prevention guidelines practiced in Australia and the WHO recommendation of introducing complementary feeding until 6 months. These conflicting ideas need to be resolved through extensive research to harmonize the age of introduction of complementary feeding to realize good nutritional outcomes from complementary feeding.

As indicated in chapter six of this report, the most exposed age was 5 months and the least exposed group was 3 months. This could be because this age group complementary composite is introduced and frequently taken, compared to the younger (3 months) and older ones who may mostly depend on breast milk and family food respectively (Achiro *et al.*, 2023b). Therefore, while harmonizing when to introduce complementary feeding, children's exposure to food contaminants needs to be taken into consideration to realize good nutritional outcomes from complementary feeding.

The current study revealed that nearly all of the consumed food in the studied household was grown; only a small portion was purchased (Table 13). The finding suggests that the foods consumed in this community are relatively safe since this study further revealed that the studied community perceived that their grown produce is relatively safe compared to what is purchased. This may apply to poor households who might not buy relatively safe quality agricultural produce sold in the market. The implication is that, in case of crop failure, this community may suffer food insecurity coupled with the utilization of unsafe food. In agreement with our findings, other studies revealed that rural areas in low- and middle-income countries extensively depend on their grown produce for household consumption (Adeyeye *et al.*, 2022; Ejeromedoghene *et al.*, 2020; Gebre & Rahut, 2021). This scenario is important as the current study revealed that poor households can only have quality and safety when grown. Therefore, the government

should strengthen the capacity of households to grow their food and diversify their livelihood activities to enhance resilience in case of crop failure. Chapter four of this report indicates low contamination by *Aspergillus spp* (less than a quarter of the total samples).

Concerning gender roles and responsibilities, caregivers reported that in nearly half of the male-headed households, decision-making on income usage and responsibility for household needs was done by the male gender (Table 13). This could have been because the female gender earns low income due to their unpaid care and domestic work (Guloba *et al.*, 2018). Women (94%) only decided on food acquisition and preparation. This was typical of rural African households where women chiefly decide on food since they are gatekeepers of food and nutrition security. And, mainly men decide on household income. Gender roles and responsibilities have changed over time. Initially, in an African setting, specifically in northern Uganda, the husband/family head was responsible for all the household decision-making and provision of household standard of living. Similarly, in Tanzania, Anderson *et al.* (2017) indicated that men held more power in terms of household decision-making and provision except in specialized areas. This may have negative implications on household food safety and food and nutrition security in case of crop failure since the gender that makes the critical decision on household food consumption may not be fully involved in household income usage. Thus, decisions on household income should be collectively made to ensure food safety and food and nutrition security as a whole.

8.5 Food safety knowledge of the caregivers

Though the majority of the respondents had sufficient food safety knowledge, many did adhere to food hygiene practices such as washing hands with soap and clean water before preparing and eating food or feeding the child, ensuring a clean working environment, cleaning utensils, covering food well to avoid cross-contamination, and keeping food in a clean dry place (Table 14). This could partly be explained by the low level of education attained by the caregivers. A similar scenario was reported by Webb and Morancie (2015) whereby most of the participants knew the importance of hygiene practices such as proper hand washing but only a small number practiced it wellfew (Figure 9). This reflected weakness could be due to time constraints and inadequate resources needed to perform the task reported by Brannon *et al.* (2009). Similar constraints were also echoed by the current study participants as barriers to good hygiene practices.

These obstacles likely contributed to 100% contamination by total and aflatoxin B1 along the processing points across the two seasons as described in chapter five of this report. Therefore, barriers to good food hygiene practices thus need to be addressed and social-demographic factors such as frequent food safety training should be encouraged to enhance knowledge, Ncube *et al.* (2020) acknowledged that hygiene practices such as hand washing need validation by observation because a significant deviation in the observation checklist from the reported practice was discovered. Da Cunha *et al.* (2019) affirmed that observed practice is influenced by knowledge and attitude and is closer to actual practice. This was limited in our study, so future studies should incorporate the two approaches. Kunadu *et al.* (2016) indicated that most of the participants in their study reported washing their hands with antibacterial soap after visiting a toilet and before food preparation to prevent food-borne illnesses. This deviation could be due to the formal environment (restaurant) where their study was conducted unlike the typically rural households considered in this study. This calls for documentation of recommended codes of practices as general principles of food hygiene and good manufacturing practices in a household environment. This study showed that although more than half of the caregivers were aware of the food safety authority, the same proportion of the caregivers did not know their roles as the authority in ensuring food safety. Therefore, policymakers should reinforce functional food safety departments at all levels.

8.6 Caregivers' food safety attitude

Largely, caregivers had a positive attitude toward food safety as a concern at home. More than half of caregivers had confidence in the food safety authority. The caregivers had a positive attitude toward the safety of the homegrown and purchased raw materials used for making malted millet–sesame–soy composite (Table 15). Although the FGDs pointed out that “as long as you have money, you can always buy good quality safe food, sometimes even better than own grown”. Safari *et al.* (2017) attested to this statement that food safety attitudes could not only be influenced culturally/socially but also economically. This study found that nearly three-quarters (70%) of the caregivers were willing to pay extra prices for safe raw materials for making malted-millet-sesame soy composite. Only less than a quarter of them were not willing because of financial constraints. More than a third of the caregivers have a negative attitude toward the safety of purchased raw materials for making malted millet–sesame–soy composite. This could be because quality raw materials are usually highly priced compared to poor-quality ones. However, the willingness to pay an extra price is dependent on the economic situation of the

household. Therefore, households need to be economically empowered to utilize safe foods. The finding from this study replicated Meysenburg *et al.* (2014) findings indicating that food handlers reported that “the only thing you can control is what you have in your house”. The caregivers’ positive attitude needs to be translated into good food hygiene practices by breaking the barriers such as economic constraints and caregivers’ workload and addressing socio-economic factors and the decision on household income that influence attitude. The male gender’s decision on household income positively influences attitude. This could be because they own more resources and earn more money. The female gender thus needs to be empowered to produce safe foods not only for home consumption but to access both the local and international market by meeting the standard limits for contaminants such as aflatoxins as elaborately explained in chapter five, sub-section 5.4. This is because caregivers who are female are doorkeepers of food safety as revealed by this study.

8.7 Food Safety self-reported practices

In terms of postharvest handling, the majority of caregivers did not dry their agricultural produce on the bare ground since they had personal or shared/borrowed tarpaulin for drying agricultural produce. This finding is reflected in chapter four, sub-section 6.4 and chapter 5, sub-section 5.4 of this thesis whereby overall results showed low contamination due to careful drying of raw materials. Tarpaulin ownership was limited because they were quite expensive for a rural household setting. After drying agricultural products, most households did not store their produce on raised ground predisposing them to mold growth, rodents, and insect infestations that compromise food quality and safety (Table 16). This practice could have resulted into cross contamination thus hindering clear cut determination of contamination levels across the processing points as observed in sub-sections 4.4 and 5.4. Only a small percentage of the households stored the produce on raised ground (Table 16). Thus, there is a need to sensitize the community about the danger of improper postharvest management and training on best practices.

Most of the agricultural produce was stored by stockpiling on polypropylene sacks and baskets in grass-thatched houses that were used for sleeping by the household members due to fear of thieves as reported by the community (Table of 16). This practice should be discouraged because it predisposes the produce to cross-contamination, so the local authority in rural communities should ensure social security. Our finding is comparable to that of Viola (2017), who found three- quarters of the products are stored in rooms at home after harvesting for fear of thieves.

Environmental conditions of the households are improper for storage of produce. The uncontrolled conditions may encourage proliferation of mycotoxin producing fungi. This may explain prevalence of both total aflatoxins and aflatoxin B₁ in all the samples including those that toxigenic *Aspergillus* species was not detected as shown in sub-section 5.4 and 4.4 whereby sample without detection of *Aspergillus* species. The caregivers revealed that only a smaller percentage of the households stored their agricultural produce in the local community store (Table 16). Community storage of the product should be encouraged because the facility offers better services that reduce contamination, thus ensuring food quality and safety. More than half of the caregivers reported storing their processed composite for 2 to 7 days. The caregivers reported that they were trained to keep the composite for a short duration to prevent the proliferation of spoilage agents. Other caregivers stored for 8 to 30 days and only very few stored for more than 30 days. These varying storage days are, however, challenging in case they need to access a wider market. This means irrespective of the source of raw materials, the exact shelf-life needs to be determined.

Concerning time and temperature control, nearly half of the caregivers took an estimated 15 to 30 min to cook MMSSC (the locally made complementary food) and only a smaller proportion did not know the time taken to cook the composite (Table 16). In this rural setting, cooking is done arbitrarily. The cooked porridge is usually stored for a range of 1 to 24 hr. The stored porridge is usually re-warmed by boiling and warming for a very few (2–3) minutes before taking it (Table 16). Warming by boiling should be encouraged because, in case of any germination of spores, the vegetative form can be destroyed by boiling. Although in case of toxin production resulting from contamination at pre-harvest and postharvest, mostly during improper storage of raw materials and flour, simple boiling may not eliminate hazards. Therefore, proper pre- and postharvest management should be encouraged.

Porridge should be properly stored to eliminate hazards. Warming for very few (2–3) minutes, however, should be discouraged as it does not destroy the vegetative form of the microorganism but provide favorable environment for their proliferation. A greater percentage of the caregivers does not warm the leftover porridge because they store the leftover porridge in a vacuum flask to keep it hot until use (Table 16). Several studies have reported an enormous challenge of temperature and time control to many food handlers (Wodnik *et al.*, 2018; Kwol *et al.*, 2020; Ncube *et al.*, 2020; da Vitória *et al.*, 2021). Frequent trainings should be offered to food handlers

regarding temperature control to avoid temperature abuse that can lead to proliferation of microbial contaminants that may lead to food-borne diseases.

8.8 Association of caregivers' characteristics with food safety knowledge and attitude

Currently, results from multivariate binary logistic regression models substantiated that many of the socio-demographic characteristics could not give a significant association between sufficient food safety knowledge and a positive attitude toward food safety. However, gender role in decision-making on household income was a significant predictor for sufficient knowledge and positive attitude. The model further revealed that households with the male gender taking the lead in decision-making on household income were associated with twofold increase in the level of food safety knowledge, which could have been because men earn more income than women and so enabling them to support food safety programs could enhance the household's food safety knowledge (Guloba *et al.*, 2018). But since the study revealed that mainly women decide on food acquisition and preparation, they should be empowered and participate equally in household income decision-making. Conversely, households with the male gender taking the lead in decision making on household income are associated with a virtually twofold decrease in a positive attitude toward food safety. The decrease in positive attitude could have been because most caregivers play a lesser role in household income. And so they cannot decide on appropriate food safety measures that require finances.

Furthermore, the frequency of food safety training and households with children who have ever suffered from food-borne illness significantly predicted sufficient food safety knowledge. Whereby, caregivers who frequently attended food safety training had more than half an increase in food safety knowledge than their counterparts who only attend once. Additionally, households with children who had ever suffered from food-borne illness had a nearly one-fold increase in food safety knowledge than those who had never. Equally, households with children aged 1 to 17 years had significantly sufficient food safety knowledge and positive attitude with more than half (0.58)-fold increase in food safety knowledge compared to those who had only younger children aged under 5 years. Also, a quarter (0.254)-fold increase in a positive attitude toward food safety was observed in a household with children aged 1 to 17 years. The above predictors could plausibly be explained by the experience in food safety that a caregiver gained during training and over the years of child bearing which build their self efficacy and positive attitude (Meysenburg *et al.*, 2014). Nee and Sani (2011) and Abdul-Mutalib *et al.* (2012), however, found

deviant results, indicating an insignificant correlation between experience in food-handling operation and increased food safety knowledge and attitude. In this study, however, a significant positive correlation between food safety knowledge and attitude was observed. Literature revealed that good predictors of safe food- handling practices are sufficient food safety knowledge and a positive attitude (Kunadu *et al.*, 2016; Al-Kandari *et al.*, 2019). However, according to Kunadu *et al.*(2016); Nassanga *et al.*(2018), and Ncube *et al.*(2020), knowledge may not necessarily be translated into appropriate practices, and self-reported practices may not be the actual practice. Therefore, self-reported practices need to be verified with an observation checklist.

Generally, association of frequency of trainings and experience of the caregivers regarding food safety could partly explain why there was less contamination along the processing points. Studies have revealed that good predictors of safe food- handling practices are sufficient food safety knowledge and a positive attitude (Kunadu *et al.*, 2016; Al-Kandari *et al.*, 2019; (Ncube *et al.*, 2020). However, other literature reported that, knowledge may not necessarily be translated into appropriate practices, and self-reported practices may not be what is actually done(Kunadu *et al.*, 2016; Nassanga *et al.*, 2018; Ncube *et al.*, 2020). Therefore, self-reported practices need to be verified with an observation checklist.

8.9 Themes generated from FDG based on the health belief model

8.9.1 Perceived susceptibility and severity

FGDs based on health belief model constructs as shown in Table 18 revealed that children were more susceptible to food-borne illness compared to other age groups in the household possibly due to their underdeveloped immune systems (Agostoni *et al.*, 2009; Du Plessis *et al.*, 2013). Chapter six (6.4) strongly support this finding by showing that different age groups were exposed to both total and aflatoxin B₁. The FGDs further revealed that adults generally suffered stomach aches and diarrhea but children suffered vomiting, loss of appetite, and fever in addition to stomach aches and diarrhea. These findings are strongly supported by Meysenburg *et al.* (2014), indicating that children and the elderly were more susceptible to food-borne illness due to their weaker immune systems.

Furthermore, the caregivers believed that their household members have ever suffered from symptoms such as vomiting, stomach ache, and diarrhea resulting from consuming contaminated food. These conditions were reported to last from 1day to more than 14days in some cases. This implied that the caregivers knew some of the consequences of eating contaminated foods. However, findings presented in Table 18 indicate that the caregivers had a low perception of susceptibility and severity to food-borne illnesses. This could have resulted from inadequate and late diagnosis of food-borne illnesses and lack of experience with food-borne illnesses. Another reason includes ignorance of the serious health consequences of food contamination such as stunting in children and a more life-threatening condition such as liver cancer and hemolytic uremic syndrome (Buzby, 2001; Meysenburg *et al.*, 2014). This could have resulted from the respondents' failure to recognize that they are susceptible to food-borne illness and the high degree of severity of infection. Caregivers' low perceptions of their susceptibility to FBI and its severity have been a result of not acknowledging the reality due to fear of undesired outcomes. Thus, this opportunistic bias may lead to invalid conclusions and this can be overcome by rigorous methods of data collection which may be costly (DeCoster *et al.*, 2015). Among the children who had the above symptoms, the majority was treated and got healed without treatment. Treatment was obtained from the medical unit and others used self-medication.

In this particular study, caregivers did not understand the dangers of food contamination as posed by unhealthy food handlers. Similar findings by (Al-Kandari *et al.*, 2019; Tuglo *et al.*, 2021) showed that food handlers lacked adequate knowledge of the risks caused by unhealthy primary food preparers. Although factors such as food contamination due to poor agricultural practice and cross-food contamination from the food handlers who had illness as diarrhea, cough, and flu have been reported to increase the risk of food-borne illnesses (Zyoud *et al.*, 2019; Al-Kandari *et al.*, 2019; (Ncube *et al.*, 2020) training on safe foods should focus on the unknown threats to caregivers.

8.9.2 Perceived benefits

Caregivers acknowledged that the overall goal of safety practices is to prevent children and other household members from getting food-borne illnesses. The food safety practices pointed out by the members included: hand washing after visiting the latrine, before preparing food, and before feeding the child, not drying agricultural produce on bare ground, cleaning utensils before preparing food, covering left-over food, in a saucepan, jug, or flask, and re-warming leftover

foods. Although these food safety practices were known by caregivers, many other food hygiene practices were not adhered to. These include hand-washing with detergent and clean water, washing hands before preparing food and feeding the child, not drying agricultural produce on the bare ground, storing the produce on raised ground, and reheating leftover food. These conflicting food safety practices are expected because according to the health belief model, the lower the perceived susceptibility and severity, the lesser the perceived benefits of taking action (food safety practices) and vice versa (Pourtaheri *et al.*, 2018). The low perceived susceptibility observed could have influenced the findings presented in chapter 5 (5.3 and 5.4) of this dissertation whereby, all the samples were contaminated within the acceptable east African community standard. But, not conformance to the EU standard and the exposure levels were above the acceptable limits. This calls for sensitization about the exposure risk of consuming contaminated food.

8.9.3 Perceived barriers

Caregivers believed barriers to practicing food safety include inadequate sanitation facilities such as pit latrines and drying equipment, especially Tarpaulin/polyethylene bags. Concerning the identified barrier, more than half of the households share a pit latrine, and also some caregivers dry their agricultural produce on the bare ground. Furthermore, women's workload was considered a big challenge. Caregivers reported that the women get exhausted and take shortcuts to prepare food and feed the hungry crying children. This finding is comparable to that of Meysenburg *et al.* (2014) showing no difference from employees in a formal setting (restaurants) who fail to adhere to set food safety practices due to time constraints and limited resources (Webb & Morancie, 2015).

Another barrier stated by the caregivers was inadequate knowledge to prepare children's food. For example, the time needed to cook a safe porridge, whereby they just prepare according to convenience, "When the bubbles in the porridge clear and the good aroma starts, I will know the porridge is ready. But some times when the child starts crying, nobody can wait for the above features." In support of our findings, most participants in various studies did not know the critical temperatures and time requirement in the control of microorganisms (Kwol *et al.*, 2020; Webb & Morancie, 2015). In agreement with our findings, (Kinabo *et al.*, 2017) found that a lack of adherence to food safety guidelines could be due to time constraints and limited resources; whereas Anon (2003) emphasizes that improper time and temperature control is one of the

causes of food-borne diseases. To promote food safety within rural communities, there is a need for intervention to address the highlighted barriers to food hygiene practices.

8.9.4 Self-efficacy

High self-efficacy refers to the ability of a person to competently execute the action to avert health threats such as food-borne illness (Rosenstock *et al.*, 1988). The study revealed caregivers' high self-efficacy in food safety practices. For instance, most of the caregivers have safe water source and have or share a pit latrine. Virtually all of the caretakers voiced washing their hands with detergent and clean water after visiting the pit latrine.

Many of the caregivers were willing to pay an extra price for safe raw materials for locally making malted millet–sesame–soy composite. This finding indicates that as long as a household is economically stable, it will be in a position to acquire safe food. But those who cannot afford costly safe food will always go for cheap unsafe food. This phenomenon was explained by Safari *et al.* (2017) as food safety knowledge, attitude, and practices can be influenced culturally, socially, and economically, such as food prices among others. This is parallel to the finding by Meysenburget *al.* (2014), who indicated that “the only thing you can control is what you have in your house.”

8.9.5 Cues to action

Some cues to action found in this study include passing important messages concerning food safety knowledge and practices from generation to generation (parents/caregivers to the children), acquiring formal rights from schools, and medical units where the mothers attend antenatal care. However, knowledge may be insufficient for behavior change; therefore, food safety programs should not only be designed to increase knowledge about food safety but also to emphasize the significance of adopting proper food hygiene practices. In the current study, caregivers pointed out the following as perceived triggers for the recommended food safety actions: posters in hospitals and clinics showing a person with diarrhea and vomiting resulting from consumption of unsafe food due to lack of adherence to proper food hygiene practices. The posters should be put appropriately in medical units where expectant mothers and community members usually go to seek medical attention and the pictures should be brightly colored and the information translated into the local languages. Social marketing communication strategy with

frightening media information about the dangers of consuming unsafe food should be aired. This can be passed on radios and television, especially experts' talk shows on the dangers of producing and consuming contaminated foods.

8.10 The influence of status of KAP of the caregivers of weaning children and the level of total aflatoxin and aflatoxin B₁ contaminations and related exposures.

The influence of caregivers' competency in terms of KAP on the level of total aflatoxin and aflatoxin B₁ contaminations and related exposures varied in the freshly processed and processed stored MMSSC across the wet and dry seasons. As observed in chapter six, knowledge and attitude were significantly correlated and literature points that they influence practice (Ahmed *et al.*, 2024). Although Achiro *et al.* (2023) found that sufficient knowledge may not necessarily reflect recommended food hygiene practice. Specifically, total aflatoxin contaminations varied along the processing points across the two seasons with the highest contamination with total aflatoxin observed in households with insufficient food safety knowledge. This could have been because knowledge influences practice which might have led to high contamination and increased aflatoxin levels in households with insufficient food safety knowledge. In support to these findings, da Cunha *et al.* (2019) affirmed that knowledge influences observed practice. However, literatures show that knowledge may not be translated in good practice (Nassanga *et al.* 2018; Ncube 2020). Specifically, the highest concentration with total aflatoxin was observed in one of the raw materials, soybean and processed stored MMSSC in households with insufficient food safety knowledge (Table 19). About other studies, this may reflect the fact that soybean is an oil seed and stored processed MMSSC are prone to aflatoxin contamination (Rajasekaran *et al.*, 2017). Sufficient knowledge in handling during processing and storage is thus required to control aflatoxin contamination.

As well, the high contaminations with total aflatoxin in processed stored MMSSC may also indicate the importance of sufficient knowledge to improve storage conditions to control the proliferation of aflatoxigenic fungi and related toxins. These findings agree with other findings which showed that storage conditions play an important role in the contamination of stored products (Mannaa & Kim, 2017; Udomkun *et al.*, 2018; Omotayo *et al.*, 2019; Ojuri *et al.*, 2019; Ncube *et al.*, 2020; Hoffmans *et al.*, 2022). Contrariwise, in households with sufficient knowledge, the highest concentration with total aflatoxin was observed in sesame. This

observation may show that control of aflatoxin in sesame may need possibly specific knowledge when handling it.

In addition, the literature points out that sufficient knowledge may not reflect actual practice and therefore knowledge should be verified with actual practice through observation, not self-reported practice (Ncube *et al.*, 2020). However, knowledge was only significantly correlated with the concentration of total aflatoxin in the processed stored MMSSC (Table 19). This may be an indication that to control aflatoxin contamination in the wet season, food handlers should have sufficient food safety knowledge regarding storage of products. In agreement with this, studies have shown that during storage in the wet season, aflatoxigenic fungi are likely to proliferate and produce aflatoxin due to favourable conditions during storage such as optimum humidity, temperature and water activity and insect infestation among others (Casquete *et al.*, 2017; Udomkun *et al.*, 2018; Massomo, 2020; Adeyeye *et al.*, 2022).

During the dry season, the highest contamination with total aflatoxin was observed in households with insufficient food safety knowledge. Whereas, along the processing points, the total aflatoxin contamination in sesame nearly doubled all others and it was the only variable that was significantly related with knowledge (Table 19). The high total aflatoxin contamination of sesame may be due to oil in sesame that promotes the proliferation of aflatoxigenic fungi and related toxins. This is evidenced by literature that showed that lipid content positively promotes the growth of aflatoxigenic fungi and related toxins. But, interplay of factors may influence the proliferation of aflatoxigenic fungi (Liu *et al.*, 2016, Rajasekaran *et al.*, 2017). This is affirmed by other studies that factors such as environmental conditions and grain/ cereal structure may also contribute to the colonization and eventual proliferation, (Seetha *et al.*, 2017; Norlia *et al.*, 2019; Adeyeye, 2020; Akello *et al.*, 2021). Therefore, in the dry season food safety knowledge in handling sesame should be emphasized to control contamination of oily cereals like sesame by aflatoxigenic fungi and related toxins, (Liu *et al.*, 2016).

The influence of status of food safety knowledge on the level of aflatoxin B₁ concentration along the processing points across the wet and dry seasons is shown in Table 20. In both the wet and dry seasons, households with insufficient food safety knowledge had higher contamination with aflatoxin B₁ than those with sufficient food safety knowledge except for sesame and freshly processed and stored MMSSC in the wet and dry seasons respectively. This finding suggests that the interaction of factors such as favourable environmental conditions, and grain/cereal structure

among others may contribute to aflatoxin contamination in both the wet and dry seasons. Therefore, specific attention/awareness should be paid to the raw materials such as sesame in the wet season and freshly processed MMSSC and stored MMSSC in the dry season respectively. This is attested by Achiro *et al.* who found that high contamination occurred in sesame in the wet season (Achiro *et al.*; 2023). In agreement with the findings, other scholars found that oily seeds/grains are more prone to aflatoxin contamination, especially in the wet season due availability of optimal proliferation conditions (Casquete *et al.*, 2017; Udomkun *et al.*, 2018; Massomo, 2020; Adeyeye *et al.*, 2022) Though Akello *et al.* argued that small gains and cereals are less prone to aflatoxin contamination due to the decreased surface for microbial colonization, (Akello *et al.* 2021).

Additionally, in the dry season, contamination of stored MMSSC by aflatoxin was more pronounced. This could have resulted from poor storage conditions (Casquete *et al.*, 2017). Nonetheless, food safety knowledge did not significantly associate with aflatoxin B₁ levels along the processing points across the wet and dry seasons.

Regarding attitude as shown in Table 21, the pairwise comparison of attitude and the contamination levels of total aflatoxin specified that in the wet season, households with positive attitude had nearly three quarter of the raw materials having higher total aflatoxin contamination levels compared to households with negative food safety knowledge. This may indicate that contamination by aflatoxin may not be influenced by attitude towards food safety although literature showed that attitude may directly influence practices that influence food contamination (da Cunha *et al.*, 2019; Kunaduet *et al.*, 2016). Contrarily in the dry season, all the raw materials and freshly processed MMSSC had higher total aflatoxin levels in households with negative attitudes while stored MMSSC had nearly equal levels of total aflatoxin contamination in both households with positive and negative attitudes. The results revealed that attitude may influence contamination of the raw material and freshly processed MMSSC as affirmed by literature (Ncube *et al.*, 2023). However, no significant linkage was observed between attitude and levels of total aflatoxin concentrations along the processing points across the wet and dry season was observed.

Regarding Aflatoxin B₁ in both the wet and dry seasons, as indicated in Table 22 households with negative food safety attitudes mainly had higher contamination with aflatoxin B₁ than those with positive food safety attitudes except for soybeans and freshly processed MMSSC and stored

products in the wet and dry seasons respectively. This finding reveals that attitude may influence aflatoxin B₁ contamination except for soybeans and freshly processed MMSSC and stored products that may not be influenced by attitude. Nonetheless, attitude towards food safety was not significantly associated with aflatoxin B₁ levels along the processing points in the wet season. In the dry season, the contamination levels of aflatoxin B₁ in soybean were significantly related with attitude, ($P = 0.007$). This may be explained by the fact that an array of factors has an influence on aflatoxin contamination not necessarily attitude (Liu *et al.*, 2016; Mannaa & Kim, 2017; Aron *et al.*, 2017; Bhardwaj *et al.*, 2022). The significant linkage between aflatoxin contamination levels in soybeans and attitude may reveal that during the dry season, specific attention should be given to soybeans to control contamination.

The influence of food hygiene practices on contamination levels of total aflatoxin along the processing points across the wet and dry seasons as shown in Table 23. It indicates that in the wet season, higher levels of total aflatoxin contaminations were registered in households that did not practice food hygiene compared to those that practice food hygiene except for freshly processed MMSSC that had higher levels of total aflatoxin concentration observed in household who practice to food hygiene practices. This finding is in agreement with other scholars indicating that practicing food hygiene has an influence on contamination levels of total aflatoxins in the wet season due to favorable climatic conditions (Mannaa & Kim, 2017; Jallow *et al.*, 2021; Lee *et al.*, 2022; Bhardwaj *et al.*, 2022). During the dry season, higher concentrations of total aflatoxin were observed in raw materials in households that practiced food hygiene. This could be because of the inaccuracy of reporting by respondents whereby self-reported practice may not be the actual practice as affirmed by earlier studies, (Nassanga *et al.*, 2018; Ncube *et al.*, 2020). And so, self-reported practices need to be verified by observation. Contrarily, in the processed MMSSC, higher levels of total aflatoxin contamination were observed in households that did not practice food hygiene. However, food hygiene practice did not significantly relate with the level of total aflatoxin along the processing points across the wet and dry season.

Furthermore, food hygiene practices of the caregivers and the contamination levels of aflatoxins B₁ along the processing points across the wet and dry seasons were compared as shown in Table 24. In the wet season, all the households that did not practice food hygiene had higher contamination with aflatoxin B₁. According to this finding, adherence to food hygiene practices and good postharvest practices may be one of the control strategies of total aflatoxin control. In

line with this finding authors have pointed out that food hygiene practices and postharvest practices have a linkage to food safety and control of food contamination in particular (Aron *et al.*, 2017; Echodu *et al.*, 2019 ; Ojuri *et al.*, 2019; Mollay *et al.*, 2021; Hoffmans *et al.*, 2022; Namubiru *et al.*, 2022). In the dry season, most households who did not practice food hygiene had higher contamination with aflatoxin B₁ than those who practice food hygiene. Nonetheless, in the wet season, food hygiene practice did not significantly relate with the contamination levels of aflatoxin B₁ along the processing points but in the dry season, this lack of relatedness may indicate that the reported practice may not be that actual practices as reported by Nucbeet *et al.*, 2020; Mshida *et al.*, 2020). Therefore, such self-reported practices need to be verified with observation in order to make concrete conclusions. Conversely, the concentration levels of aflatoxin B₁ in soybeans were significantly related with food hygiene practices in the dry season, (Table 25). This may point out that during the dry season, special attention should be paid to soybeans in order to avoid their contamination with aflatoxin B₁.

As shown in Table 25, the influence of status of food safety knowledge on exposure to total aflatoxin and aflatoxin B₁ in the freshly processed and stored MMSSC across the wet and dry seasons shows that in both the wet and dry seasons, households with sufficient food safety knowledge have low exposure levels to total aflatoxin. This could be explained by the fact that knowledge is closely associated with the practice (Namubiru *et al.*, 2022). Accordingly, authors have linked good food hygiene practices to low aflatoxin contamination (Udomkun *et al.*, 2018). Other authors argued that knowledge may not necessarily be translated into good practice (Nassanga *et al.*, 2018, Ncube *et al* 2020; Achiro *et al* 2023). Thus the gap that leads to the non-translation of knowledge into practice need to be bridged, (Yimenu *et al.*, 2022). In the wet season, households that consumed processed stored MMSSC had higher exposure to total aflatoxin (Table 25) than those that consumed freshly processed MMSSC (Table 25). This could be explained by the fact that contamination can occur during storage as a result of many factors including cross contamination and poor storage conditions among others. Exposure to total aflatoxin in the wet season was significantly associated with knowledge in households that consumed freshly processed and processed stored MMSSC (Table 25) respectively. There is a need for sufficient food safety knowledge during processing and storage to address the challenge of exposure to aflatoxin in the dry season. Inversely in the dry season, equal exposure to total aflatoxin was observed in households that consumed freshly processed products processed and processed stored products (Table 25) respectively. Exposure to total aflatoxin in households that

consumed freshly processed and processed stored MMSSC were both significantly influenced by food safety knowledge (Table 25) respectively.

In contrast, in both the wet and dry seasons, households with insufficient food safety knowledge were more exposed to aflatoxin B₁ than those with sufficient food safety knowledge. However, knowledge only influenced exposure to aflatoxin B₁ in households that consumed freshly processed MMSSC in the dry season (Table 25). Therefore, addressing exposure may require sufficient knowledge both during processing and storage. This could be that specific knowledge may be required to control aflatoxin B₁. According to scholars, knowledge may not be translated into practice leading to non-food hygiene practices and proper postharvest practices that may result in food contamination by aflatoxin B₁ and subsequent exposure. However, knowledge was only related with exposure to aflatoxin B₁ in households that consumed freshly processed MMSSC in the dry season (Table 25). Thus, to control exposure to aflatoxin B₁ resulting from the consumption of freshly processed MMSSC in the dry season, food hygiene practices during processing need to be emphasized. Literature attests to this that contamination can occur during processing due to several factors including cross-contamination and the use of contaminated raw materials among others (Mannaa & Kim, 2017; Miklós *et al.*, 2020; Acur *et al.*, 2020).

Regarding attitude as indicated in Table 26, in both the wet and dry seasons, households with negative food safety attitudes were more exposed to total aflatoxin and aflatoxin B₁. Households that consumed the freshly processed and processed stored MMSSC were more exposed to total aflatoxin in the wet season. And, those who consumed processed stored MMSSC in the wet season were more exposed to aflatoxin B₁. However, in both seasons, exposure to total aflatoxin and aflatoxin B₁ was not influenced significantly by attitude towards food safety. This finding points out that, in both the wet and dry seasons, exposure to the total aflatoxin and aflatoxin B₁ may be insignificantly influenced by attitude. This is attested by many scholars who found that attitude may not influence practice and practice is closely linked to food contamination and their resultant exposure (Neme & Mohammed, 2017; Mahuku *et al.*, 2019).

Furthermore, the linkage between food hygiene practices and exposure to total aflatoxin and aflatoxin B₁ in Table 27 showed that in both the wet and dry seasons, higher exposure levels to total aflatoxin and aflatoxin B₁ were registered in households that did not practice food hygiene compared to those who practice food hygiene. This means that exposure to total aflatoxin and aflatoxin B₁ may be directly influenced by food hygiene practices as attested by scholars who

found that food hygiene practices may influence food contamination and the subsequent exposure to the related hazards (Seetha *et al.*, 2017; Aron *et al.*, 2017; Echodu *et al.*, 2019; Mollay *et al.*, 2021). Also, this study indicated that food hygiene practice only significantly influence exposure to total aflatoxin and aflatoxin B₁ in households that consumed freshly processed products in the wet season and those that consumed processed stored products in the dry season. Therefore, strategies to control exposure to total aflatoxin and aflatoxin B₁ may require specific practices along the processing points during the wet and dry seasons. This may be due to different factors that play a (Lahouar *et al.*, 2016; Rajasekaran *et al.*, 2017).

8.11 Conclusion

This study established that caregivers in Amuru and Nwoya districts of Uganda trained in the local production of malted millet–sesame–soy composite have sufficient food safety knowledge and a positive attitude. However, there was poor practice food hygiene among caregivers.

The identified socio-demographic factors that hinder adherence to food safety practices included inadequate sanitation facilities such as pit latrines and drying equipment, especially Tarpaulin/polyethylene bags. Furthermore, women's workload was considered a great hindrance. And another barrier was inadequate knowledge to prepare children's food.

Generally, this study indicated low contamination of locally made complementary food composite with *Aspergillus* species along the processing points across the wet and dry seasons. Highest contamination was observed in millet and stored processed products and least contamination was observed in freshly processed products and sesame.

The study revealed that *A. flavus* and *A. parasiticus* were more frequently detected in millet and soybean. *A. niger* on the other hand was more frequent in millet. This study has demonstrated that TAF and AFB₁ contamination in MMSSC varied along processing points from raw materials, stored, and freshly prepared composite in descending order. The variation in TAF and AFB₁ along the processing points was higher in the wet than dry season except in the stored composite. Estimated Daily intake for both total and AFB₁ exceeded the Codex PMTDI with no variation in the wet and dry seasons. Based on food safety standards, all the raw materials, processed, and stored products met the EU, US-FDA, and EAC standards limit for general consumption but were below EU standards for processed cereal baby food. Therefore, exposure

levels in this study were above the aflatoxin PMTDI of $1.0 \text{ ng kg}^{-1}\text{bw day}^{-1}$ and $0.4 \text{ ng kg}^{-1}\text{bw day}^{-1}$ for children who are not exposed to Hepatitis B Virus (HBV) and those that are infected with HBV respectively.

Further, food safety knowledge influenced total aflatoxin contamination in processed stored MMSSC and in the sesame in the wet season and in the freshly processed MMSSC in the dry season. Additionally, knowledge only influenced exposure to aflatoxin B₁ in households that consumed freshly processed MMSSC in the dry season. Food hygiene practice influenced exposure to total aflatoxin and aflatoxin B₁ in households that consumed freshly processed MMSSC in the wet season and those that consumed processed stored MMSSC in the dry season.

8.12 Recommendation

To address food safety issues at the rural domestic level where food is produced for both consumption and sales, food safety knowledge and attitude should be strengthened through delivering food safety education sustainably based on the health belief model. This will increase the perceived susceptibility and severity of the caregivers and other food handlers to food-borne illness while increasing their perceived benefits and reducing barriers to adhering to safe food-handling practices.

The government need to urgently strengthen medical centers to include food safety training during nutrition education training for mothers/caregivers attending antenatal care to eliminate food contamination from garden to folk through good agronomic practices, good pre- and postharvest management, and proper food preparation taking into consideration time and temperature control.

Furthermore, the government through the Uganda Nutrition Action Plan in collaboration with Ministry of Agriculture Animal industry and Fisheries (MAAIF) needs to enforce by-laws on postharvest handling, especially abolishing drying produce on the bare ground. Women's workload of domestic and garden work should be addressed by conducting research that provides appropriate agricultural technologies that save time.

Also, more effort is needed to reduce cross-contamination along the processing points of locally made MMSSC while prioritizing raw materials such as millet, soybean, and stored processed

products across the wet and dry seasons. This should be done alongside shelf-life determination of the locally made complementary food to empower the rural to access local and international markets.

Additionally, EAS for Aflatoxin should be formulated taking into consideration the acceptable limits for vulnerable populations such as children. As well, a concerted effort to ensure quality raw materials and proper storage is needed to reduce contamination and exposure of the children consuming locally made MMSSC to total aflatoxin and AFB₁ taking sesame and soybean as priority ingredients and formulated composite based on the season. Control of contamination of locally made complementary food composite by genus *Aspergillus* and their metabolites; the aflatoxin requires a multi-sectorial approach involving producers, regulating bodies, policymakers, food producers, and researchers.

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APPENDICES

Appendix 1: English version of Informed Consent Document

Study Title: Microbial Safety of Locally Formulated Millet-Sesame-Soy Complementary Food Composite in Nwoya and Amuru Districts of Northern Uganda

Principal Investigator: Eunice Achiro (PhD Student)

Introduction and consent

Good morning /afternoon, my name is..... (the interviewer mentions his/her name).
I am part of the team from Gulu University conducting research on the topic: ***Microbial Safety of Locally Formulated Millet-Sesame-Soy Complementary Food Composite in Amuru and Nwoya Districts in Northern Uganda.***

Study Purpose: The aim of this study is to ascertain the microbial safety of locally formulated Millet-Sesame-Soy complementary food composite among households in Amuru and Nwoya districts of northern Uganda so as to realize child good nutrition outcome from locally formulated complementary food composite. A baseline survey will be conducted to establish the level of knowledge, attitude and practice (KAP) the of caregivers of children aged 6-23 months regarding microbial safety of locally formulated complementary food composite in Amuru and Nwoya districts in northern Uganda. In addition, the consumption pattern of the millet-sesame-soy composite together with weight and height of the children aged (6-23months) will also be measured. Findings from this study shall make a contribution to risk management & policy formulation for control of food-borne infection and intoxication. Additionally, information on Knowledge, Attitude levels& practices of the caregivers towards achieving microbial safety of locally formulated complementary food composite will be useful in understanding the behaviors of the caregivers regarding the risk of unsafe food handling practices which is an essential element in the prevention of food-borne diseases.

Sponsors of the research project: The research project implementation is funded by African Development Bank Project under Gulu University staff development programme.

Procedures: Your participation in this study will entail giving information about your household in relation to knowledge, attitude, and practices regarding the microbial safety of Malted Millet-Sesame-Soy composite (MMSSC). Except for the control groups, participants will also give information on consumption patterns of MMSSC, provide MMSSC for microbial analysis and consent for their children's weight and height to be measured.

Participants in the study: You have been purposively selected to participate in this study because you are taking care of a child (children) aged 6 -23months. 184 caretakers of children (aged 6-23months) and 93 children (aged 6-23months) will participate in the study and about 1kg of MSSC will be bought from each participant. The whole process will take about 45 minutes to 1 hour.

Risks/discomforts: There is no anticipated risk of harm or discomfort that will arise from both you and your child participation in this study. Participants shall not incur the cost of MSSC to be sampled. But, there may be discomfort to the child/children during measuring the weight and height and the inconvenience of time spent.

Benefits:

Participants' time will be compensated and appreciated by giving each participant UGX. 5,000. Additionally, they will get feedback on findings from the study. Participants shall also benefit when favorable policies regarding food safety risk management are formulated subsequent to recommendations from this study.

Confidentiality: Information provided by the participants will be kept confidential and used for study purpose only. Confidential information will only be accessed by the principal investigator. Hard and soft copy of the collected information will be kept under restricted access. Identification information such as names and location will be coded to ensure anonymity.

Alternative: You are free not to participate in this study if you are not interested and you will not lose any benefit in case you do not participate.

Cost: There will not be any additional cost incurred as a result of participating in this study.

Questions: In case you have any question (s), further inquiry related to this study, you can contact the principal investigator, **Ms. Achiro Eunice** on telephone number **+256777364886** /email: **euchiro07@gmail.com**.

Statement of vulnerability: Participation in the research study is voluntary and you may join on your own free will. You have a right to withdraw from the study at any time without penalty.

If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson, Gulu University Research Ethics Committee, Dr. Gerald Obai on telephone number +256772305621; email: **lekobai@yahoo.com/lekobai@gmail.com**; or the Uganda National Council for Science and Technology, on plot 6 Kimera Road, Ntinda, Kampala on Tel +256414705500.

Statement of consent: Individual interview

The interviewer, ... has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw from the study at any time. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name of Participant.....

Signature/Thumbprint of participant.....Date.....

Witness name.....

Signature/Thumbprint.....Date.....

Name.....

Signature of interviewer.....Date.....

Statement of consent: Focus Group Discussion

The interviewer, ... has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw from the study at any time. I understand that by signing this form, I do not waive any of my legal rights

but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name of Participant.....

Signature/Thumbprint of participant.....Date.....

Witness name.....

Signature/Thumbprint.....Date.....

Name.....

Signature of interviewer.....Date.....

Appendix 2: Luo (Acholi) version of Informed Consent Document

Appendix 2: Luo (Acholi) version of Informed Consent Document

Dul ma Aryo: Yeyo ikom waraga man.

Wii lok: Bedo pee pa kwidi ma tino tinoi kom wit cam ma ki yubu ki gang ma calo kal, nyim ki soya iAmuru gin ki Nwoya district ikumalo me Uganda.

Ladit maloyo kikweda man: Eunice Achiro (Latin Kwan me PhD).

Nyute ki yeyo.

Icoonining/ iriinining, annyinga..... (Lamii lapeny Waco nyinge).

An atye but dul ma gu aa I Gulu university matye ka timo kikweda ikom: **bedo pee pa kwidi ma tino tino ikom wit cam ma ki yubui gang ma calo kal, niym ki soya iAmuru gin ki Nwoya district ikumalo me Uganda.**

Tyenlok me kwanman: miti me kwan man ocung ikom nyang I kombedo pee pa kwidi ma tinoikom wit cam ma ki yubui gang ma calo kal, niym ki soya i Amuru gin ki Nwoya district ikumalo me Uganda. Wek wa ngee adwogi me cam ma beco pii latin/lutino mu aa ki ikom cam ma ki rubu ki gang. Kikweda irwom ma ping bi time me nyang ikom ngec, cwiny ki tim ma lugwok lutino me dwe 6-23 ma lupe ki bedo pee pa kwidi ma tino ikom wit cam ma giyubu ki gang ma calo kal, niym ki soya iAmuru gin ki Nwoya district ikumalo me Uganda.Me medo iwii

meno kit ma ki camo kwede nyuka man, kacel ki pek ki bo pa lutino me dwe 6-23 ki bi pimo bene. Adwoki me kwan man ki bi tiyo kwede me yubucik ma neno lok kom peko ma ki nongo ikom cam ma pe opore. Ma pat ki menoni, ngec ikom nyang, cwiny ki timpa ogwok lutino ma lupe ki kit me gwoke ikom kwidi matino ma nonge inyuka ma kiyubu ki bel, nyim ki soya. Pire bi bedo tek me nyang kit ma lugwok lutino gi kwokwede ma lupe ki tim me gwoko cam ma pire tekiyoo me gwoke ikom twoo ma aa ikom cam.

Joo ma ocung inge ekikweda man: African development Bank projekii tee yub me dongo rwom pa lutic me Gulu University.

Kit ma yub man obi woto kwede: Bedo tye ma megii Kwan man bi miyo ngec malupe ki gang ma me gi iikomngec, cwiny ki tim malupe ki kit me gwoke ki kwidi ma tino tino matwero nonge iwit cam me nyuka. Ma pat ki joo ma pe ki pwonyo, joo ma bi bedo iyup man gi bi miyo ngec iyoo ma gin tiyo kwede ki bedo pee pa kwidi ma tino ikom wit cam ma ki yubu ki gang ma calo kal, niym ki soya (MSSC), Joo magi bi miyo cam ma kibikwedo kwidi matino karacel ki miyo yeyo me pimo pek ki bo pa lutino gi.

Joo ma obibedo I ki kweda man: Ki yero in kityenlok me bedo ikikweda man pyeni in itye ka gwoko Latin (lutino) ocakedwe 6-23. Ogwok lutino 184 (mwaka me dwe 6-23) karacel ki lutino 93 (mwaka me dwe 6-23) gi bi bedo I kikweda man bene, ki bi wilo bedo iyup man gi bi miyo ngec iyoo ma gin tiyo kwede ki bedo pee pa kwidi ma tino ikom wit cam ma gi yubu ki gang ma calo kal, niym ki soya (MMSSC) maromo kilo acel-acel ki bot joo ma bi bedoi yup man. Yup man bi tero ikine me dakika 45 me ooica waacel.

Peko/ ayela-yela: Petye pekomo me awano ma ki bi keto tek-teko nyooayela-yela ma ki bi keto ikom in ki latini ma obi obedoi yup man. Joo ma obi bedo I yup man pe gu bi culupii (MMSSC) ma ki bi tiyokwede I yup man. Ento ayela-ayela mo manok twero bedo tye ikom latin/lutino I cawa me pimo pekgi, ki bogi maromo keloagala-gala me cawa.

Ber pa yup man: Ngatacel bi nongo cilling 5000/= me pwoc ki cawa ma enobalo, ma pat ki meno bene ki bi nongo adwoki me ngo ma ki nongo ikomkikweda man, ngatacel ma obedo I yup man o bi nongo ber ne ka cik ma opore ma kwako gwoko cam ki yupu ma pore ki yeyo ikom tam ma lwakomiyo ma aa ikom ki kweda man.

Gwok ma opore: Ngec ma ngat acel omiyo ikom kikweda man ki bi gwoko maber ci ki bi tiyo kwede me Kwan kiken. Ngec ma pigi tego luki kweda ma dongo-dongo kiken ma obi bedo ki twero me nongo ne, ngec ma ki koko ni matye ikaratac ki I computer ki bi gwoko kama pe ki room nongo ne atar. Nying ki kabedo ma ki tiyo kwede me nongo ngec ki bi kano ne woko.

Yoo mukene: Itye agonya pe me bedoi Kwan man ka I peke ki miti iye ki bene pe I birwenyo gimo kiken pi bedo pe ni I yup man.

Wel: Wel pe bi mete ma calo adwogi pe me bedo ni ikikweda man.

Lapeny: Ka I nongo ni itye ki lapenymo, onyoo imito nongo ngec matut ikom kikweda man iromo goyo cim bot ladit ma loyo kikweda man Mego Achiro Eunice I nama cim man, +256777364886 onyo, email: euchiro07@gmail.com.

Yeyo me bedo ikwan man: Bedo ikwan man tyeicwiny me dyere, itye agonya me aa ki ikwan man Labongo kit pwod mokiken.

Ka itye ki lokmo makwoko twero mamegi ki bedo ikwan man, ci walegi me kupe ki won kom me Gulu University Research Ethics Committee, Dr. Gerald Obai I nama cim man, +256772305621 onyo email: lekobai@yahoo.com/lekobai@gmail.co monyo bot Uganda National Council pi Science ki Technology, i plot nama 6 Kimera road, Ntinda, Kampala inama cim +256414705500.

Dwon me yeyo: Lapeny pi ngata celacel.

La mii lapeny,.....otita kore ki kore ngo ma bi time, peki, ber ne ki twero pa an ma abibedo ikwan man. Anyiang maber ni mok ma mega me bedo ikwan man pe bi bedo ki a dwogi mokiken. Ikare me tic ki ngec man lok koma ki bi gwoko imung. Atye ki ngec ni aromo aa woko ki ikwan man cawa mo kiken. Anyang ni keto cinga I karatac man pe turu twero na ento bi nyuto ni a nongo ngec malupe ki kikweda man ma atye ka dyere me bedo iyee. Bwot karatac man ki bi mina.

Nying la gam la peny.....

Cing /din opany.....Nino dwe.....

Nying caden.....

Cing/din opany Nino dwe.....

Nying la mii lapeny

Cing /din opany Nino dwe.....

Dwon me yeyo: Lapeny pi Dul.

La mii lapeny,.....otita kore ki kore ngo ma bi time, peki, ber ne ki twero pa an ma abibedo ikwan man. Aniang maber nimok ma mega me bedo ikwan man pe bi bedo ki a dwogi mo kiken. Ikare me tic ki ngec man lok koma ki bi gwoko imung. Atye ki ngec ni aromo aa woko ki ikwan man cawa mo kiken. Anyang ni keto cinga I karatac man peturu twero na ento bi nyuto ni a nongo ngec malupe ki ki kweda man ma atye ka dyere me bedo iyee. Bwot karatac man ki bi mina.

Nying la gam la peny.....

Cing /din opany Nino dwe.....

Nying caden.....

Cing/din opany Nino dwe..... Nying la mii lapeny

Cing /din opany Nino dwe.....

Dong aromo cako miyo lapeny?

Appendix 3: Focus group discussion (FDG) guide

Microbial food safety knowledge

- 1) Awareness of Microbial contaminants and Control (Good Hygiene Practice and Good Manufacturing Practice).
- 2) Are you aware of food-borne pathogens and associated foods (Toxigenic fungi, *Bacillus cereus*, *Aspergillus flavus*, *Escherichia coli*, *Salmonella*, *Clostridium botulinum*, *Staphylococcus aureus*, *E.coli*, *Hepatitis A virus*, *Vibrio cholerae* and *Campylobacter jejuni* among others?)
- 3) How do you get Information on food safety?
- 4) Who is responsible for ensuring food safety?
- 5) Where do you think food infection/intoxication (food poisoning) occur?
(Restaurants, hotels, schools, marketplaces and homes among others)
- 6) Do you know any regulatory authority responsible for food safety?
- 7) If yes, do you have confidence in food safety authorities?
- 8) If yes, why?
- 9) If no, why?

10) Do you know the cooking temperature and time taken for porridge to be ready? (Demonstration activities will verify the information and determine the actual parameter that cannot be well explained by the participants).

11) Do you know the importance of temperature control during cooking (on microbial growth and survival and hence a valuable tool in keeping food safety at the household level)?

Attitude toward food safety

12) Is food safety an important issue at home? (Food safety benefits).

13) What is the distinct food safety hazard which is most feared? (whether biological/microbiological, chemical and others such as genetically modified foods). (Food safety threats).

14) Rate the most significant perceived food safety risk e.g. pesticides residues, Microbial contaminants etc.

15) What have you done to overcome the food safety threats? (core to action)

16) What prevents one from doing the above to overcome the food safety threats? (Barrier to action).

17) Do you feel that you have the capacity to do things the right way and even train others in order to ensure food safety at home (self-efficacy)?

Food hygiene handling practices

18) How do you prepare/process millet-sesame-soy composite?

19) How do you store the processed and cooked composite (temperature and water activity)?

20) What container do you use (Pot, covered bucket, uncovered bucket, poly-ethene bag, sack etc)?

21) How long do you store the cooked millet-sesame-soy composite (Porridge)?

22) How long do you store the processed composite (Flour)?

23) How long do you cook millet-sesame –soy composite porridge?

24) Have you ever served left porridge at room temperature (29–32°C) longer than 2 hours?

25) If yes, did you reheat?

26) How do you reheat leftovers? (Until Hot and steamy, sufficiently warm and do not reheat (eat cold).

Sanitation practices

27) How do you clean the kitchen?

28) How do you keep the flour?

- 29) How do you keep the prepared porridge?
- 30) What are the safety practices of preventing cross-contamination of foods (Hand washing, clean utensils, cleaning raw materials)?

THANK YOU FOR YOUR PARTICIPATION! YOU MAY ASK ANY QUESTION IF YOU HAVE ANY.

Appendix 4:Section A: Household Demographics & Socio-Economic Data

Instructions: Circle the number corresponding to the response that the caregiver gives. Record the appropriate response in areas where choices have not been given. All ‘Any other’ responses should be specified)

	QUESTION	RESPONSE	CHOICE
Q1	Household identification number		
Q2	Sex of household head	Male Female	1 2
Q3	Age of mother/ caregiver	 Years	
Q4	Marital status	Single Married Separated Widowed	1 2 3 4
Q5	Occupation of the household head (skip to A6 if the mother is the household head)	Not employed Employed (salaried) Small-scale trading Casual labor Any other(specify)	1 2 3 4 5
Q6	Occupation of mother	Not employed/house wife Employed (salaried) Small scale trading Casual labor Any other (specify)	1 2 3 4 5
Q7	Education level of the household head	No formal education	1

		Primary	2
		Secondary	3
		Tertiary	4
Q8	The education level of the caregiver	No formal education	1
		Primary	2
		Secondary	3
		Tertiary	4
Q9	Household size (people who usually eat from the same pot/house)	 people	
Q10	How many children do you have? (1-17 years)	children	
Q11	How many children are below 5 years of age?	children	
Q12	How many children are below two years?	children	
Q13	The main source of family income	Formal employment	1
		Casual labor	2
		Small-scale business	3
		Sale of agriculture produce	4
		Any other (specify)	5
Q14	How is food obtained in the household? (Probe for all responses)	Farming	1
		Purchase	2
		Food aid/donation	3
		Transfers	4
		Any other (specify)	5
Q15	Who has the primary responsibility of providing food for the household?	Father	1
		Mother	2
		Grandparent	3
		Relatives	4
		Any other (specify)	5
Q16	What is the estimated percentage of	Man	1

	household income that is allocated to food? / What is the estimated amount of money that you spend on food per month?	woman	2
Q17	Who usually decides how family income is used?	Husband/Partner Wife/mother Any other (specify)	1 2 3
Q18	Who usually decides on what food to be cooked each day in the household?	Husband/Partner Wife/mother Any other (specify)	1 2 3
Q19	The residential village of the respondent	A B C D	1 2 3 4

Appendix 5 Section B: Child's Data

If there is more than 1 child 6-23 months in the household, identify each child's mother or primary caregiver starting with the youngest and arrange to interview her once. This Section of the interview schedule is completed after you have completed the questionnaire for the first child.

	Question	Response	Choice
Q1	Child Identification Number	Child's code	
Q2	Sex of the child	Male Female	1 2
Q3	Child's date of birth (If there is no documentary source, probe using memorable dates /calendar of events until a mother provides the most accurate answer)	Date: ____ / ____ / 20....	
Q4	Source of birth date	Child health card Mother/caregiver	1 2

		Any other source (specify)	3
Q5	Source of the birth e.g., hospital, home	Hospital Home	1 2
Q6	Order of birth of the child (Whether the child is the 1 st , 2 nd , 3 rd , born e.t.c)		
Q7	Birth interval (Spacing between the children)		
08	The weight of the child at birth		
09	Actual child weight		
10	Actual child height		
11	Is your child still breastfeeding	Yes No	1 2
12	Number of times the child is fed on porridge in a day		
12	Number of times the child is fed on porridge in a week		
13	Number of weeks the child is fed on porridge in months		
14	Number of months in a year		
15	At what age did you introduce this porridge		
16	On introduction of the porridge did the child suffer from Vomiting/ stomach upset and or diarrhea?		
17	If yes, how often?		
18	Was the child treated and where?		
19	What is the source of water for domestic use?	Borehole Well Protected spring Dam Tap water Others (specify)	1 2 3 4 5 6

20	Do you have a pit latrine?		
21	If yes, how many households share the latrine?		
22	If nowhere do you dispose the human waste?		
23	Do you wash hands after visiting the latrine?		
24	Do you wash hands when preparing porridge?		
25	Do you wash hands when feeding the child?		

Appendix 6: Section C, Food Safety Knowledge of the Caregiver

Q1. What do you understand by the term safe food?

- a) Nutritious food
- b) Food free from contaminants
- c) Others (specify)

Q2. How many times have you participated in any training on food safety?

- a) Never
- b) One training
- c) 2-3 training
- d) More than 3 training

Q3. What is your source of information regarding food safety?

- a) Doctor/ health worker
- b) Friends and relatives
- c) Radio
- d) Others specify

Q4. Why is it important to prepare safe food?

- a) Protect children from food-borne diseases
- b) Others specify

Q5. How do you dry the raw materials?

- a) On the ground
- b) On the mat/tarpaulin
- c) Others

Q6. How long do you cook the porridge? To be verified during a demonstration activity.

- a) 15minutes,
- b) 30minutes 1 hour

c) Others (Specify)

Q7. Do you store the cooked porridge?

Q8. If yes, for how long?

Q9. How do you warm it?

a) Boil

b) Warm

c) Other (specify)

Appendix 7 Section D: Food-frequency Questionnaire

How often in the past one month did you give Millet Sesame composite (MSSC)	Less than 1 per wk	7 Per wk	7 per wk	4 per wk	5 per wk	6 per wk	1 per day	2 per day	3 or more a day (specify)	Missing/ Don't Know (M/DK)
	0	1	2	4	5	6	7	8	9	10
1. How much do you serve/meal										
2. What ingredient do you add on MSSC and what quantity	Ingredients 1..... quantity..... 2..... quantity..... 3.....quantity.....									

Appendix 8: Section E: Attitude to food safety

This section is about community's food safety attitude. Please respond to the statement below by ticking the most appropriate option for you using the following scale; D =

Disagree NS = Not Sure A = Agree

		D	NS	A
1	Food safety an important issue at home (Food safety benefits).			
2	I fear feeding my child on foods contaminated by small organisms than chemicals			
3	I fear feeding my child on foods contaminated by chemicals than small organisms than chemicals			
5	The most significant perceived food safety risk is small organisms that cause diseases			
6	The most significant perceived food safety risk is chemical contaminants.			
7	The most significant perceived food safety risk is physical contaminants such as sand and metals.			
8	Freshness and absence of spoilage are considered food safety			
9	Home grown raw materials used for making malted millet sesame soy composite are safe			
10	Purchased raw materials used for making malted millet sesame soy composite are safe			
11	Malted millet sesame soy composite porridge is a safe product for children aged 6-23months			
12	Processing and preparation procedures of malted millet sesame soy composite are a great concern to food safety			

13	I am willing to pay extra price for a safe raw materials for making malted millet sesame soy composite			
14	I am aware of food safety authority			
15	I know the roles of food safety authority			
16	I have confidence in food safety			
17	I know the different measures of increasing food safety			
18	I know the basic rules for food hygiene			
19	My child has ever suffered from food borne illness			

Appendix 9: Recommended International Code of Practice General Principles of Food Hygiene CAC/RCP 1-1969, REV. 4-20031

Introduction

People have the right to expect the food they eat to be safe and suitable for consumption. Food-borne illness and food-borne injury are at best unpleasant; at worst, they can be fatal. But there are also other consequences. Outbreaks of food-borne illness can damage trade and tourism, and lead to loss of earnings, unemployment, and litigation. Food spoilage is wasteful, costly and can adversely affect trade and consumer confidence. International food trade and foreign travel are increasing, bringing important social and economic benefits. But this also makes the spread of illness around the world easier. Eating habits too, have undergone a major change in many countries over the last two decades and new food production, preparation and distribution techniques have developed to reflect this. Effective hygiene control, therefore, is vital to avoid the adverse human health and economic consequences of food-borne illness, food-borne injury, and food spoilage. Everyone, including farmers and growers, manufacturers and processors, food handlers and consumers, have a responsibility to assure that food is safe and suitable for consumption. These General Principles lay a firm foundation for ensuring food hygiene and should be used in conjunction with each specific code of hygienic practice, where appropriate, and the guidelines on microbiological criteria. The document follows the food chain from primary production through to final consumption, highlighting the key hygiene controls at each stage. It recommends a HACCP-based approach wherever possible to enhance food safety as described in *Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its*

Application (Annex). The controls described in this General Principles document are internationally recognized as essential to ensure the safety and suitability of food for consumption. The General Principles are commended to Governments, industry (including individual primary producers, manufacturers, processors, foodservice operators, and retailers) and consumers alike.

Section 1 - objectives

1.1 The codex general principles of food hygiene:

- Identify the *essential* principles of food hygiene applicable *throughout the food chain* (including primary production through to the final consumer), to achieve the goal of ensuring that food is safe and suitable for human consumption;
- Recommend a HACCP-based approach as a means to enhance food safety;
- Indicate *how* to implement those principles; and
- Provide a *guidance* for specific codes which may be needed for - sectors of the food chain; processes; or commodities; to amplify the hygiene requirements specific to those areas.

Section 2 - scope, use, and definition

2.1 Scope

2.1.1 The food chain

This document follows the food chain from primary production to the final consumer, setting out the necessary hygiene conditions for producing food which is safe and suitable for consumption. The document provides a baseline structure for other, more specific, codes applicable to particular sectors. Such specific codes and guidelines should be read in conjunction with this document and *Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its Application* (Annex).

2.1.2 Roles of Governments, industry, and consumers

Governments can consider the contents of this document and decide how best they should encourage the implementation of these general principles to:

- Protect consumers adequately from illness or injury caused by food; policies need to consider the vulnerability of the population, or of different groups within the population;
- Provide assurance that food is suitable for human consumption;
- Maintain confidence in internationally traded food; and
- Provide health education programmes which effectively communicate the principles of food hygiene to industry and consumers.

The industry should apply the hygienic practices set out in this document to:

- Provide food which is safe and suitable for consumption;
- Ensure that consumers have clear and easily-understood information, by way of labeling and other appropriate means, to enable them to protect their food from contamination and growth/survival of food-borne pathogens by storing, handling and preparing it correctly; and
- Maintain confidence in internationally traded food.

Consumers should recognize their role by following relevant instructions and applying appropriate food hygiene measures.

2.2 Use

Each section in this document states both the objectives to be achieved and the rationale behind those objectives in terms of the safety and suitability of food.

Section III covers primary production and associated procedures. Although hygiene practices may differ considerably for the various food commodities and specific codes should be applied where appropriate, some general guidance is given in this section. Sections IV to X set down the general hygiene principles which apply throughout the food chain to the point of sale. Section IX also covers consumer information, recognizing the important role played by consumers in maintaining the safety and suitability of food. There will inevitably be situations where some of the specific requirements contained in this document are not applicable. The fundamental question in every case is “what is necessary and appropriate on the grounds of the safety and suitability of food for consumption?” The text indicates where such questions are likely to arise by using the phrases “where necessary” and “where appropriate”. In practice, this means that, although the requirement is generally appropriate and reasonable, there will nevertheless be some situations where it is neither necessary nor appropriate on the grounds of food safety and suitability. In deciding whether a requirement is necessary or appropriate, an assessment of the risk should be made, preferably within the framework of the HACCP approach. This approach allows the requirements in this document to be flexibly and sensibly applied with a proper regard for the overall objectives of producing food which is safe and suitable for consumption. In so doing it takes into account the wide diversity of activities and varying degrees of risk involved in producing food. Additional guidance is available in specific food codes.

2.3 Definitions

For the purpose of this Code, the following expressions have the meaning stated:

Cleaning - the removal of soil, food residue, dirt, grease or other objectionable matter.

Contaminant - any biological or chemical agent, foreign matter, or other substances not intentionally added to food which may compromise food safety or suitability.

Contamination - the introduction or occurrence of a contaminant in food or food environment.

Disinfection - the reduction, by means of chemical agents and/or physical methods, of the number of micro-organisms in the environment, to a level that does not compromise food safety or suitability.

Establishment - any building or area in which food is handled and the surroundings under the control of the same management.

Food hygiene - all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Hazard - a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

HACCP - a system which identifies, evaluates, and controls hazards which are significant for food safety.

Food handler - any person who directly handles packaged or unpackaged food, food equipment, and utensils, or food contact surfaces and is therefore expected to comply with food hygiene requirements

Food safety - assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Food suitability - assurance that food is acceptable for human consumption according to its intended use.

Primary production - those steps in the food chain up to and including, for example, harvesting, slaughter, milking, fishing.

Section 3 - Primary production

Objectives:

Primary production should be managed in a way that ensures that food is safe and suitable for its intended use. Where necessary, this will include:

- Avoiding the use of areas where the environment poses a threat to the safety of food;
- Controlling contaminants, pests, and diseases of animals and plants in such a way as not to pose a threat to food safety;
- Adopting practices and measures to ensure food is produced under appropriately hygienic conditions.

Rationale:

To reduce the likelihood of introducing a hazard which may adversely affect the safety of food, or its suitability for consumption, at later stages of the food chain.

3.1 Environmental hygiene

Potential sources of contamination from the environment should be considered. In particular, primary food production should not be carried on in areas where the presence of potentially harmful substances would lead to an unacceptable level of such substances in food.

3.2 Hygienic production of food sources

The potential effects of primary production activities on the safety and suitability of food should be considered at all times. In particular, this includes identifying any specific points in such activities where a high probability of contamination may exist and taking specific measures to minimize that probability. The HACCP-based approach may assist in the taking of such measures.

Producers should as far as practicable implement measures to:

- Control contamination from air, soil, water, feedstuffs, fertilizers (including natural fertilizers), pesticides, veterinary drugs or any other agent used in primary production;
- Control plant and animal health so that it does not pose a threat to human health through food consumption, or adversely affect the suitability of the product; and
- Protect food sources from fecal and other contamination.

In particular, care should be taken to manage wastes, and store harmful substances appropriately. On-farm programmes which achieve specific food safety goals are becoming an important part of the primary production and should be encouraged.

3.3 Handling, storage, and transport

Procedures should be in place to:

- Sort food and food ingredients to segregate material which is evidently unfit for human consumption;
- Dispose of any rejected material in a hygienic manner; and
- Protect food and food ingredients from contamination by pests, or by chemical, physical or microbiological contaminants or other objectionable substances during handling, storage, and transport. Care should be taken to prevent, so far as reasonably practicable, deterioration and spoilage through appropriate measures which may include controlling temperature, humidity, and/or other controls.

3.4 Cleaning, maintenance and personnel hygiene at primary production

Appropriate facilities and procedures should be in place to ensure that:

- Any necessary cleaning and maintenance is carried out effectively; and
- An appropriate degree of personal hygiene is maintained.

Section 4 - Establishment: design and facilities

Objectives:

Depending on the nature of the operations, and the risks associated with them, premises, equipment and facilities should be located, designed and constructed to ensure that:

- Contamination is minimized;
- Design and layout permit appropriate maintenance, cleaning, and disinfection and minimize air-borne contamination;
- Surfaces and materials, in particular, those in contact with food, are non-toxic in intended use and, where necessary, suitably durable, and easy to maintain and clean;
- Where appropriate, suitable facilities are available for temperature, humidity, and other controls; and
- There is effective protection against pest access and harbourage.

Rationale:

Attention to good hygienic design and construction, appropriate location, and the provision of adequate facilities, is necessary to enable hazards to be effectively controlled.

4.1 Location

4.1.1 Establishments

Potential sources of contamination need to be considered when deciding where to locate food establishments, as well as the effectiveness of any reasonable measures that might be taken to protect food. Establishments should not be located anywhere where, after considering such protective measures, it is clear that there will remain a threat to food safety or suitability. In particular, establishments should normally be located away from:

- Environmentally polluted areas and industrial activities which pose a serious threat of contaminating food;
- Areas subject to flooding unless sufficient safeguards are provided;
- Areas prone to infestations of pests;
- Areas where wastes, either solid or liquid, cannot be removed effectively.

4.1.2 Equipment

Equipment should be located so that it:

- Permits adequate maintenance and cleaning;
- Functions in accordance with its intended use; and
- Facilitates good hygiene practices, including monitoring.

4.2 Premises and rooms

4.2.1 Design and layout

Where appropriate, the internal design and layout of food establishments should permit good food hygiene practices, including protection against cross-contamination between and during operations by foodstuffs.

4.2.2 Internal structures and fittings

Structures within food establishments should be soundly built of durable materials and be easy to maintain, clean and where appropriate, able to be disinfected. In particular, the following specific conditions should be satisfied where necessary to protect the safety and suitability of food:

- The surfaces of walls, partitions, and floors should be made of impervious materials with no toxic effect in intended use;
- Walls and partitions should have a smooth surface up to a height appropriate to the operation;
- Floors should be constructed to allow adequate drainage and cleaning;
- Ceilings and overhead fixtures should be constructed and finished to minimize the buildup of dirt and condensation, and the shedding of particles;
- Windows should be easy to clean, be constructed to minimize the buildup of dirt and where necessary, be fitted with removable and cleanable insect-proof screens. Where necessary, windows should be fixed;
- Doors should have smooth, non-absorbent surfaces, and be easy to clean and, where necessary, disinfect;
- Working surfaces that come into direct contact with food should be in sound condition, durable and easy to clean, maintain and disinfect. They should be made of smooth, non-absorbent materials, and inert to the food, to detergents and disinfectants under normal operating conditions.

4.2.3 Temporary/mobile premises and vending machines

Premises and structures covered here include market stalls, mobile sales, and street vending vehicles, temporary premises in which food is handled such as tents and marquees. Such premises and structures should be sited, designed and constructed to avoid, as far as reasonably practicable, contaminating food and harboring pests.

In applying these specific conditions and requirements, any food hygiene hazards associated with such facilities should be adequately controlled to ensure the safety and suitability of food.

4.3 Equipment

4.3.1 General

Equipment and containers (other than once-only use containers and packaging) coming into contact with food, should be designed and constructed to ensure that, where necessary, they can be adequately cleaned, disinfected and maintained to avoid the contamination of food. Equipment and containers should be made of materials with no toxic effect in intended use. Where necessary, equipment should be durable and movable or capable of being disassembled to allow for maintenance, cleaning, disinfection, monitoring and, for example, to facilitate inspection for pests.

4.3.2 Food control and monitoring equipment

In addition to the general requirements in paragraph 4.3.1, equipment used to cook, heat treat, cool, store or freeze food should be designed to achieve the required food temperatures as rapidly as necessary in the interests of food safety and suitability, and maintain them effectively. Such equipment should also be designed to allow temperatures to be monitored and controlled. Where necessary, such equipment should have effective means of controlling and monitoring humidity, air-flow and any other characteristic likely to have a detrimental effect on the safety or suitability of food. These requirements are intended to ensure that:

- Harmful or undesirable micro-organisms or their toxins are eliminated or reduced to safe levels or their survival and growth are effectively controlled;
- Where appropriate, critical limits established in HACCP-based plans can be monitored; and
- Temperatures and other conditions necessary for food safety and suitability can be rapidly achieved and maintained.

4.3.3 Containers for waste and inedible substances

Containers for waste, by-products and inedible or dangerous substances, should be specifically identifiable, suitably constructed and, where appropriate, made of impervious material. Containers used to hold dangerous substances should be identified and, where appropriate, be lockable to prevent malicious or accidental contamination of food.

4.4 Facilities

4.4.1 Water supply

An adequate supply of potable water with appropriate facilities for its storage, distribution and temperature control, should be available whenever necessary to ensure the safety and suitability of food. Potable water should be as specified in the latest edition of WHO Guidelines for Drinking Water Quality, or water of a higher standard. Non-potable water (for use in, for example, fire control, steam production, refrigeration and other similar purposes where it would

not contaminate food), shall have a separate system. Non-potable water systems shall be identified and shall not connect with, or allow reflux into, potable water systems.

4.4.2 Drainage and waste disposal

Adequate drainage and waste disposal systems and facilities should be provided. They should be designed and constructed so that the risk of contaminating food or the potable water supply is avoided.

4.4.3 Cleaning

Adequate facilities, suitably designated, should be provided for cleaning food, utensils, and equipment. Such facilities should have an adequate supply of hot and cold potable water where appropriate.

4.4.4 Personnel hygiene facilities and toilets

Personnel hygiene facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained and to avoid contaminating food. Where appropriate, facilities should include:

- Adequate means of hygienically washing and drying hands, including wash basins and a supply of hot and cold (or suitably temperature controlled) water;
- Lavatories of appropriate hygienic design; and
- Adequate changing facilities for personnel.

Such facilities should be suitably located and designated.

4.4.5 Temperature control

Depending on the nature of the food operations undertaken, adequate facilities should be available for heating, cooling, cooking, refrigerating and freezing food, for storing refrigerated or frozen foods, monitoring food temperatures, and when necessary, controlling ambient temperatures to ensure the safety and suitability of food.

4.4.6 Air quality and ventilation

Adequate means of natural or mechanical ventilation should be provided, in particular to:

- Minimize air-borne contamination of food, for example, from aerosols and condensation droplets;
- Control ambient temperatures;
- Control odours which might affect the suitability of food; and
- Control humidity, where necessary, to ensure the safety and suitability of food.

Ventilation systems should be designed and constructed so that air does not flow from contaminated areas to clean areas and, where necessary, they can be adequately maintained and cleaned.

4.4.7 Lighting

Adequate natural or artificial lighting should be provided to enable the undertaking to operate in a hygienic manner. Where necessary, lighting should not be such that the resulting colour is misleading. The intensity should be adequate to the nature of the operation. Lighting fixtures should, where appropriate, be protected to ensure that food is not contaminated by breakages.

4.4.8 Storage

Where necessary, adequate facilities for the storage of food, ingredients and non-food chemicals (e.g. cleaning materials, lubricants, fuels) should be provided.

Where appropriate, food storage facilities should be designed and constructed to:

- Permit adequate maintenance and cleaning;
- Avoid pest access and harbourage;
- Enable food to be effectively protected from contamination during storage; and
- Where necessary, provide an environment which minimizes the deterioration of food (e.g. by temperature and humidity control).

The type of storage facilities required will depend on the nature of the food. Where necessary, separate, secure storage facilities for cleaning materials and hazardous substances should be provided.

SECTION 5 - Control of operation

Objective:

To produce food which is safe and suitable for human consumption by:

- Formulating design requirements with respect to raw materials, composition, processing, distribution, and consumer use to be met in the manufacture and handling of specific food items; and
- Designing, implementing, monitoring and reviewing effective control systems.

Rationale:

To reduce the risk of unsafe food by taking preventive measures to assure the safety and suitability of food at an appropriate stage in the operation by controlling food hazards.

5.1 Control of food hazards

Food business operators should control food hazards through the use of systems such as HACCP.

They should:

- **Identify** any steps in their operations which are critical to the safety of food;
- **Implement** effective control procedures at those steps;
- **Monitor** control procedures to ensure their continuing effectiveness; and

- **Review** control procedures periodically, and whenever the operations change.

These systems should be applied throughout the food chain to control food hygiene throughout the shelf-life of the product through proper product and process design.

Control procedures may be simple, such as checking stock rotation calibrating equipment or correctly loading refrigerated display units. In some cases, a system based on expert advice, and involving documentation, may be appropriate.

5.2 Key aspects of hygiene control systems

5.2.1 Time and temperature control

Inadequate food temperature control is one of the most common causes of food-borne illness or food spoilage. Such controls include time and temperature of cooking, cooling, processing, and storage. Systems should be in place to ensure that temperature is controlled effectively where it is critical to the safety and suitability of food.

Temperature control systems should take into account:

- The nature of the food, e.g. its water activity, pH, and likely initial level and types of microorganisms;
- The intended shelf-life of the product;
- The method of packaging and processing; and how the product is intended to be used, e.g. further cooking/processing or ready-to-eat.

Such systems should also specify tolerable limits for time and temperature variations.

Temperature recording devices should be checked at regular intervals and tested for accuracy.

5.2.2 Specific process steps

Other steps which contribute to food hygiene may include, for example:

- Chilling
- Thermal processing
- Irradiation
- Drying
- Chemical preservation
- Vacuum or modified atmospheric packaging

5.2.3 Microbiological and other specifications

Management systems described in paragraph 5.1 offer an effective way of ensuring the safety and suitability of food. Where microbiological, chemical or physical specifications are used in any food control system, such specifications should be based on sound scientific principles and state, where appropriate, monitoring procedures, analytical methods, and action limits.

5.2.4 Microbiological cross-contamination

Pathogens can be transferred from one food to another, either by direct contact or by food handlers, contact surfaces or the air. Raw, unprocessed food should be effectively separated, either physically or by time, from ready-to-eat foods, with effective intermediate cleaning and where appropriate disinfection.

Access to processing areas may need to be restricted or controlled. Where risks are particularly high, access to processing areas should be only via a changing facility. Personnel may need to be required to put on clean protective clothing including footwear and wash their hands before entering.

Surfaces, utensils, equipment, fixtures, and fittings should be thoroughly cleaned and where necessary disinfected after raw food, particularly meat and poultry, has been handled or processed.

5.2.5 Physical and chemical contamination

Systems should be in place to prevent contamination of foods by foreign bodies such as glass or metal shards from machinery, dust, harmful fumes and unwanted chemicals. In manufacturing and processing, suitable detection or screening devices should be used where necessary.

5.3 Incoming material requirements

No raw material or ingredient should be accepted by an establishment if it is known to contain parasites, undesirable micro-organisms, pesticides, veterinary drugs or toxic, decomposed or extraneous substances which would not be reduced to an acceptable level by normal sorting and/or processing. Where appropriate, specifications for raw materials should be identified and applied.

Raw materials or ingredients should, where appropriate, be inspected and sorted before processing. Where necessary, laboratory tests should be made to establish fitness for use. Only sound, suitable raw materials or ingredients should be used. Stocks of raw materials and ingredients should be subject to effective stock rotation.

5.4 Packaging

Packaging design and materials should provide adequate protection for products to minimize contamination, prevent damage, and accommodate proper labeling. Packaging materials or gases used must be non-toxic and not pose a threat to the safety and suitability of food under the specified conditions of storage and use. Where appropriate, reusable packaging should be suitably durable, easy to clean and, where necessary, disinfect.

5.5 Water

5.5.1 In contact with food

Only potable water should be used in food handling and processing, with the following

exceptions:

- For steam production, fire control and other similar purposes not connected with food; and
- In certain food processes, e.g. chilling, and in food handling areas, provided this does not constitute a hazard to the safety and suitability of food (e.g. the use of clean sea water).

Water recirculated for reuse should be treated and maintained in such a condition that no risk to the safety and suitability of food results from its use. The treatment process should be effectively monitored. Recirculated water which has received no further treatment and water recovered from the processing of food by evaporation or drying may be used, provided its use does not constitute a risk to the safety and suitability of food.

5.5.2 As an ingredient

Potable water should be used wherever necessary to avoid food contamination.

5.5.3 Ice and steam

Ice should be made from water that complies with section 4.4.1. Ice and steam should be produced, handled and stored to protect them from contamination.

Steam used in direct contact with food or food contact surfaces should not constitute a threat to the safety and suitability of food.

5.6 Management and supervision

The type of control and supervision needed will depend on the size of the business, the nature of its activities and the types of food involved. Managers and supervisors should have enough knowledge of food hygiene principles and practices to be able to judge potential risks, take appropriate preventive and corrective action, and ensure that effective monitoring and supervision takes place.

5.7 Documentation and records

Where necessary, appropriate records of processing, production, and distribution should be kept and retained for a period that exceeds the shelf-life of the product. Documentation can enhance the credibility and effectiveness of the food safety control system.

5.8 Recall procedures

Managers should ensure effective procedures are in place to deal with any food safety hazard and to enable the complete, rapid recall of any implicated lot of the finished food from the market.

Where a product has been withdrawn because of an immediate health hazard, other products which are produced under similar conditions, and which may present a similar hazard to public health, should be evaluated for safety and may need to be withdrawn. The need for public warnings should be considered.

Recalled products should be held under supervision until they are destroyed, used for purposes other than human consumption, determined to be safe for human consumption, or reprocessed in a manner to ensure their safety.

Section 6 - Establishment: maintenance and sanitation

Objective:

To establish effective systems to:

- Ensure adequate and appropriate maintenance and cleaning;
- Control pests;
- Manage waste; and
- Monitor effectiveness of maintenance and sanitation procedures.

Rationale:

To facilitate the continuing effective control of food hazards, pests, and other agents likely to contaminate food.

6.1 Maintenance and cleaning

6.1.1 General

Establishments and equipment should be kept in an appropriate state of repair and condition to:

- Facilitate all sanitation procedures;
- Function as intended, particularly at critical steps (see paragraph 5.1);
- Prevent contamination of food, e.g. from metal shards, flaking plaster, debris, and chemicals.

Cleaning should remove food residues and dirt which may be a source of contamination. The necessary cleaning methods and materials will depend on the nature of the food business. Disinfection may be necessary after cleaning. Cleaning chemicals should be handled and used carefully and in accordance with manufacturers’

instructions and stored, where necessary, separated from food, in clearly identified containers to avoid the risk of contaminating food.

6.1.2 Cleaning procedures and methods

Cleaning can be carried out by the separate or the combined use of physical methods, such as heat, scrubbing, turbulent flow, vacuum cleaning or other methods that avoid the use of water, and chemical methods using detergents, alkalis or acids.

Cleaning procedures will involve, where appropriate:

- Removing gross debris from surfaces;
- Applying a detergent solution to loosen soil and bacterial film and hold them in solution or suspension;

- Rinsing with water which complies with section 4, to remove loosened soil and residues of detergent;
- Dry cleaning or other appropriate methods for removing and collecting residues and debris; and
- Where necessary, disinfection with subsequent rinsing unless the manufacturers' instructions indicate on a scientific basis that rinsing is not required.

6.2 Cleaning programmes

Cleaning and disinfection programmes should ensure that all parts of the establishment are appropriately clean, and should include the cleaning of cleaning equipment.

Cleaning and disinfection programmes should be continually and effectively monitored for their suitability and effectiveness and where necessary, documented.

Where written cleaning programmes are used, they should specify:

- Areas, items of equipment and utensils to be cleaned;
- Responsibility for particular tasks;
- Method and frequency of cleaning; and
- Monitoring arrangements.

Where appropriate, programmes should be drawn up in consultation with relevant specialist expert advisors.

6.3 Pest control systems

6.3.1 General

Pests pose a major threat to the safety and suitability of food. Pest infestations can occur where there are breeding sites and a supply of food. Good hygiene practices should be employed to avoid creating an environment conducive to pests. Good sanitation, inspection of incoming materials and good monitoring can minimize the likelihood of infestation and thereby limit the need for pesticides. *CAC/RCP 1-1969, Rev.4- 2003 Page 16 of 3.*

6.3.2 Preventing access

Buildings should be kept in good repair and condition to prevent pest access and to eliminate potential breeding sites. Holes drains and other places where pests are likely to gain access should be kept sealed. Wire mesh screens, for example on open windows, doors, and ventilators, will reduce the problem of pest entry. Animals should, wherever possible, be excluded from the grounds of factories and food processing plants.

6.3.3 Harborage and infestation

The availability of food and water encourages pest harbourage and infestation. Potential food sources should be stored in pest-proof containers and/or stacked above the ground and away

from walls. Areas both inside and outside food premises should be kept clean. Where appropriate, refuse should be stored in covered, pest-proof containers.

6.3.4 Monitoring and detection

Establishments and surrounding areas should be regularly examined for evidence of infestation.

6.3.5 Eradication

Pest infestations should be dealt with immediately and without adversely affecting food safety or suitability. Treatment with chemical, physical or biological agents should be carried out without posing a threat to the safety or suitability of food.

6.4 Waste management

Suitable provision must be made for the removal and storage of waste. Waste must not be allowed to accumulate in food handling, food storage, and other working areas and the adjoining environment except so far as is unavoidable for the proper functioning of the business.

Waste stores must be kept appropriately clean.

6.5 Monitoring effectiveness

Sanitation systems should be monitored for effectiveness, periodically verified by means such as audit pre-operational inspections or, where appropriate, a microbiological sampling of environment and food contact surfaces and regularly reviewed and adapted to reflect changed circumstances.

Section 7 - Establishment: personal hygiene

Objectives:

To ensure that those who come directly or indirectly into contact with food are not likely to contaminate food by:

- Maintaining an appropriate degree of personal cleanliness;
- Behaving and operating in an appropriate manner.

Rationale:

People who do not maintain an appropriate degree of personal cleanliness, who have certain illnesses or conditions or who behave inappropriately, can contaminate food and transmit illness to consumers.

7.1 Health status

People known, or suspected, to be suffering from, or to be a carrier of a disease or illness likely to be transmitted through food, should not be allowed to enter any food handling area if there is a likelihood of their contaminating food. Any person so affected should immediately report illness or symptoms of illness to the management.

Medical examination of a food handler should be carried out if clinically or epidemiologically indicated.

7.2 Illness and injuries

Conditions which should be reported to management so that any need for medical examination and/or possible exclusion from food handling can be considered, include:

- Jaundice;
- Diarrhea;
- Vomiting;
- Fever;
- Sore throat with fever;
- Visibly infected skin lesions (boils, cuts, etc.);
- Discharges from the ear, eye or nose.

7.3 Personal cleanliness

Food handlers should maintain a high degree of personal cleanliness and, where appropriate, wear suitable protective clothing, head covering, and footwear. Cuts and wounds, where personnel are permitted to continue working, should be covered by suitable waterproof dressings. Personnel should always wash their hands when personal cleanliness may affect food safety, for example:

- At the start of food handling activities;
- Immediately after using the toilet; and
- After handling raw food or any contaminated material, where this could result in contamination of other food items; they should avoid handling ready-to-eat food, where appropriate.

7.4 Personal behaviour

People engaged in food handling activities should refrain from behavior which could result in contamination of food, for example:

- Smoking;
- Spitting;
- Chewing or eating;
- Sneezing or coughing over unprotected food.

Personal effects such as jewellery, watches, pins or other items should not be worn or brought into food handling areas if they pose a threat to the safety and suitability of food.

7.5 Visitors

Visitors to food manufacturing, processing or handling areas should, where appropriate, wear protective clothing and adhere to the other personal hygiene provisions in this section.

Section 8 - Transportation

Objectives:

Measures should be taken where necessary to:

- Protect food from potential sources of contamination;
- Protect food from damage likely to render the food unsuitable for consumption; and
- Provide an environment which effectively controls the growth of pathogenic or spoilage micro-organisms and the production of toxins in food.

Rationale:

Food may become contaminated, or may not reach its destination in a suitable condition for consumption, unless effective control measures are taken during transport, even where adequate hygiene control measures have been taken earlier in the food chain.

8.1 General

Food must be adequately protected during transport. The type of conveyances or containers required depends on the nature of the food and the conditions under which it has to be transported.

8.2 Requirements

Where necessary, conveyances and bulk containers should be designed and constructed so that they:

- Do not contaminate foods or packaging;
- Can be effectively cleaned and, where necessary, disinfected;
- Permit effective separation of different foods or foods from non-food items where necessary during transport;
- Provide effective protection from contamination, including dust and fumes;
- Can effectively maintain the temperature, humidity, atmosphere and other conditions necessary to protect food from harmful or undesirable microbial growth and deterioration likely to render it unsuitable for consumption; and
- Allow any necessary temperature, humidity and other conditions to be checked.

8.3 Use and maintenance

Conveyances and containers for transporting food should be kept in an appropriate state of cleanliness, repair and condition. Where the same conveyance or container is used for transporting different foods, or non-foods, effective cleaning and, where necessary, disinfection should take place between loads.

Where appropriate, particularly in bulk transport, containers and conveyances should be designated and marked for food use only and be used only for that purpose.

Section 9 - Product information and consumer awareness

Objectives:

Products should bear appropriate information to ensure that:

- Adequate and accessible information is available to the next person in the food chain to enable them to handle, store, process, prepare and display the product safely and correctly;
- The lot or batch can be easily identified and recalled if necessary.

Consumers should have enough knowledge of food hygiene to enable them to:

- Understand the importance of product information;
- Make informed choices appropriate to the individual; and
- Prevent contamination and growth or survival of food-borne pathogens by storing, preparing and using it correctly.

Information for industry or trade users should be clearly distinguishable from consumer information, particularly on food labels.

Rationale:

Insufficient product information, and/or inadequate knowledge of general food hygiene, can lead to products being mishandled at later stages in the food chain. Such mishandling can result in illness, or products becoming unsuitable for consumption, even where adequate hygiene control measures have been taken earlier in the food chain.

9.1 Lot identification

Lot identification is essential in product recall and also helps effective stock rotation. Each container of food should be permanently marked to identify the producer and the lot. Codex General Standard for the Labeling of Prepackaged Foods (CODEX STAN 1-1985, Rev. 1(1991)) applies.

9.2 Product information

All food products should be accompanied by or bear adequate information to enable the next person in the food chain to handle, display, store and prepare and use the product safely and correctly.

9.3 Labelling

Prepackaged foods should be labeled with clear instructions to enable the next person in the food chain to handle, display, store and use the product safely. Codex General Standard for the Labeling of Prepackaged Foods (CODEX STAN 1-1985, Rev. (1991)) applies.

9.4 Consumer education

Health education programmes should cover general food hygiene. Such programmes should enable consumers to understand the importance of any product information and to follow any

instructions accompanying products, and make informed choices. In particular consumers should be informed of the relationship between time/temperature control and food-borne illness.

Section 10 - Training

Objective:

Those engaged in food operations who come directly or indirectly into contact with food should be trained, and/or instructed in food hygiene to a level appropriate to the operations they are to perform.

Rationale:

Training is fundamentally important to any food hygiene system.

Inadequate hygiene training, and/or instruction and supervision of *all* people involved in food related activities pose a potential threat to the safety of food and its suitability for consumption.

10.1 Awareness and responsibilities

Food hygiene training is fundamentally important. All personnel should be aware of their role and responsibility in protecting food from contamination or deterioration. Food handlers should have the necessary knowledge and skills to enable them to handle food hygienically. Those who handle strong cleaning chemicals or other potentially hazardous chemicals should be instructed in safe handling techniques.

10.2 Training programmes

Factors to take into account in assessing the level of training required include:

- The nature of the food, in particular its ability to sustain growth of pathogenic or spoilage micro-organisms;
- The manner in which the food is handled and packed, including the probability of contamination;
- The extent and nature of processing or further preparation before final consumption;
- The conditions under which the food will be stored; and
- The expected length of time before consumption.

10.3 Instruction and supervision

Periodic assessments of the effectiveness of training and instruction programmes should be made, as well as routine supervision and checks to ensure that procedures are being carried out effectively.

Managers and supervisors of food processes should have the necessary knowledge of food hygiene principles and practices to be able to judge potential risks and take the necessary action to remedy deficiencies.

10.4 Refresher training

Training programmes should be routinely reviewed and updated where necessary. Systems should be in place to ensure that food handlers remain aware of all procedures necessary to maintain the safety and suitability of food.

Appendix 10:

Table 28: Table for determining sample size from a given population

N	S	N	S	N	S
10	10	220	140	1200	291
15	14	230	144	1300	297
20		240	148	1400	302
25	24	250	152	1500	306
30	28	260	155	1600	310
35	32	270	159	1700	313
40	36	280	162	1800	317
45	40	290	165	1900	320
50	44	300	169	2000	322
55	48	320	175	2200	327
60	52	340	181	2400	331
65	65	360	186	2600	335
70	59	380	191	2800	338
75	63	400	196	3000	341
80	66	420	201	3500	346
85	70	440	205	4000	351
90	73	460	210	4500	354
95	76	480	214	5000	357
100	80	500	217	6000	361
110	86	550	226	7000	364
120	92	600	234	8000	367
130	97	650	242	9000	368
140	103	700	248	10000	370
150	108	750	254	15000	375
160	113	800	260	20000	377
170	119	850	265	30000	379
180	123	900	269	40000	380
190	127	950	274	50000	381
200	132	1000	278	75000	382
210	136	1100	286	100000	384

Note. *N* is population size and *S* is sample size

Appendix 11: Permission from Chief Administrative Officer of Amuru District


GULU UNIVERSITY

**FACULTY OF AGRICULTURE AND ENVIRONMENT
DEPARTMENT OF FOOD SCIENCE AND POST HARVEST
TECHNOLOGY**
P. O. Box 166 Gulu, Uganda. Tel: +256 471 432 518 Email: fae@gu.ac.ug

March 13, 2019

The Chief Administrative Officer
Amuru District

Dear Sir,

RE: Permission to carryout academic research in Amuru district

I hereby write to request for the above. I am a student of Gulu University pursuing Doctor of Philosophy in Agricultural and Applied Biosciences, majoring in Food Safety and Quality Management.

My research topic is: **"Microbial Safety of Locally Formulated Millet-Sesame-Soy Complementary Food Composite in Nwoya and Amuru Districts in Northern Uganda"** The aim of the study is to ascertain the microbial safety of locally formulated Millet-Sesame-Soy complementary food composite among households in Amuru and Nwoya districts of northern Uganda so as to realize child good nutrition outcome from locally formulated complementary food composite. I will conduct a survey to collect data on Knowledge, Attitude and Practices of caregivers of children aged 6-23months regarding food safety and carryout food sample analysis to determine microbial contaminants and exposure of the children aged 6-23months to microbial toxins.

Attached is the Gulu University Research Ethics Committee clearance.

Your consideration will highly be appreciated.

Thank you.

Yours Sincerely,
Achiro Eunice
Principal Investigator, PhD Student (REG NO. 16/U/001/PHD)

Handwritten notes:
Permission granted to meet communities for purposes of study.
14/3/2019

Stamp:
CHIEF ADMINISTRATIVE OFFICER
AMURU DISTRICT
14 MAR 2019
P.O. BOX 1074, GULU

Appendix 12: Permission from Chief Administrative Officer of Nwoya District



REPUBLIC OF UGANDA



NWOYA DISTRICT LOCAL GOVERNMENT
OFFICE OF THE CHIEF ADMINISTRATIVE OFFICER

TEL: 0392176298

P.O. BOX 1033, GULU.

Email: nwoya2010district@gmail.com

In correspondence to this subject
Please Quote CR/220/1

20th March, 2019

Ms. Achiro Eunice
Gulu University
Faculty of Agriculture and Environment
Department of Food Science and Post Harvest Technology
P.O Box 166,
GULU

RE: PERMISSION TO CARRY OUT RESEARCH IN NWOYA DISTRICT

The above subject matter refers.

Reference is made to your request dated 13th March, 2019 requesting for permission to carryout academic research in Nwoya District entitled (Microbial Safety of Locally Formulated Millet-Sesame-Soy Complementary Food Composite in Nwoya and Amuru District in Northern Uganda".

This therefore, is to inform you that permission is hereby granted for the above research, please liaise with the District Production Officer for necessary guidance.



Asaba Innocent Birekeyaho

CHIEF ADMINISTRATIVE OFFICER/ NWOYA

CC: District Chairperson/ **Nwoya**
CC: Resident District Commissioner/ **Nwoya**
CC: District Production Officer/ **Nwoya**
CC: Ag. Principal Human Resource Officer/ **Nwoya**
CC: File

Appendix 13: Ethical clearance – GUREC

GULU P.O. Box 166 Gulu Uganda Website: www.gu.ac Email: guluuniversity.rec@gmail.com		UNIVERSITY Tel: +256-4714-32096 Fax: +256-4714-32913 Mob: +256772305621/776812147
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RESEARCH ETHICS COMMITTEE

February 15, 2019

APPROVAL NOTICE

Ms Eunice Achiro,
Gulu University

Re: Application No. GUREC-027-19

Type of review:
☒ Initial review
☐ Amendment
☐ Continuing review
☐ Termination of study
☐ SAEs
☐ Other, Specify: _____

Title of proposal: **"Microbial safety of locally formulated millet-sesame-soy complementary food composite in Nwoya and Amuru District in Northern Uganda"**

I am pleased to inform you that at the 48th convened meeting on 29th November 2018, the Gulu University Research Ethics Committee (**GUREC**) voted to approve the above referenced application.

Approval of the research is for the period of **29th November 2018 to 28th November 2019**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the **GUREC** for re-review and approval prior to the activation of the changes. The **GUREC** application number assigned to the research should be cited in any correspondence.



GULU UNIVERSITY
INSTITUTIONAL REVIEW COMMITTEE
APPROVED
★ 15 FEB 2019 ★
FACULTY OF MEDICINE
P. O. Box 166, Gulu

3. Any unanticipated problems involving risks to participants must be promptly reported to the **GUREC**. New information that becomes available which could change the risk: benefit ratio must be submitted promptly for the **GUREC** review.
4. Only approved and stamped consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The **GUREC** may conduct audits of all study records, and consent documentation may be part of such audits.
5. Regulations require review of an approved study not less than once per 12-month period. Therefore, a continuing review application must be submitted to the **GUREC** eight (8) weeks prior to the above expiration **date of 28th November 2019** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely manner may result in suspension or termination of the study, at which point new participants may not be enrolled and currently enrolled participants must be taken off the study.
6. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following documents have been approved in this application by the **GUREC**:

	Document	Language	Version	Version Date
1	Protocol	English	Version 2.0	15 th February 2019
2	Data Collections tools	English	Version 2.0	15 th February 2019
3	Informed consent Document	English/Luo	Version 2.0	15 th February 2019



For; Chairperson
Gulu University Research Ethics Committee

Appendix 14: Ethical clearance – UNCST



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: SS 4958

23rd April 2019

Ms. Eunice Achiro
Gulu University
Gulu

Dear Ms. Achiro,

Re: Research Approval: Microbial Safety of Locally Formulated Millet – Sesame – Soy Complementary Food Composite in Nwoya and Amuru Districts in Northern Uganda

I am pleased to inform you that on **03/04/2019**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **03/04/2019 to 03/04/2022**.

Your research registration number with the UNCST is **SS 4958**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval prior to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: <http://www.uncst.go.ug>



Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	N/A	February 2019
2.	Informed consent documents	English and Luo	N/A	February 2019
3.	Focus group discussion guide	English	N/A	February 2019
4.	Data collection tools	English	N/A	February 2019
5.	Recommended international code of practice general principles of food hygiene CAC/RCP 1 – 1969, REV. 4 – 20031	English	N/A	February 2019

Yours sincerely,

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair: Gulu University, Research Ethics Committee

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
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WEBSITE: <http://www.uncst.go.ug>

Appendix 15: Plagiarism Check Certificate

GULU
P.O. Box 166
Gulu, Uganda



UNIVERSITY

Tel: +256-471-32924
Fax: +256 471343095
Emails: ul@gu.ac.ug
URL: www.gu.ac.ug

OFFICE OF THE UNIVERSITY LIBRARIAN

PLAGIARISM CHECK

Declaration for Originality Form

This form must be completed and signed for all scholarly work produced.

Name: **ACHIRO EUNICE (MS.)**

Personal File No.(staff) Reg. (student). **REG. No. 16/U/001/PHD**

Faculty/Institute: **AGRICULTURE AND ENVIRONMENT, GULU UNIVERSITY**

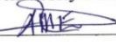
Department: **FOOD SCIENCE AND POSTHARVEST TECHNOLOGY**

Title and bibliographic description of the work

**AFLATOXICOLOGICAL RISK OF HOME-MADE COMPLEMENTARY FOOD
FORMULAE AND CAREGIVERS' COMPETENCY FOR MITIGATION: THE CASE
OF AMURU AND NWOYA DISTRICTS, UGANDA**

Declaration.

1. I understand what plagiarism is and I am aware of the University policy on this regard,
2. I declare that this ...Thesis, ... (Dissertation, Proposal, essay, assignment, paper, report etc.) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my work has been used, this has been properly acknowledged and referenced in accordance with Gulu University requirements,
3. I have not sought or used the services of any professional agency to produce this work,
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own,
5. I understand that any false claim of this work shall result in disciplinary action in accordance with University Policy on Plagiarism.

Signature 

Date **09/12/2024**

Originality Report

The article/thesis/document titled above have been subjected to plagiarism check and the level was found to be.....**20%** (percent) this is classified as:

- ☒ Minor
☐ Moderate
☐ Serious

Yours sincerely,



Manana N Herbert
Ag. University Librarian

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