MICROBIAL LOAD ASSESSMENT OF EDIBLE TERMITES (*Macrotermes spp*) TRADED IN OPEN AIR MARKETS FOR FOOD SAFETY

By

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DECLARATION

I, Inyambo Mumbula do hereby declare that this thesis is my original work and has not been presented for an award of a diploma or conferment of degree in this or any other university or institution.

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DEDICATION

I dedicate this work to Mom and Dad for seeing me through school and encouraging me to study. God bless you, I love you.

ABSTRACT

Edible termites and other have long been a delicacy in Zambia and the rest of Africa. Most of the consumed insects are wild harvested and traded in open air markets where they fetch a decent price. Due to their innate microbial content, natural habitat exposure, several handling points in the value chain, edible termites are prone to microbial contamination. This study aimed at identifying food safety and hygiene practices of edible termite traders and sought to enumerate total viable counts (TVC) and *Enterobactericeae* in open air market traded termites. A cross sectional descriptive design was used and 26 edible termite traders were purposively selected to assess food safety knowledge and common practices in Chisokone market in Kitwe district Zambia. A Fishers exact test revealed significant relationships between, gender and knowledge on purpose for wearing gloves (p= 0.027); Age and knowledge on food poisoning resulted from eating food containing biological or chemical toxins (p=0.041), cross contamination as the transfer of harmful microorganism (p=0.013) and covering food as prevention for cross contamination (p=0.040); Education level and knowledge on control of bacterial growth by reducing temperature (p=002), and reasons for drying insects before storage (p=0.036). Other demographic variables had no significant relationship (p>0.05) with the knowledge items. Microbiological enumerations of samples collected from three sites of Serenje at three handling points (collection point, after transport and display) revealed higher loads of Total Viable Counts (TVC) and *Enterobacteriaceae* than those recommended for minced meat 5.7- 6.7 log cfu/g. Analysis of Variance (ANOVA) of the effect of handling points and sites on microbial counts indicate that both handling points and sites had significant effect (*p<0.05)* on the microbial load of open air traded termites. A Tukey honest significant difference (HSD) showed that the largest increase in TVC was during the transportation period (*p*<0.05) even though a significant increase (*p*=0.027) was recorded during marketing. The largest increase in *Enterobacteriaceae* was during marketing (*p*<0.05). Transportation also showed a significant increase in *Enterobacteriaceae* (p= 0.028). The isolates identified in this study include *Staphylococcus aureus, Escherichia coli,* Yeast *spp.* other than *C. albicans* and *Zygomycetes spp.* Findings of this study can be used by public health authorities to formulate directed training programs on food safety and hygiene practices for edible termite traders. Traders can use the information to adopt practices that can help reduce the microbial loads at each handling points.

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ACRONYMS AND ABBREVIATIONS

ANOVA	-	Analysis of Variance
AOAC	-	Association of Analytical Chemists
a_w	-	Water Activity
CAC	-	Codex Alimentarius Commission
CAR	-	Central African Republic
CBU	-	Copperbelt University
CCOHS	-	Canadian Centre for Occupational Health and Safety
CDC	-	Centre for Disease Control
CFU	-	Coliform Forming Units
COVID19	-	Corona Virus Disease of 2019
DRC	-	Democratic Republic of Congo
ERC	-	Ethics Review Committee
FAO	-	Food and Agriculture Organization
HACCP	-	Hazard Analysis Critical Control Points
HSD	-	Honest Significant Difference
ISO	-	International Standards Organisation
JOOUST	-	Jaramogi Oginga Odinga University of Science and Technology
KES	-	Kenyan Shillings
ODK	-	Open Data Kit
MHA	-	Mueller Hinton Agar
pH	-	Potential of Hydrogen
SDA	-	Sabourauld Dextrose Agar
Sp.	-	Specie
Spp.	-	Species
TDRC	-	Tropical Diseases Research Centre
TVC	-	Total Viable Counts
USD	-	United States Dollar
WHO	-	World Health Organization
ZAR	-	South African Rand

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Food security, the access to sufficient, safe and nutritious food which meets the dietary needs and food preferences for an active and healthy life for all people at all times (Napoli, *et al*, 2011) continue to worsen globally (Lartey, *et al*, 2018). Distribution obstacles, global climate, a lack of successful local agriculture, an inability or disinterest to act by local officials (Luchuo, *et al*, 2013) and an increasing population at alarming rates of 82 million people per year (Worldometers, 2019) are the major drawbacks to food security. These inadequacies, encourage unsustainable production of food (through modern agricultural technologies) in developed countries and contribute to prevalent malnutrition especially Protein-Energy Malnutrition in developing countries (Luchuo, *et al*, 2013). Luckily, the world currently is encouraging entomophagy-the practice of eating insects, as solution to the current dilemma of animal protein food security and its sustainable production. Discussions of entomophagy as an important practice in food security were initialized by a report which quantified the role of edible insects as food in food security (Huis, et al., 2013). Use of edible insects is a sustainable source of animal protein in that it meets the intended nutritional needs at lesser environmental costs compared to conventional sources of animal protein (Dobermann *et al*., 2017), they are ubiquitous and provide various options to choose from.

Insects are abundant life forms and the most heterogeneous of all arthropods. More than 80% of animal life forms are insects (Chakravorty, 2014). Though insects are thought of as damaging pests and others are implicated as disease vectors, a number of them are edible. Nearly 2000 edible species are described in 113 countries and eaten by 300 ethnic groups (Tiencheu & Hilaire, 2017). These species include 31% of the Coleopterans (beetles), 18% Lepidopteron (caterpillars), 14% Hymenopterans (bees), 13% Orthoptera (locusts), 10% Hemiptera (cicadas), 2% Diptera (flies), Isoptera (termites) and Odonata (dragonflies) at 3 % and other orders make up 6% of consumed insects world over. In Africa however, van Huis (2003) reports 250 edible insect species with the majority being Lepidopteron, Orthoptera, Coleopterans, Isopterans and others in that order. This research however, set to study the edible termites and determines the microbial load of edible termites (*Macrotermes spp*.) of the order Isoptera sold in open air markets which is little studied.

Macrotermes is a genus with about 330 termite species indigenous to most of Africa and tropical Asia (Egan, *et al*, 2021). They are uniquely large species with up to 15 cm long queens in the case of *Macrotermes natalensis.* Most of the *Macrotermes* species build mounds with a few being subterranean (TermiteWeb, 2019). Their diet comprises dead plant material in the form of wood, soil or animal dung (Ogedegbe & Eloka, 2015). Such a diet predisposes them to heavy microbial loads. In Africa, the genus *Macrotermes* makes the most commonly used as food for humans and feed for livestock such as poultry and Fish. This genus is widely spread across the continent more than other genera. They are consumed at all stages, adult alates, soldiers, workers and queens. The winged adults (alates) are the most consumed stage in Africa (Fombong & Kinyuru, 2018) harvested after emergence from their termitaries for their nuptial flights.

Harvesting of edible termites is seasonal, in Ghana months of June and July, in Cameroon- March and April, in Kenya- March to May and September to December (Fombong & Kinyuru, 2018). Generally, termites are collected from the beginning of rain season at the end of the dry seasons when they emerge from their termitaria. Different methods used to trap them include placing a basin of water beneath a light source to which they are attracted then trapped. Soldiers are collected by sticking grass blades into the termite mounds to which the termites stick and are pulled out (Fombong & Kinyuru, 2018). Other harvesters break the mounds to find the queen. These then are sold in village markets or transported to town open air markets (Siulapwa, *et al*, 2014). Processing for preservation includes sun drying, blanching, frying, roasting and smoking or steaming. Salting, though significant in extending shelf life, has only been reported to have been used for taste (Fombong & Kinyuru, 2018).

Even though the long history of insect eating in developing countries suggests that insects harvested for human consumption do not cause any significant health problems, with less proof otherwise (Spiegel & Noordam, 2013), the Codex Alimentarius Commission (CAC) reported that food safety of edible insects had not been studied extensively (Huis, et al., 2013). Edible insect nutritional value is comparable to commonly eaten animal protein sources (Kouřimská, *et al*, 2016) as such; this nutritional value forms a conducive breeding and growth environment for microorganisms (Prescott, *et al*, 2002). This makes uncooked insects more vulnerable and a microbiological hazard (Bernard *et al*., 2014) unless proper handling and food hygiene practices are observed. Edible insects are equally a potential microbial hazard due to the presence of their own microbiota and their possibility to serve as vectors of human disease causing agents (microorganisms) (Fraqueza & Patarata, 2017).

The increasing interest in insects for food and feed does not correspond to the available literature to understand hazards related to those edible insects. There is need for studies that validate the identification procedures of potential hazards or emerging potential hazards and measures to reduce such threats (Fraqueza & Patarata, 2017). In the same research, Fraqueza & Patarata, (2017) reveals a number of potential microbiological hazards including families and genera of Enterobacteriaceae (*Proteus, Escherichia*), *Pseudomonas, Staphylococcus, Streptococcus, Bacillus, Micrococcus, Lactobacillus, Acinetobacter* and *Listeria spp*. These have been identified on insect species such as Crickets (*Acheta domesticus*), and Mopane worm (*Imbrasia bellina/Gonimbrasia bellina*) (Fraqueza & Patarata, 2017). Apart from bacterial infections, edible insects are susceptible to fungal toxicity. Some Aflatoxin producing pathogens are entomopathogenic and also colonize insects after harvesting (Kachapulula, *et al*., 2018), therefore unsafe exposure and unhygienic handling of edible insects by traders can result into a microbial hazard for the consumer. Wanjiku, (2018) identified three types of fungi; *Aspergillus spp., Alternarie spp.* and *Penicillium spp* on samples of black soldier fly larvae and house crickets.

A consumption of food stuff including insects that contain high doses of disease causing agents may result into food borne illnesses. Foodborne illnesses have continued to be threatening in both first and third world countries. With globalization, it has become easy to transmit these diseases across borders with food as the main vehicle (Kaferstein, *et al*, 1997). In most cases, foodborne illnesses are caused by contaminants, food allergies and/or improper food handling practices during production, trade, preparation or even consumption (Victoria, 2015). The World Health Organization (WHO) defines food safety as the "condition and measures that are necessary during production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, whole some and fit for human consumption" (WHO, 2020). Food safety has remained a matter of concern for all professionals in the food service industry due to its ability to have substantial costs on individuals and the economy of a country in cases of foodborne disease outbreak.

Take for instance, the WHO investigated 31 global hazards which caused about 600 million foodborne illnesses in 2010 (WHO, 2015). Other than taking 420,000 lives, these foodborne illnesses resulted into 33 million Disability Adjusted Life Years (DALYs). In this, Africa was the highest burden bearer of foodborne disease per population. While all ages are victims of this scourge, children under five and people living in low-income countries are at highest risk (Sockett and Rodgers, 2001) of foodborne illnesses. Though mainly not responsible for the contamination of the food, children under five accounted for over 40% of the global foodborne disease burden in 2010 (WHO, 2015).

This disease burden can be considerably reduced with sufficient knowledge in food safety and hygiene and its practice by the food handlers from farm to folk. In most African countries (as reported for Nigerian rural and sub urban regions), lack of essential facilities and adequate sensitization or awareness on the importance of food safety and its observance may answer for the high foodborne illness burden experienced in Africa (Onyeaka, *et al* 2021). Though procedures and practices such as the WHO's Five Keys to Safer Food, Hazard Analysis Critical Control Point (HACCP) and several sanitation standards proposed to ensure food safety have been developed, their adoption and enforcement in Africa is limited (Tall, n.d.). In food production and marketing, quality of raw material, personal hygiene of the food handler, the sanitation of the environment where the food is stored and exposed for sale as well as the implements used in preparation and marketing are the major components of sanitation (Schmidt, 2011). Once these standards are compromised, food can be contaminated with harmful components which may result in food poisoning

1.2 Statement of the Problem

Studies have shown the occurrence of potential microbiological hazards of both bacteria and fungi in edible insects such as crickets, grasshoppers and caterpillars (Braide & Nwaoguikpe, 2011; Grabowsky & Klein, 2017). Nevertheless, Codex Alimentarius Commission (CAC) reported that food safety of edible insects had not been studied extensively (van Huis, *et al.*, 2013). Especially, the microbial food safety of edible *Macrotermes spp.* which make up the widely used 'termite species in both human and/or livestock diet in most of Africa including Zambia is less understood.

Though Africa is amongst the leading continents in entomophagy, most of which is wild harvested it has the least studies on their safety. The need to understand the microbial load of edible termites is made apparent by outbreaks of diarrheal diseases recorded in nalolo, (Kateule, et al., 2021) Lusaka (Siziya, 2012) and Kitwe (Nyanga & Siziya, 2019) districts of Zambia.

Edible termites are transported long distances to the business hotspots (Siulapwa *et al*, 2014) characterized with 'extreme overcrowding and high environmental risks' which may potentially be a source of microbial contamination and a factor for food safety legislation. Though microbes can be normal flora, contamination can arise from storage environments, implements, packaging material and trader handling practices. As such trader hygiene is cardinal. Personal hygiene, knowledge levels, and food safety practices of food handlers remain a matter of concern at Chisokone market (Battersby & Watson, 2019), this compromise is suspected to be so due to high population densities and overcrowding which may lead to unsanitary conditions around food items traded.

1.3 Objectives

1.3.1 General Objectives

To assess microbial load of edible termites (*Macrotermes spp*.) and trader food safety knowledge and practices in open air markets for food safety.

1.3.2 Specific Objectives

Specific objectives of the study include to:

1. Assess the common food safety and hygiene knowledge and practices of edible termite traders in Chisokone open air market Kitwe.
2. Determine the prevalence of indicator microorganisms in open air traded edible termites in Serenje District.

1.4 Research Questions

1. What are the common food safety and hygiene practices of edible termites' trader in Chisokone open air market Kitwe?
2. What is the prevalence of indicator microbes in open air traded edible termites in Serenje District?

1.5 Significance of the Study

This study contributes to the body of knowledge in understanding the microbial load of the Open air traded edible termites. The knowledge of the microbial load will alert the consumer on the preparation practices necessary to minimize possible microbial hazards that may arise from termites. Traders will benefit from the study as they will best understand possible sources of microbial contamination and be well placed to adopt safe handling and food hygiene practices. Assessment of the microbial load of edible termites provides information necessary for informed decision making on the choice of the food stuff; its preparation, storage and disposal. Information derived from this study allows for enhanced food safety legislation from policy makers or ministries in charge of public health.

This information, whether used by traders, consumers or legislators will enhance food security by ensuring that edible termites remain safe and wholesome.

1.6 Scope

Termites are expected to emerge at the beginning of the rain seasons right at the end of the dry season. Therefore, data collection for Open air traded edible termites is seasonal. Given the period and time for completion, Zambia's rain season commencement is placed within the favorable period of data collection around the month end of November. Furthermore, the choice of the study area (Chisokone market) is strategic in that it is a central market of the Copperbelt province and serves a large population of consumers. Due to this, the market is characterized with 'extreme overcrowding and high environmental risks' (Battersby & Watson, 2019) which may potentially be a source of microbial contamination and a factor for food safety legislation in the country.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 The Need for Entomophagy

The demand for an alternative and more sustainable food (especially protein) sources to feed the growing population has continued to increase. Food sources such as edible algae, fungi and/or *in vitro* meat have been considered (Pali-Schöll, *et al,* 2018). Also, the world currently is discussing the practice of entomophagy as solution to the current dilemma of animal protein food security and sustainable production. This is evidenced in a report on the Contribution of Forest Insects to Food Security which quantified the role of edible insects as food thereby initiating discussions on entomophagy as an important practice in food security (Huis, et al., 2013).

Entomophagy as a practice comes with many opportunities, among them are; Nutrition, Although significant variations are found in different species and age groups of edible insects, edible insects generally provide sufficient amounts of energy and protein, meet amino acid amounts required in humans, have high monounsaturated and/or polyunsaturated fatty acids, and are rich in micronutrients such as copper, iron, magnesium, manganese, phosphorous, selenium and zinc, as well as riboflavin, pantothenic acid, biotin and, in some cases, folic acid (Huis, et al., 2013) at quantities either richer or equal to conventional animal protein sources. Emissions, Studies considering husbandry contributions to emissions have found that insects produce far fewer Green House gases than large livestock (Dobermann *et al*, 2017). Land and water use: the growing concerns of sustainable agriculture are that there will not be sufficient resources (land and water) for both the approximately 9 billion predicted people and agricultural needs. The higher feed efficiency of edible insects suggest that they require less feed, and water for their metabolic processes and hence sustainably use the resources in comparison to conventional livestock (Dobermann *et al.*, 2017).

2.2 World practice of Entomophagy

Entomophagy is not a new phenomenon, the practice dates as far back as the eighth century BCE (Huis, et al., 2013). Currently, over 2 billion people consume insects all over the world. The practice is more common in Africa than any other part of the world (Niassy & Sunday, 2017). Niassy & Sunday, (2017) report the Democratic republic of the Congo, the Central African Republic, Cameroon, Uganda, Zambia, Zimbabwe, Nigeria, and South Africa as dominant insect eating countries and caterpillars, termites, crickets and palm weevils being the most eaten insects. These insects are consumed either raw or fried or roasted (Das, 2020). Termites (Isoptera) in Africa make the most consumed insects behind only Caterpillars (Lepidoptera), Locusts (Orthoptera) and Beetles (Coleoptera) (Huis, 2003).

In Asia, entomophagy is also reported to have been part of the accepted diet. In Thailand for instance, insect eating has become more widespread over the few years. In this country some insects are sold in the markets to be used as ingredients and enjoyed as snacks. This has resulted in the development of established food chains (Yen A. I., 2015). The Asian pacific countries such as Australia, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Papua New Guinea, South Korean, Vietnam and Thailand are involved in either Semi domestication or Farming of edible insects for subsistence and commercial use (Yen A. I., 2015).

In 2010 developed countries legislation regarding insects in food products was on the continuum of zero tolerance in the United Kingdom to allowing maximum permissible levels in the United States (Yen, *et al*, 2010). Now, though still largely unconventional, developed countries have also began considering entomophagy as means to support their growing population's nutritional requirements (Tao & Li, 2018). A worldwide record of edible insects and their distribution is provided by Jongema, (2017). Information in table 1. Provides percentage summary of the most commonly consumed insects around the world.

Table 1: Worlds edible insect species by orders and percentage

Order	Example	%	Order	Example	%
Coleopteran	Beetles	31	Isoptera	Termites	3
Lepidoptera	Caterpillars	18	Odonata	Dragonflies	3
Hymenoptera	Bees, wasps and ants	14	Diptera	Flies	2
Orthoptera	Grasshoppers, locusts, and crickets	13	Others		6
Hemiptera	Cicadas, Leafhoppers, planthoppers, scale insects and true bags	10			

Source: (Van Huis, 2013)

2.3 Termites in Economic Lenses

Termites are mainly known for their destructive nature. This knowledge has often masked the important role termites play in decomposition of plant organic matter, formation of soils, energy and nutrient flow. Other than there ecosystem stability importance, termites have long been part of traditional diets as source of nutrition (entomophagy) and medicinal resources (entomotherapy)(Esther *et al*., 2015).

2.3.1 Termites as pests

As pests, termites are known to cause destruction to crops, forestry, rangelands and structures. The most important genera of termites known to have a pest effect include *Odontermes, Macrotermes, Pseudacanthotermes, Microtermes, Ancistrotermes, Allodontermes, Amitermes, Trinervitermes* and *Hodotermes.* For instance, *Coportermes curvignathus* was found to be the main termite pest species of palm plantations and Agricultural plants. A research, however, showed that Mound building *Macrotermes* species, in particular *M. bellicosus* were soil feeders and appeared to be non pests of crops (Ogedegbe & Eloka, 2015). This result contrasts those of Bigger (1991), who suggests that *Macrotermes spp* are serious pests of agricultural plants especially cassava. These views are reconciled because *Macrotermes* spp are only opportunistic pests. They attack the base of crops planted close to mounds though they are soil feeders.

Generally, termites are pests of agricultural plants, plantation forests, wooden structures (woody homes, fences) *etc*. Even though chemical control of these termite pests exist, more sustainable controls such as biological control are suggested (Verma & Sindhu, 2011). Entomophagy and/or entotherapy could be beneficial means of termite pest control.

2.3.2 Ecosystem services of termites

Termites as described above have roles that are diversified from just destruction of valuables. Apart from being sources of food, termites have other ecological importance. They offer support, regulatory, provisioning and cultural services. Termites have earned themselves a name as 'Ecosystem engineers' due to their bioturbating activities as they build mounds (Ogedegbe & Eloka, 2015). Through these activities, they aid in soil formation, nutrient cycling, waste decomposition and detoxification**.** Mound soils are known to be rich in calcium, magnesium, potassium and sodium and phosphorous. Soil type (silt, clay & fine sand) and organic matter composition of termite mounds combined with their micronutrients could improve yields three fold when used as a fertilizer (Van Huis, 2017).

Other services include, geophargy-the consumption of soil for animals. Termite mounds provide 14% of the recommended dietary allowance of iron in pregnancy. In Kenya, 25% of pregnant women have preferences for termite soil (Van Huis, 2017). Elephants are also reported to eat the termite soils. Culturally, termite mounds are also known to have religion and superstition significance.

2.3.3 Termites as food and feed

There are over 45 termite species used as food and feed around the world. These are distributed across four families; Termitidae (39 *spp.*, 87%), Rhinotermitidae (7 *spp.*, 6%), Kalotermitidae (2 *spp.*, 4%), and Hodotermitidae (2 *spp.*, 4%) (Fombong & Kinyuru, 2018).

In Africa, the genus *Macrotermes* makes the most commonly used as food and feed and is widely spread across the continent amongst termites. The species *M. falciger* and *M. bellicosus* are the widely eaten species in at least 12 and 13 countries respectively across the continent (Fombong & Kinyuru, 2018). The edible termites are consumed at various stages. These stages include the Queen, Soldiers and alates (winged adults) (Fombong & Kinyuru, 2018). Most of the consumed edible insects in Africa are wild harvested (Murefu, *et al,* 2019). In Zambia, many tribes collect alates of species such as *M. falciger* during their nuptial flights after emerging from their termitaries after the early rains. After harvesting, the insects are transported to the urban markets in the Copperbelt, Lusaka and Livingstone where they fetch a handsome price (Siulapwa *et al*, 2014). The edible winged termites' seasonal availability in Zambia includes the months of November, December and January only.

2.3.4 Termites a source of income

Edible termites have a direct and indirect contribution to livelihood of many rural families. The direct contribution to income of a household is through the 'handsome' prices they fetch over some other animal protein sources. For instance Netshifhefhe, *et al*, (2018) found that a kilogram of termites sold at 100 South African Rand (ZAR100) (6.52USD) was higher than that of beef chuck, pork chops and whole chicken which sold at ZAR73.67/Kg, ZAR78.59/kg and ZAR43.69/kg respectively. The edible termite value was only lower in prices to dried beef, sausages, and Lamb leg and beef rump steak. However, a Kilogram of edible termites could feed at least 15 people far more than fresh chicken, beef, pork or dried meats could feed (Netshifhefhe *et al*., 2018). In Southern Africa, Trade in edible insects (including termites) is economically important (Pal & Roy, 2014). Siulapwa, *et al*, (2014) report that those involved in trading edible insects in Zambia are able to take their children to school from this practice.

Edible termites are therefore a better option for trade not only for their superior protein, but also for their economic value. Indirectly, termite mounds built at homes are owned by those households. The mounds are only used for anything at the permit of that household (Arnold Van Huis, 2017). Around these mounds families cultivate their crops and vegetables which they sale or consume at home. The households in Africa decide to use the mound soil as building material for houses, furnaces *etc*. these, when sold provide income for individual households (Van Huis, 2017). While promotion of edible termites as food and feed is cardinal for their nutrition, therapeutically and income benefits, their sustainable harvest and production are matters worth discussion (Netshifhefhe *et al*., 2018).

2.4 Contamination of Edible Insects- including termites

The acceptability of Edible insects as Food has been extensively studied (Murefu, *et al* , 2019). Fombong & Kinyuru, (2018) confirm the dearth of research made on how large scale farming of insects can be done. They however recognise that food safety and hygiene issues have received lesser attention hence the need for this study. Wild harvested edible insects have even a higher risk of contamination (Murefu, *et al*, 2019).

2.5 Microbiological Load of edible insects

2.5.1 Gut Microbiota

Edible insect microbial load is made up of surface and gut microbiota. Surface microbiota results from contact with environmental surfaces. Amadi *et al.* (2005) have shown that the microbial loads of edible insect gut could be higher than the skin microbial loads (Haubruge, *et al*, 2017). Insect gut microbiota may be normal flora to the insect, aiding in its metabolism, promoting efficient digestion and protecting the host from other potentially harmful microbes. Amidst these benefits, insect gut microbes may engage in opportunistically harmful interactions with the host and/or pose as a health risk when consumed by humans or livestock (Yun *et al*., 2014). Insect fasting in processing before being killed by freezing, reduces the gastrointestinal loads of residual food in the insect gut and, consequently, the insect microbial loads (Haubruge *et al*., 2017).

2.5.2 Indicator organisms

Organisms that are used as a sign of quality or hygienic status in a food, water or environment are referred to as indicator organism. The presence of these indicator organisms insinuates insanitation and public health concerns. Indicator organisms have been elucidated to evaluate quality and safety of commodities (Tortorello, 2003). Use of indicator organisms as a test for quality and safety of water and foods extends to edible insects. Imathiu, *et al*, (2018) quantified the Total Viable Counts (TVC), Enterobacteriaceae and molds and yeasts in *Ruspolia differens.* The European Union Regulation (EC) N0. 1441/2007 for indicator organisms used also for edible insects of 5.7 and 6.7 log cfu/g was exceeded by the TVC ranging between 7.0 to 8.5 log cfu/g.

Another study of Haubruge *et al.,* (2017), determined TVC and molds and yeasts to ascertain the microbiological load of Edible Insects found in Belgium. In this research, heat treatments such as blanching and sterilization significantly reduced the indicator organism quantities from higher than recommended levels to those acceptable (Haubruge *et al*., 2017). Wanjiku, (2018) identified Three types of fungi; *Aspergillus spp., Alternaria spp.* and *Penicillium spp* on samples of Black soldier fly larvae and House Crickets. Members of genus *Aspergillus* produce mycotoxins such as aflatoxins which are carcinogenic in nature. Finally, the presence of indicator organisms is a matter of concern by mere presence or if they exceed certain quantities (Tortorello, 2003).

2.5.3 Pathogenic bacteria

Food contaminated with pathogenic bacteria is a serious public health risk. There are a number of food pathogenic bacteria which include, *Salmonella*, Strains of *E.coli, Listeria spp, Shigella spp, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum, etc.* Imathiu et al., (2018) recognizes *Salmonella*, *E.coli* and *Listeria* as common food pathogens. These pathogens cause disease by multiplication within the host organism or by secretion of toxin which disrupt normal body functioning (Roberts & Greenwood, 2003).

Salmonella is the most common foodborne illness causative agent for animal based foods such as raw meat and meat products. It is specially a food risk for food that is slightly heated or none at all. *E.coli* has long been noted to occur as normal flora, however, pathogenic strains do exist, and these strains are known to produce Shiga toxins which can cause severe foodborne diseases. The most common known pathogenic *E.coli* is of the 0157:H7 strain (World Health Organisation, 2018) . Most pathogenic bacteria are gastrointestinal survivors and are therefore sensitive to heat, this is why a heat treatment could significantly reduce them (Imathiu *et al.,* 2018).

Bacterial contaminants which include those of genera *Proteus, Escherichia* (Enterobacteriaceae), *Pseudomonas, and Staphylococcus, Streptococcus, Bacillus, Micrococcus, Lactobacillus, Acinetobacter* and *Listeria spp* have been noticed in edible termites (Fraqueza & Patarata, 2017).

Studies on microbial load of edible insects including edible termites are rare. Contamination however can occur through the interaction of the edible termites with the soil, during handling, storage or before consumption. A case of botulism resulting from consumption of *Clostridium botulinum* contaminated termites resulted into death of five (5) individuals reported in Kenya

(Fombong & Kinyuru, 2018). 1980). In this case, plastic bags were reportedly used to store the insects at anaerobic conditions in 4 days of transportation an environment which favored the growth of microbes. Processing of *Ruspolia differens* using conventional methods such as deep frying, toasting and smoking are effective in the removal of pathogens such as *Salmonellae* and *Listeria spp* (Imathiu *et al*., 2018).

2.6 Processing and preservation of edible termites

Though termites can be consumed raw and immediately as they emerge from the termitaries, processing plays an important role in palatability and acceptability of termite products. Processing is important also in the preservation of edible insects for later consumption or for markets. Furthermore, processing improves on the safety of edible insects from possible contaminants and health risks (Fombong & Kinyuru, 2018).

There are different processing methods that are used by varying communities. In Africa, roasting, smoking or simply sun drying is some of the common processing practices of edible termites. Termites have high oil contents, to remove the oil, people of the Central African Republic (CAR) and the Democratic Republic of Congo (DRC) squeeze the termites into a tube to form a colorless oil for frying (Fombong & Kinyuru, 2018). Table 2 is a summary of processing practices often used in Africa.

Table 2: Edible termites processing methods

Country	Processing method	Reason for Method	Reference
DRC & CAR	Squeezing in bottle	Removal of oil	(Fombong and Kinyuru, 2018)
Botswana	Roasted in hot ash and sand	For consumption	
Nigeria	Washing, salting and mild roasting/frying	Flavoring for consumption	
Benin	Remove wings then fry in palm oil	Preservation and consumption	
Kenya	Roasting, sun drying then grind	Value addition into crackers and muffins using conventional methods	
South Africa	Eaten raw from mounds	Consumption	(Netshifhefhe *et al*, 2018)
	Boil, fry, grill, roast and sun dry	Preservation and consumption	

Processing methods have been demonstrated to have the ability to reduce the microbial load of edible insects. The microbial load of insects is a result of the insect itself and cross contamination from its environment (including traders). Edible insects are often contaminated through contact with the soils during sun drying and during poor storage (Haubruge *et al*., 2017). For instance, Wanjiku, (2018) showed that most drastic increase in Total Viable Counts occurred under ambient storage conditions for adult House cricket meal (*Acheta domesticus*) in a period of 0-45 days. In a study, Haubruge *et al*., (2017) demonstrated that boiling reduces the number of enterobactericae and roasting reduced the bacterial spore load. Processing of *Ruspolia differens* using conventional methods such as deep frying, toasting and smoking are effective in the removal of pathogens such as *Salmonella*e and *Listeria spp* (Imathiu *et al*., 2018). The table below shows processing methods of various insect species and their corresponing microbial load reductions.

Table 3: Microbial load reduction by processing methods

Processing method	Insect	Microbial load reduced	Reference
Boiling	Meal worm, termites, House crickets, caterpillar	Enterobacteriaceae, yeast and mold	(Haubruge *et al*., 2017)
Roasting	Meal worm, termites, House crickets, caterpillar	Bacterial spores, yeast and mold	
Blanching	Mealworm and termites	Vegetative cells, yeast and mold	
Freeze drying and sterilization	House cricket	Total Aerobic counts, yeast and mold	
Deep frying, toasting and smoking	*Ruspolia differens*	*Salmonella* and *Listeria spp.*	(Imathiu *et al*., 2018)

2.7. Food safety and hygiene knowledge and practices of edible insect traders

The intent of the food industry is to provide acceptable nourishing food to its consumers. Consumers are equally in order to demand safe food to eat from the industry (EUFIC, 2013). This provision of wholesome and acceptable contaminant free foods can be challenging because contamination happens at all stages (Production to consumption) in the value chain of any food commodity. Food contamination may occur at any point during its journey through production, processing, distribution, and preparation (CDC, 2017). Mudey, *et al,* (2010) Show that the risk of contamination is dependent to a large extent on food hygiene practice and knowledge of food handlers, their health status and personal hygiene.

Foodborne illnesses have continued to be threatening in both first and third world countries. With globalization, it has become easy to transmit these diseases across borders with food as the main vehicle (Kaferstein, *et al,* 1997). In most cases, foodborne illnesses are transmitted by intrinsic food factors and also external factors such as hygiene practices of the handlers and the environment were in the food is produced, traded, prepared or even consumed. With this, food hygiene is not the only necessity in providing a safe food. The term Food Safety is now popularly used in place of food hygiene to denote that food is harmless when consumed. The World Health Organization (WHO) defines Food safety as the "condition and measures that are necessary during production, processing, Storage, distribution and preparation of food to ensure that it is safe, sound, wholesome and fit for human consumption" (WHO, 2020). Food safety has remained a matter of concern for all professionals in the food service industry due to its ability to have substantial costs on individuals and the economy of a country in cases of foodborne disease outbreak.

Take for instance, the WHO investigated 31 global hazards which caused about 600 million foodborne illnesses in 2010 (WHO, 2015). Other than taking 420,000 lives, these foodborne illnesses resulted into 33 million Disability Adjusted Life Years (DALYs). In this, Africa was the highest burden bearer of foodborne diseases per population. While all ages are victims of this scourge, children under five and people living in low-income countries are at highest risk (Sockett and Rodgers, 2001). Though mainly not responsible for the contamination of the food, children under five accounted for over 40% of the global foodborne disease burden in 2010 (WHO, 2015). This therefore shows how that food safety is of a substantial public health concern.

This disease burden can be considerably reduced with sufficient knowledge in food safety and hygiene and its practice by the food handlers from farm to folk. In most African countries as reported for Nigerian rural and sub urban regions, Lack of essential facilities and adequate sensitization or awareness on the importance of food safety and its observance may answer for the high foodborne illness burden experienced in Africa. Though procedures and practices such as the WHO's five Keys to Safer Food, Hazard Analysis Critical Control Point (HACCP) and several sanitation standards proposed that ensure food safety have been developed, their adoption and enforcement in Africa is limited. In food production and marketing, quality of raw material, personal hygiene of the food handler, the sanitation of the environment where the food is stored and exposed for sale as well as the implements used in preparation and marketing are the major components of sanitation. Once these standards are compromised, food can be contaminated with harmful components which may result in food poisoning.

2.7.1 Contamination routes of open air traded foods

Contamination routes are pathways by which food contaminants can find their way into the food. In food value chains (production, processing, marketing and consumption) contaminants may access food and its environment through Raw material, Personnel and Implements

(Croner-i, 2018). Many of these contaminants (physical, Biological and chemical) of food occur naturally in aquatic and general environments, and may be transmitted onto food before harvest, during and after processing. Furthermore, contamination of food can occur through the air. Here, dust particles or aerosols may be suspended in the air especially when contaminated surfaces or even floors are sprayed forming suspensions of microbial, chemical and/or physical droplets (Morgenstern, 2020). Water is also a vehicle for transmission of many agents of diseases (Dun-Dery, 2013) especially in open air markets.

In Africa, the open air market traded edible insects and termites in particular are wild harvested. The sanitation system in Africa is poor, 215 million people are estimated to engage in unsanitary practices such as open defecation in sub-Saharan Africa (Galan, *et al*, 2013). During the raining season, flood waters are polluted with animal and human excreta which will subsequently contaminate edible insect sources (termite mounds in the case of edible termites). This stands as a possible source of bacteria of the family Enterobacteriaceae which are gastrointestinal and discharged into the environment through defecation. High levels of fecal coliforms have been reported to occur in vegetables such as lettuce, cabbage and carrots (Newman, 2005). The fecal coliform is suspected to have originated from waste water applied to the vegetables before harvest, or during sell. This practice can cause significant microbial contamination (Malm, *et al* 2015). This risk however may be minimized for edible insects which are sold dry. Pathogenic bacteria are naturally present in aquatic environments (*Clostridium botulinum* type E, pathogenic *Vibrio sp., Aeromonas*) and the general environment (*Botulinum* type A and B, *Listeria monocytogenes),* other microorganisms are of the animal/human reservoir (*Salmonella, Shigella, E.coli*, enteric virus) (Dun-Dery, 2013). Because food contaminants are ubiquitous from farm (production) to folk (consumption), it is possible that they could be introduced into the food through any of the discussed means.

Food can be contaminated easily by surfaces they come in contact with, especially when exposed to the atmosphere. Containers, pumps or tanks used for holding or transporting food resources for open air market trade are rarely cleaned or disinfected (Croner-i, 2018). Other than transport, food resources such as edible termites are displayed on woven African baskets or sacks. These too are barely cleaned or disinfected. As shown above, food handlers can be a route of contamination. Transfer of microorganisms by personnel particularly from hands, is of vital importance (CDC, 2017). Food handler malpractices in this case open air market traders is the common risk factor for foodborne illnesses (CDC, 2017). These malpractices are reported to contribute to 97% of foodborne illness in the food service industry (Howes, *et al*, 1996).

Low infectious doses of organisms such as *Shigella* and pathogenic *Escherichia coli* have been linked to hands as a source of contamination (Dun-Dery, 2013). Poor hygiene, particularly deficient or absence of hand washing has been identified as the causative mode of transmission. Proper hand washing and disinfection has been recognized as one of the most effective measures to control the spread of pathogens, especially when considered along with the restriction of ill workers (CCOHS, 2020)

2.7.2 Procedures for food quality assurance

Ultimately, it is the implementation and application of acquired knowledge in general preventative measures that guarantees production of safe food (Dun-Dery, 2013). General preventative measures here refer to procedures, processes, controls and other precautions that exclude, prevent, minimize, and inhibit product failures so as to yield safe, suitable foods of uniform quality, according to their intended use. General Preventive Hygiene as a process and practice is part of General Preventive Management concerned with general hygiene, microbial safety and product spoilage. In large food industries, a systematic approach to identification, assessment and control of hazard during production, processing, manufacturing, preparation and use of food, water or other substances is observed. However, in open air markets, this systematic approach is not adhered to. Thus, to ensure food safety of open air traded food commodities including edible termites, good hygiene, cleaning and sanitation are necessary to secure low levels of microorganism on the food product and this should be the direct responsibility of hygiene authorities (Murefu, *et al*, 2019). Interestingly however, Huss (1997) is cited to have shown that this might be difficult in practice (Dun-Dery, 2013). He claims that "the more you clean the more you have" food Hazard. However, this phrase is used to mean, strict hygiene during manufacture of food products may decrease the risk from one pathogen and increase the risk of contamination from another (Dun-Dery, 2013). Nevertheless, the quality of food at every point of the food value chain should be paramount for all its handlers and even legislators. The safety of food is therefore assured not only by personal hygiene of handlers which prevents introduction of microorganisms but also by other practices that prevent/slow growth of existing microbes (Dun-Dery, 2013).

2.8. Conceptual Framework

The relationship between dependent and independent variables of this study are expressed in the conceptual framework below (figure 1). Through quantitative analyses of the data, outcomes to the objectives will be ascertained.

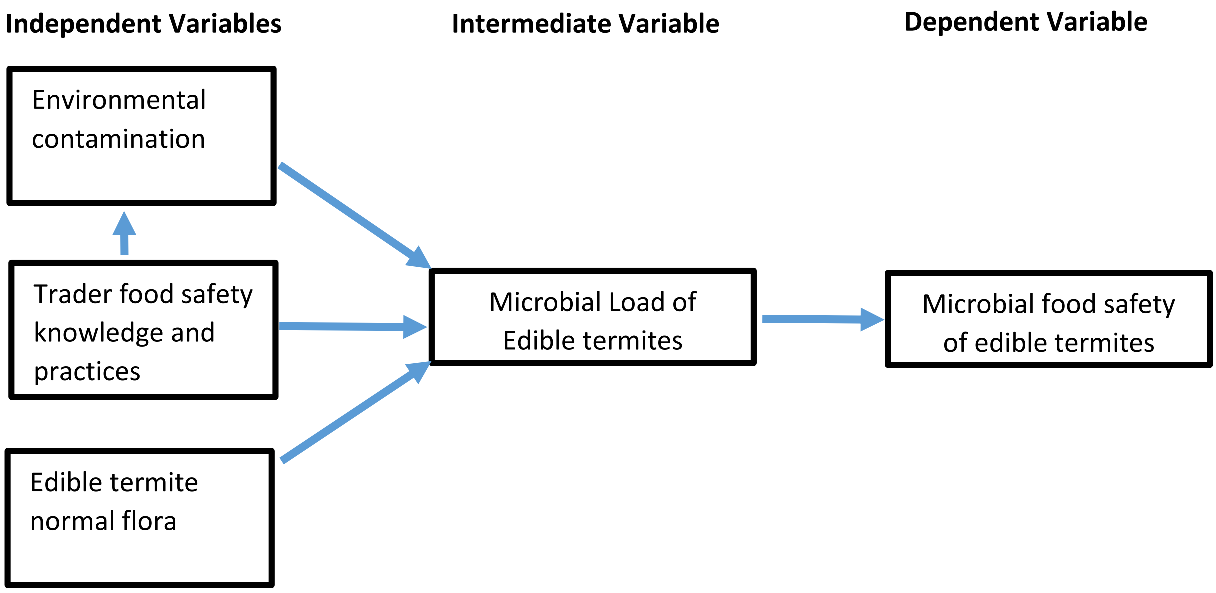


Figure 1: Conceptual framework on edible termites Microbial Load traded on Open air markets. Source; author, (2019)

The relationship between factors that influence microbial loads of edible termites and their safety are presented in the framework above. Intrinsic and Extrinsic parameters affect the microbial load of food. Physical and chemical properties of foods that either facilitate or inhibit the growth of microbes are intrinsic factors. The physiochemical properties of food can however be altered through various processing/preservation methods by traders leading to an alteration of the microbial load in the food substance. Extrinsic factors of food are those factors present in the immediate environment of the food substance. Harvesting, storage and display, atmospheric conditions and processing/preservation methods have an impact on the extrinsic parameters of the food substance.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Description of Study Sites

3.1.1 Agro-ecological Characteristics of Kitwe District

A survey was conducted in Kitwe District, Copperbelt Province of Zambia at Chisokone market. The landscape around Kitwe is a mix of woodlands, dambos, farmland and rivers. The major river (Kafue River) flows along Kitwe's eastern and southern edges (Mwitwa, *et al*, 2016). It is the second largest city in Zambia with a population at 517,543 people according to the 2010 census projected at 710,000 people in 2021 (Central Statistics Office, 2011). Kitwe city lies between 12^{o} and 13^{o} south and 27^{o} to 29^{o} east. The mean altitude of Kitwe is about 1295 meters above sea level. Kitwe district experiences both wet and dry seasons and extreme temperatures ranging between 29^{o}C and 32^{o}C in hot seasons and 9^{o}C to 14^{o}C in cold seasons (Mwitwa, *et al*, 2016).

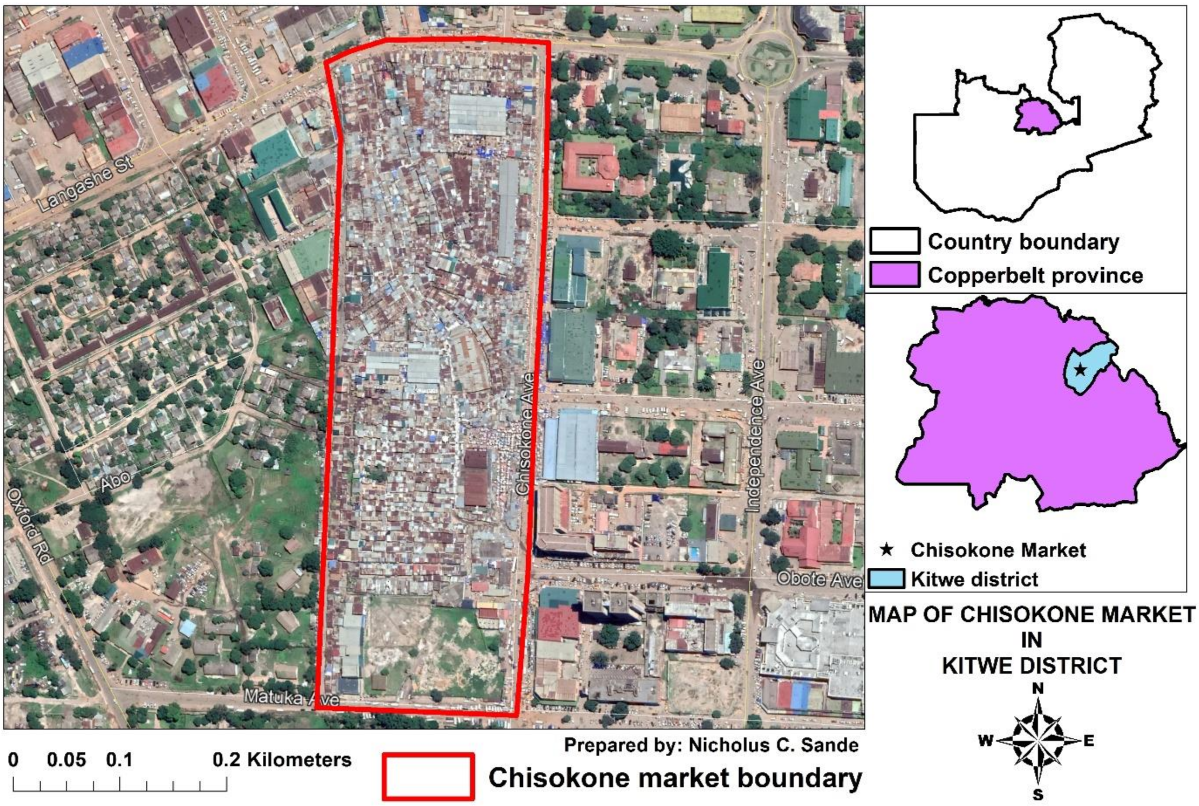


Figure 2: Map of Chiskone Market in Kitwe district

3.1.2 Agro-ecological Characteristics of Serenje District

The collection sites of edible termites in Serenje district included Kwa Kaseba (13^{o}31'00.1"S, 30^{o}21'29.8"E), Kaseba turnoff (13^{o}21'15.5"S, 30^{o}23'28.1"E) and Kwa Kamena (13^{o}27'15.5"S, 30^{o}21'29.8"E) located 50 km, 40km and 35km respectively from Serenje town in the central province of Zambia. The traders from each site transported the termites from the sites for resale at Serenje market displayed at stands 1(13°13'56.2"S, 30°14'01.6"E), stands 2 and 3 (13°14'00.5"S, 30°13'54.2"E) (Coordinates were obtained using whatsapp GPS live locations). The market is located in the town center and is characteristic of most African Open air markets on which edible insects are traded.

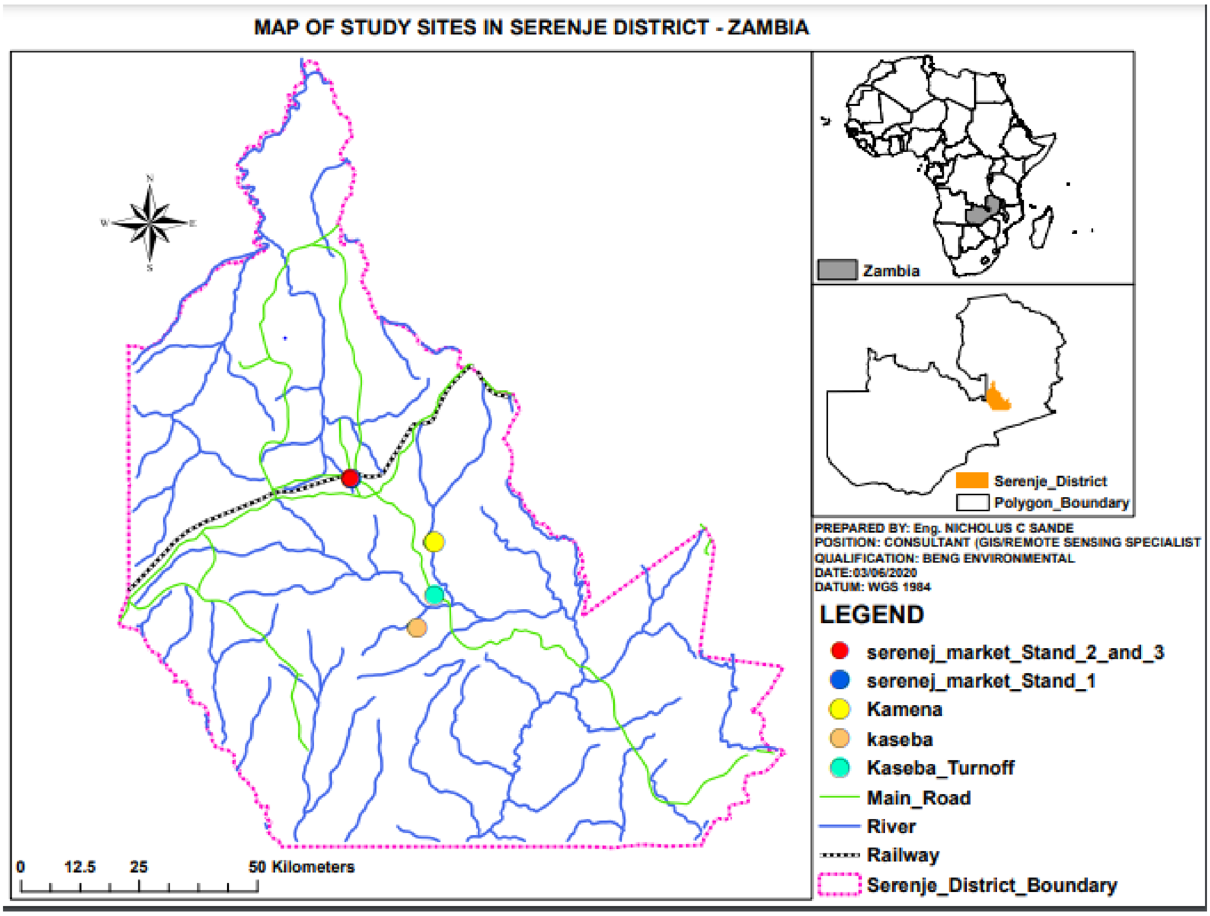


Figure 3: Map of the sample collection sites Serenje

Serenje District is located in an agro-ecological region II of the country. This region is characterized with an annual rainfall of 1000 to 1500 mm. Due to these high rainfalls, the soils are leached and have strong acidity, high aluminum toxicity, poor nutrient reserves and low nutrient retention capacity (FAO, 1998). The Districts vegetation is mainly Miombo (Lumbwe, 2012). The Miombo vegetation is a woodland characterized with Brachystegia and Julbernardia spp that form a light closed canopy (Lawton, 1978).

3.2 Objective 1: Assessment of common food safety and hygiene knowledge and practices of edible termite traders in Chisokone open air market Kitwe.

3.2.1 Study design and sample size

A cross sectional descriptive design was used with a purposive sampling criteria of all termite traders of Chisokone market of Kitwe district Zambia was conducted. The Market was purposively sampled due to its volume of edible termites traders and traffic of customers for edible termites. Complete enumeration of edible termite traders of Chisokone was performed. The local market authorities could not provide an estimate number of edible termite traders. As such, traders who displayed termites where asked to identify other traders who trade or had traded in edible termites in a snowball method until saturation. With this Method, a total of 26 traders were identified and interviewed face to face. The limitation is that we cannot be certain that 26 is exhaustive of all termite traders of chisokone market owing to recall bias amongst traders.

3.2.2 The Survey tool

A questionnaire with structured questions (Appendix 1) was used to collect data about the hygiene and food safety knowledge and practices of edible termite traders. Face to face interviews were conducted. Hygiene and Food Safety practices of the traders were identified. Demographics, knowledge on Food safety and hygiene, Legislation and Food safety and hygiene practices were the sections of the questionnaire.

3.2.3 Data Analysis

For objective one (1), Data organization, visualization and descriptive statistics was done using the Microsoft excel (2013) and Statistical Package for Social Sciences (SPSS), v20 packages for survey data. Simple frequency tables were generated and Fishers exact test was used to demonstrate relationships between categorical variables at 95% confidence interval and level of significance set at p-value ≤ 0.05 for all inferential analysis.

3.3 Objective 2: Determining prevalence of indicator microorganisms in Open air traded edible termites in Serenje District.

3.3.1 Study Design and sampling

For microbiological analysis, a Completely Randomized Block Design was set with Microbial Load as the response variable. The samples assessed for microbiological prevalence were purchased from three (3) randomly selected traders of Serenje market. Three (3) sample replicates (morning, afternoon and evening) for each sampling point (collection sites, Transport bags and display) from each trader were collected during the data collection period (09/01/20 to 15/01/20). Serenje is a termite collection hotspot.

Sources of Microbial load variations (treatments) included the collection process, transportation, and display. The different collection sites (Kwa Kaseba-Site 1, Kaseba turnoff-Site 2 and Kwa Kamena- Site 3) were the blocks. 9 samples were collected from each site that is, three (3) from each handing point. Three experimental units (morning, afternoon and evening) were established for each sampling point in order to reduce on variability in results of the microbial load of the treatments. At display, the termites were sampled 2 days after arrival in the market area. A total of 27 samples were collected. For each collection, samples were packed and sealed to prevent further contamination in a sterile bag, transported and stored at refrigeration temperatures (2-8^{o}C) in the TDRC laboratories until culture.

3.3.2 Species Identification

The termites were identified to genera level using the salient morphological characteristics of randomly selected soldier termites collected from each collection site. The heads of the soldier termites were viewed under the dissection microscope (x40) at TDRC labs to assess salient morphological characteristics which included compound eyes, mandibles shape of pronotum, fontanelle and the labrum. Identification was based on the soldier caste key to termite genera as described by Mitchell, (1980).

3.3.3 Termite Sample preparation for Microbial analysis

A sterile blender was used to homogenize 25g of termite samples in 225ml sterile normal saline. The blender was sterilized between each sample homogenization period to avoid cross contamination. A milliliter of the homogenized sample was transferred into 9ml of sterile normal saline and serial dilutions continued till a 10^{-6} concentration was obtained. For each handling point, 3 replicates of samples were collected to minimize random errors in data collection.

3.3.4 Media Preparation

3.3.4.1 Procedure for Preparation of Media

MacConkey agar (MAC), Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) was prepared by suspending 50 g, 38 g and 65 g respectively of dehydrated medium in separate

1000 ml of distilled water. The mixtures were heated to boil until the media were completely dissolved before autoclaving at 15lbs pressure (121^{o}C) for 15 minutes to sterilize. The media were left to cool to ~45 to 50^{o}C and poured into petri plates as described by Aryal, (2020).

3.3.4.2 Media Quality control- Sterility tests

Media sterility tests were performed by incubating uninoculated plates MAC and MHA at 37^{o}C for 48 hours and SDA at room temperature (25^{o}C) for 5 days (Aryal, 2020). The entire batch was used after passing sterility tests (Aryal, 2020).

3.3.4.3 Media Quality control- Performance tests

Pure strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) obtained from TDRC microbiological research and clinical laboratory were inoculated onto MAC and MHA for 24 hours at 37^{o}C as positive control for lactose fermentation, positive control for non-lactose fermentation and control for gram positive bacteria respectively. MAC agar was used when performance control plates indicated pink colonies for lactose fermenting *E. coli*, colonies without pigmentation for *P. aeruginosa* and no growth for *S. aureus.* MHA agar was used when performance control plates indicated pale straw colored colonies, straw colored colonies and cream colored colonies for *E. coli, P. aeruginosa* and *S. aureus* respectively (Aryal, 2020).

The SDA performance quality control was performed by inoculating *Candida albicans* (ATCC 16404) and *Aspergillus brasiliensis* (ATCC 16404) incubated at room temperature (25^{o}C) for 5 days. Media with cream colonies of *C. albicans*, white mycelium and black spores for *A. brasiliensis* were used (Aryal, 2020).

3.3.5 Quantitative Enumeration and isolation of Microorganism

All Samples (n=27) were inoculated on fresh Mueller Hinton Agar (MHA) and MacConkey Agar (MAC) in 90x15 mm petri dishes. Inocula of 0.1 ml from 4th to 6th dilutions were evenly spread on petri dishes using a sterile metal spreader. The MHA and MAC were incubated at 37^{o}C for 24 hours for enumeration of total viable counts and *Enterobacteriaceae* respectively.

The bacterial colonies were counted on establishment of growth and divided with the product of the dilution factor and the plated volume to obtain viable cells expressed as colony forming units per gram (cfu/g) of the sample (Jersek, 2017). The total load was then recorded as a logarithm of the coliform forming units per gram (Log_{10} cfu/g).

The microbial counts were obtained using the formula below;

$$Microbial\ Load = \frac{number\ of\ colonies\ counted}{liquid\ plated\ X\ dilution\ factor}$$ *Equation 1: Formula for determination of microbial load*

(Jersek, 2017)

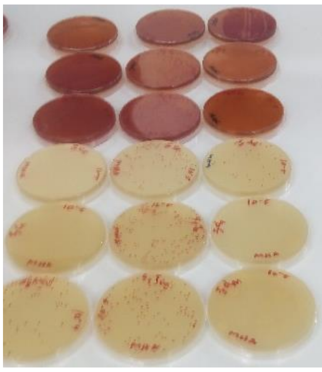
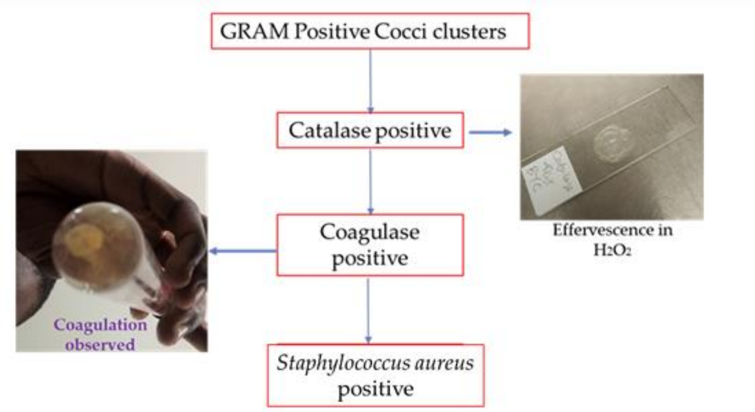


Figure 4: Plates with 10^{-4} to 10^{-6} dilution and incubated at 37°C.

3.3.6 Qualitative isolation of pathogenic microorganisms

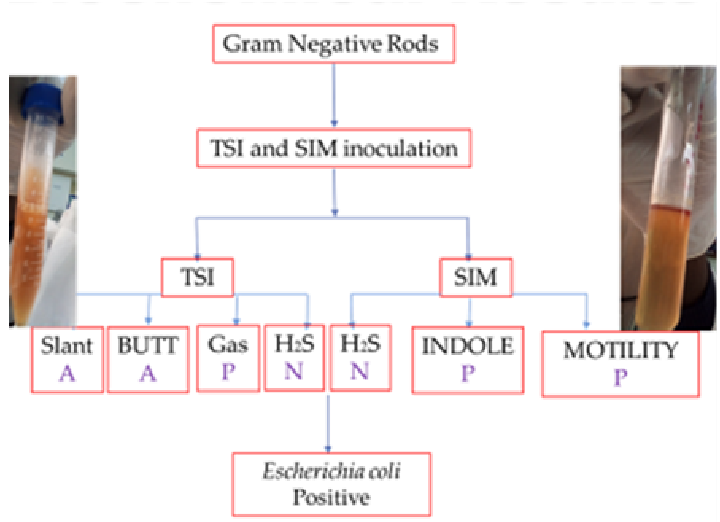
A randomly selected colony plate with growths from the display point cultures of Site 1 were examined for presence of presumptuous *Escherichia coli*, *Staphylococcus aureus* and mold and yeasts. Presumptive *E. coli*, a lactose fermenting microbe was identified macroscopically by its pink colonies on MAC and pale straw colonies that appear on MHA. Presumptive *S. aureus* colony was identified on MHA by its cream colonies that it forms on MHA. Isolates of presumptive colonies were cultured on MHA as pure cultures. Pure cultures of the presumptive isolates were further characterized by gram stain (*E.coli*- gram negative, *S. aureus*-gram positive*)* microscopic cell morphology (*E.coli*- rod shaped, *S. aureus*- cocci in clusters) and biochemical methods [TSI/SIM for *E.*coli and catalase and coagulase for *S. aureus*] (Hologic, 2016; Zhang, et al., 2016).

Sabourauld Dextrose Agar (SDA) was inoculated with randomly selected display point cultures of Site 1 and incubated for 120 hours at room temperature (20-25°C) to enumerate mold and yeasts. Presumptive yeast and mold were isolated on SDA. Identification was based on macromorphology (colony – yeast, filamentous growth- mold) and micromorphology (Single cells – yeast, branching hyphae - mold), and reverse and surface coloration of molds on SDA. Germ tube test was used to rule out *Candida albicans* through inoculation of presumptive colonies in serum and incubated at 37°C for 4 hours. The serum tube was left overnight because no germ tubes grew in the initial 4 hours.



Source: Author

Figure 5: Gram positive bacteria identification flow chart



Source: Author

Figure 6: Gram negative bacteria identification flow chart

3.3.7 Yeast and mold identification

Sabourauld Dextrose Agar (SDA) was inoculated with randomly selected display point cultures of Site 1 and incubated for 120 hours at room temperature to enumerate molds and yeasts. Presumptive yeast and molds were isolated on SDA. Identification was based on macro-and micromorphology and reverse and surface coloration of moldy colonies on SDA. Germ tube test was used to rule out *Candida albicans* through inoculation of presumptive colonies in serum and incubated a 37°C for 4 hours 24 hours after negative results. The serum tube was left overnight because no germ tubes grew in the initial 4 hours. A wet mount of a presumptive yeast colony was observed under low power magnification (X40). Molds were stained with lactophenol blue and visualized under the low power (X40) objective.

3.3.8 Data analysis

All microbial counts were expressed as $\log_{10}$ cfu/g. Statistical analysis was performed using R statistical package version 3.5.3 for microbiological data. The Boxplot and Histograms where used as the graphical representation of data to show normality. Homogeneity of variances and normality tests were performed to test ANOVA assumptions. A two way ANOVA was performed to compare microbial load means across the handling points (Collection Site, after transportation and at display point) and three sites (Site-1, Kwa kaseba, Site-2, kaseba turnoff and Site-3, Kwa Kamena). For all analyses, means were separated using Bonferroni adjustment at 95% confidence level. Statistical results are presented in section 4.4.1.

CHAPTER FOUR:

4.0 RESULTS

4.1 Demographic Information of participants

The table (4) below indicates demographic categorical characteristics of edible termite traders of Chisokone market. The characteristics includes gender, age in years covering years from 15 to greater than 41, marital status, educational status and yeas spent as an edible termite trader.

Table 4: Demographic Characteristics of Edible termite traders at Kitwe Chisokone Market

Characteristics	Frequency	Percent	Cumulative percent
Gender			
Female	18	69.2	69.2
Male	8	30.8	100.0
Total	26	100.0	
Age (years)			
15 to 20	2	7.7	7.7
21 to 30	6	23.1	30.8
31 to 40	7	26.9	57.7
>41	11	42.3	100.0
Total	26	100.0	
Marital Status			
Single	6	23.1	23.1
Married	20	76.9	100.0
Total	26	100.0	
Education level			
No Formal Education	2	7.7	7.7
Primary	12	46.2	53.8
Secondary	11	42.3	96.2
Tertiary	1	3.8	100.0
Total	26	100.0	
Years as trader			
First Year	2	7.7	7.7
1 to 5	7	26.9	34.6
6 to 10	9	34.6	69.2
>10	8	30.8	100.0
Total	26	100.0	

Demographic results of edible termite traders of Chisokone market.

As Table 4 above shows, the majority (69.2%) of participants were female compared to males (30.8%) in a ratio of 18:8 respectively. Only 7.7% of the participating traders were in age range of 15-20 years. Even though a 10 year interval age range of 21 to 30 years and 31 to 40 years had a 50% representation, participants above the age of 40 (>40) had the highest representation (n=11, 42.3%). Most of the participants (76.9%) were married and others (23.1%) were single, however, the reason for which they were single (that is divorced, widowed e.t.c) was not inquired. The majority (n=12) of the respondents had a primary school education one more than those with a secondary education (n=11). 7.7% of the respondents did not have a formal education while 3.8% of respondents had a tertiary education. Those traders who had spent less than one year (<12months) trading in edible termites were scored as first year edible termite traders. These were 7.7% (n=2). 26.9% of respondents had spent at least one year and at most five years as edible termite traders. From years 6 to 10 of trading in edible termites, 34.6% traders were recorded as the majority. The other traders (n=8, 30.8%) had spent more than 10 years (>10 years) in the edible termite trading business.

4.2 Food safety and hygiene knowledge

The food safety and hygiene knowledge of the traders was inquired focusing on cross contamination, control of microbial growth and foodborne illnesses. Table 5 summarizes the findings.

Table 5: Food safety and hygiene knowledge of edible termite traders

Response	Frequency	Percent	Cumulative Percent
Knowledge on cross contamination			
I have received food safety and Hygiene training			
No	24	92.3	92.3
Yes	2	7.7	100.0
Total	26	100.0	
Washing hands can prevent microbial contamination of food			
False	1	3.8	3.8
True	22	84.6	88.5
I don't know	3	11.5	100.0
Total	26	100.0	
Covering food can prevent cross contamination of food			
False	1	3.8	3.8
True	24	92.3	96.2
I don't know	1	3.8	100.0
Total	26	100.0	
What is the purpose of wearing gloves during handling of food?			
Protect handler from contamination	3	11.5	11.5
Protect food from contamination	16	61.5	73.1
Both of the above	7	26.9	100.0
Total	26	100.0	
People, insects and mixing of different food stuff can cause transfer of bacteria			
False	1	3.8	3.8
True	24	92.3	96.2
I don't know	1	3.8	100.0
Total	26	100.0	
Cross Contamination is the transfer of harmful microorganisms from?			
Food to food	1	3.8	3.8
Person to food	1	3.8	7.7
Surface to food	2	7.7	15.4
All of the above	22	84.6	100.0
Total	26	100.0	

Knowledge on control of microbial growth on food			
Why should the insects be dried before storage?			
Increase shelf Life	18	69.2	69.2
Prevent microbial growth	0	0	69.2
Both of the above	7	26.9	96.1
I don't know	1	3.9	100.0
Total	26	100.0	
Why should your storage room have low moisture content?			
Prevent microbial growth	17	65.4	65.4
To make it look clean	1	3.8	69.2
I don't know	8	30.8	100.0
Total	26	100.0	
Growth of most bacteria can be controlled by reducing temperature			
False	4	15.4	15.4
True	11	42.3	57.7
I don't know	11	42.3	100.0
Total	26	100.0	
Knowledge on food borne illnesses			
Food poisoning is the illness resulting from eating food containing biological or chemical toxins			
False	2	7.7	7.7
True	21	80.8	88.5
I don't know	3	11.5	100.0
Total	26	100.0	
Food Borne Diseases can result only from chemical contamination			
False	10	38.5	38.5
True	7	26.9	65.4
I don't know	9	34.6	100.0
Total	26	100.0	
Typhoid and other diarrheal diseases are illnesses caused by bacteria			
False	1	3.8	3.8
True	7	26.9	30.7
I don't know	18	69.3	100.0
Total	26	100.0	

According to table 5, out of a total of 26 respondents, 92.3% had not received any training on food safety and hygiene and only 7.7% had some kind of that training. Table 5 also shows that the majority, 84.6% (n=21) of the traders knew that Washing hands can prevent microbial contamination of food, while 11.5% (n=3) had no idea whether washing hands can or cannot prevent microbial contamination of food. 92.3% (n=24) of the traders know that covering food can prevent microbial contamination. A cumulative total of 73.1% of traders thought that gloves were worn for either protecting handlers from contamination or protecting food from contamination (n=3 and n=16 respectively) while only 26.9% (n=7) know that gloves were worn to protect both the food and the handler from contamination. Twenty four (24) respondents (92.3%) knew that people, insects and mixture of different food stuffs can cause transfer of bacteria. The apparent knowledge on cross contamination is probably because a majority 84.6% (n=22) of the traders know that cross contamination was the transfer of harmful microorganisms from food to food, person to food and surface to food.

Only 26.9% (n=7) had the knowledge that drying insects was for purposes of increasing shelf life and prevention of microbial growth while the majority 69.2% (n=18) thought that drying insects was only for purposes of increase in shelf life. However, the majority (n=17, 65.4%) recognized that lower moisture contents prevented microbial growth on food. Nonetheless, 30.8% (n=8) did not know whether or not lower moisture contents prevented microbial growth. On temperature, 42.3% (n=11) of traders thought that reduction of temperature could control growth of microbes on food and an equal number did not know whether or not reduction of temperature could control microbial growth.

With regards to food poisoning, the majority 80.8% (n=21) know that food poisoning is caused by both biological and chemical toxins. However, only 38.5% (n=10) of edible termite traders know that food borne diseases are not only a result of chemical contamination while 34.6% (n=9) did not know whether chemical contamination of food was the only reason for foodborne diseases or not. Further, only 7 (26.9%) of the edible termite traders knew that typhoid and other diarrheal illnesses were caused by bacteria and the majority 69.2% (n=18) had no idea whether bacteria caused the diarrheal illnesses or not.

4.3 Food safety and hygiene practices

Edible termite traders of Chisokone market trade in more food items than one. Figure 6 shows proportions of edible termite traders and the food items they trade in.

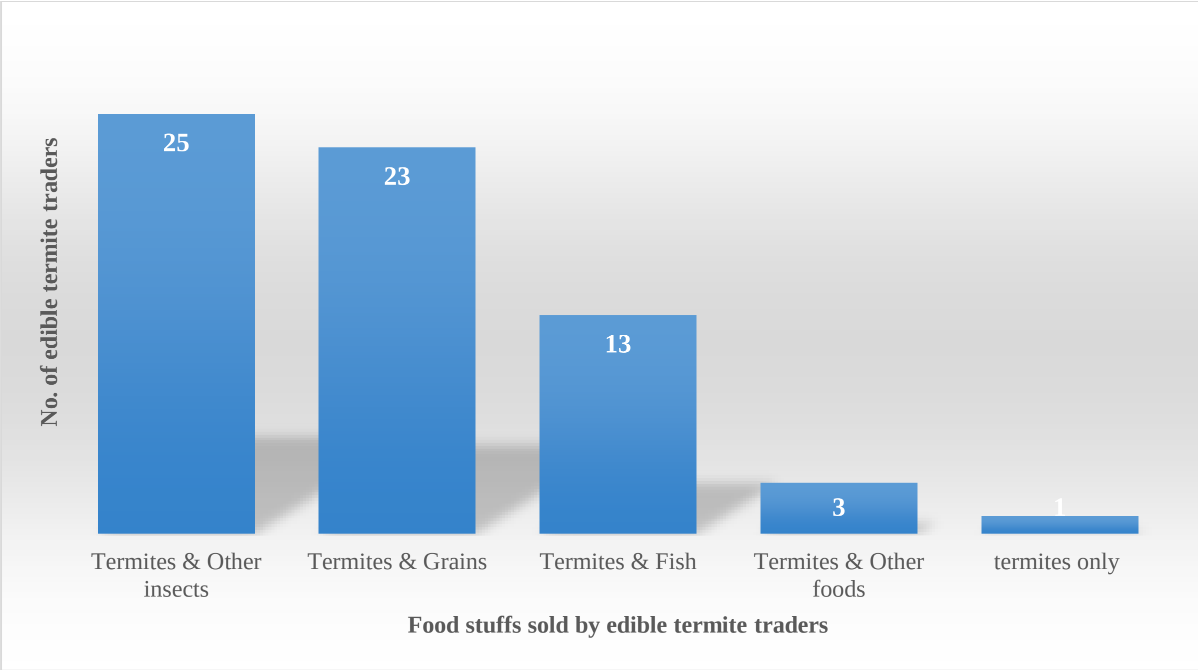


Figure 7: Other food stuffs sold by edible termites' traders

As presented in figure 6, 25 edible termite traders also sold other edible insects. 23 of the edible termite traders also sold grains. The edible termite traders that also sold Fish were 13 whereas 3 edible termite traders sold other foods. Only one trader exclusively traded in edible termites.

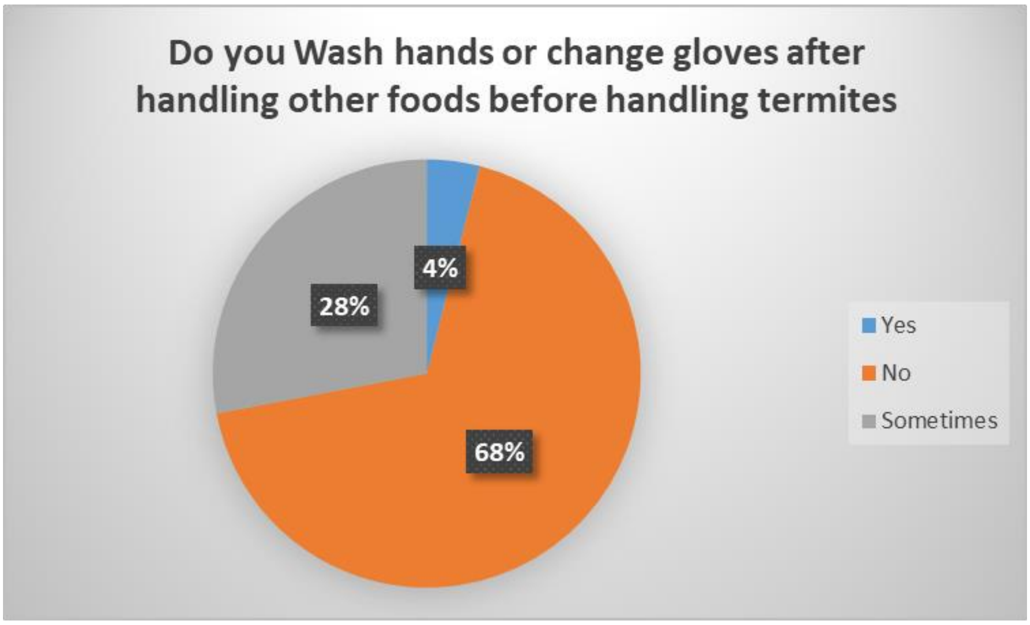


Figure 8: Figure shores washing of hands or glove changing before handling of edible termites

Majority (68%) of the edibale termites' traders did not wash their hands or change gloves after handling other foods and money before handling edible termites. Only 4% (n=1) indicated that they either washed their hands or changed gloves when alternating between commodities they sold.

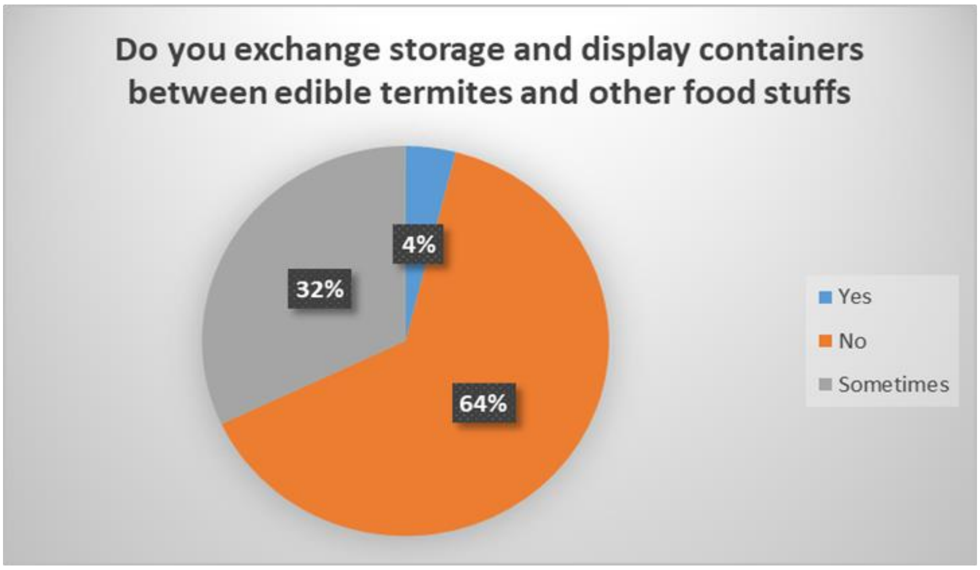


Figure 9: Exchange of storage and display containers between edible termites and other food stuffs

Most (64%) of the traders did not exchange storage and display containers between edible termites and other food stuffs sold. 4% (n=1) of traders used the same storage and display containers for their commodities while 32% would occasionally exchange the containers amongst commodities.

Table 6: Food safety and hygiene practices of edible termites traders

Question	Category	Response (n)	Percentage (%)
How often do you wash your hands with soap and water when handling your insects	Never	14	56
	Sometimes	8	32
	Always	3	12
How often do you wear gloves when handling your insects	Never	18	69
	Always	1	4
	When customer demands	7	27
How often do you use a mask when handling your insects	Never	21	81
	Always	1	4
	When customer demands	4	15
How often do you clean your implements before sale	Never	1	4
	Sometimes	8	31
	Always before use	7	27
	When I see dirt	10	38
How often do you clean your storage and sale area	Never	1	4
	Sometimes	2	8
	Always	22	84
	When demanded by Law	1	4

According to table 6, a large percentage (56%) of edible termite traders never washed their hands with soap when handling the termites. An even higher percentage (69%) of the traders never used gloves. Interestingly, 27% (n=7) indicated that they used gloves only when demanded by a customer. On wearing masks, 81% (n=21) never use masks during sell of termites while 4 (15%) could use them when demanded by customers. Table 6 further shows that, most traders (84%, n=22) always cleaned their storage and sale area while 1 (4%) stated that they only cleaned when the law demanded so.

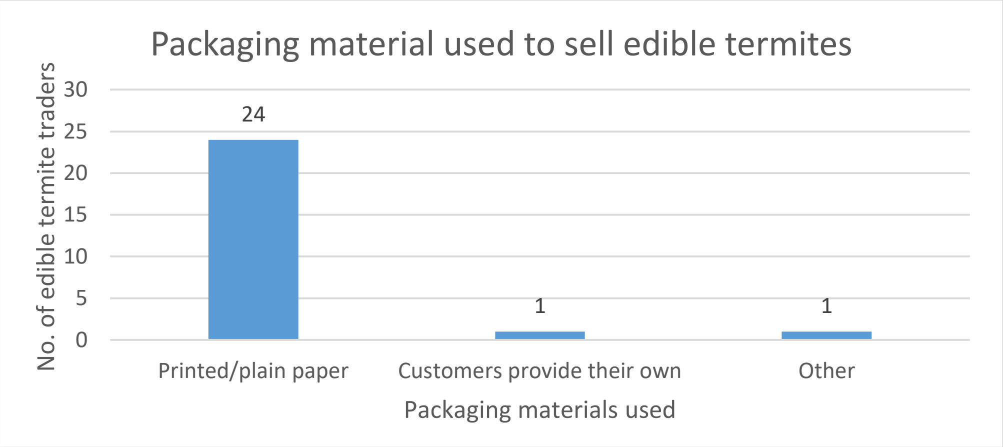


Figure 10: Packaging material used to sell edible termites

As in figure 10, 92% (n=24) of the traders used printed/plain papers to sell their edible termites while an equal percentage (4%, n=1) used other forms of packaging material and let customers provide their own.

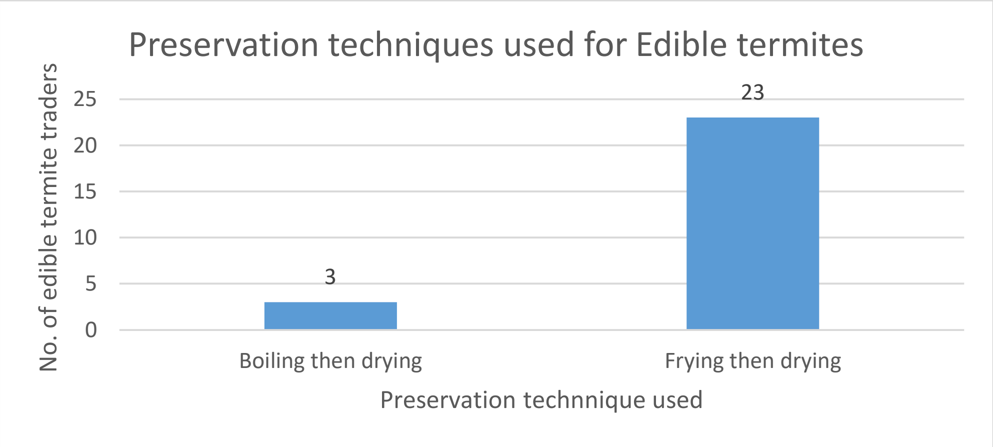


Figure 11: Preservation techniques used for Edible termites

Majority of the traders (88%, n=23) at Chisokone market fry the termites in their own body fats then dry termites to preserve them. Others (12%, n=3) boil them before drying them. None of the traders sold fresh termites due to their short shelf life in their unprocessed state.

Table 7: Association between demographic variables and categorical knowledge items

Variable	Level	Responses				p-value*
		Purpose of wearing gloves				
		Protect handler from contamination	Protect Food from contamination	Both of them		
Gender	Female	0 (0.0%)	13 (72.2%)	5 (27.8%)		0.027
	Male	3 (37.5%)	3 (37.5%)	2 (25.0%)		
		Cross contamination is the transfer of harmful microorganism from?				
		Food to food	Person to food	Surface to food	All of them	
Age	15 to 20	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0.013
	21 to 30	1 (16.7%)	0 (0.0%)	0 (0.0%)	5 (83.3%)	
	31 to 40	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (100.0%)	
	>41	0 (0.0%)	0 (0.0%)	1 (9.1%)	10 (90.9)	
		Food poisoning is the illness resulting from eating food containing biological or chemical toxins				
		False	True	I don't know		
Age		1 (50.0%)	0 (0.0%)	1 (50.0%)		0.041
	21 to 30	0 (0.0%)	6 (100.0%)	0 (0.0%)		
	31 to 40	1 (14.3%)	5 (71.4%)	1 (14.3%)		
	>41	0 (0.0%)	10 (90.9%)	1 (9.1%)		
		Covering the food can prevent cross contamination				
		False	True	I don't know		
Age	15 to 20	1 (50.0%)	1 (50.0%)	0 (0.0%)		0.040
	21 to 30	0 (0.0%)	5 (83.3%)	1		
	31 to 40	0 (0.0%)	7 (100.0)	0 (0.0%)		
	>41	0 (0.0%)	11 (100.0%)	0 (0.0%)		
		Growth of most bacteria can be controlled by reducing temperature				
		False	True	I don't know		
Education Level	No formal education	2 (100.0%)	0 (0.0%)	0 (0.0%)		0.002
	Primary	1 (8.3%)	2 (16.7%)	9 (75.0%)		
	Secondary	1 (9.1%)	8 (72.7%)	2 (18.2%)		
	Tertiary	0 (0.0%)	1 (100.0%)	0 (0.0%)		

		Why should the insects be dried before storage				
		Increase Shelf life	Prevent microbial growth	Both of them	I don't know	
Education Level	No formal education	0 (0.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0.036
	Primary	10 (83.3%)	0 (0.0%)	2 (16.7%)	0 (0.0%)	
	Secondary	8 (72.7%)	0 (0.0%)	3 (27.3%)	0 (0.0%)	
	Tertiary	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	

*Fishers exact test values used

As shown in Table 7, A Fishers exact test revealed significant relationships between, Gender and knowledge on purpose for wearing gloves (p= 0.027); Age and knowledge on Food poisoning being a result of eating food containing biological or chemical toxins (p=0.041), cross contamination as the transfer of harmful microorganism (p=0.013) and covering food as prevention for cross contamination (p=0.040); Education level and knowledge on control of bacterial growth by reducing temperature (p=002), and reasons for drying insects before storage (p=0.036). Other demographic variables had no significant relationship (p>0.05) with the knowledge items.

4.4 Prevalence of indicator microbes in edible termites

4.4.1 Quantitative analysis of microbial loads

Total Viable Counts and Enterobacteriaceae were quantified as indicator organisms of probable contamination. The mean microbial load of both Total Viable Counts and Enterobacteriaceae was expressed as Log_{10} cfu/g and are presented in table 8 for each handling point from all 3 sites.

Table 8: Mean ($\bar{X}$±sd) microbial load of Total Viable Counts and Enterobacteriaceae per handling point of each site (n=3)

Microbial Load counts (log_{10} cfu/g)	Site 1			Site 2			Site 3		
	Collection Site	After Transport	At Display	Collection Site	After Transport	At Display	Collection Site	After Transport	At Display
Total Viable Count	8.379± 0.40^{a}	9.057± 0.13^{ac}	9.287± 0.08^{c}	7.201± 0.42^{a}	7.592± 0.13^{ac}	8.430± 0.26^{c}	6.870± 0.41^{a}	7.508± 0.30^{ac}	7.827± 0.45^{c}
Enterobacteriaceae	7.462± 0.61^{a}	8.108± 0.26^{ac}	8.537± 0.06^{c}	6.950± 0.73^{a}	7.427± 0.33^{ac}	8.168± 0.09^{c}	6.641± 0.57^{a}	7.036± 0.15^{ac}	7.686± 0.40^{c}

Numbers with different superscripts per site are significantly different

A two way analysis of variance showed that sites and handling points had a significant effect (p<0.0001 for both) on the microbial load means of each load type (Total Viable Counts and Enterobacteriaceae) without interactions. A Tukey Honest Significant Difference (HSD) showed that microbial mean loads significantly increased from the collection site to the display point for both Enterobacteriaceae and TVC. The largest increase in TVC was during the Transportation period (p=0.006) even though a significant increase (p=0.027) was recorded during transport. The largest increase in Enterobacteriaceae was during the marketing period (p=0.008). Transportation of the edible termites from collection sites to the marketing centers also showed a significant increase in Enterobacteriaceae with a p=value of 0.028. There was no significant difference in microbial load means between site 2 and 3 in both total viable counts (p=0.123) and Enterobacteriaceae (p=0.099). Total counts and Enterobacteriaaceae loads were significantly different between site 1 and site 2, and Site 1 and Site 3 with P<0.0001 for total counts, and P=0.023 (Site1 – Site2) and P<0.0001 (Site1-Site3) for enterobacteriaceae.

4.4.2 Qualitative isolation of Pathogenic microorganisms

Results from the qualitative analysis of the open air marketed edible termites showed that both bacteria and fungi are involved in the contamination of traded edible termites. The microscopic and biochemical characteristics of the isolated bacteria and fungi are presented in Table 11 and 12 respectively. These show that *E.coli, S. aureus,* yeasts other than *C. albicans* and *Zygomycetes spp*. are contaminants of wild harvested and open air traded edible termites.

Table 9: Presumptive identification of microbes from colony appearance and microscopic morphology

Colony appearance	**Media**	**Lactose fermentation**	**Gram reaction**	**Microscopic morphology**	**Presumptive identity**	**Probable source of contamination**
Small White Colony	MHA	N/A	-	Short single Rods	*Escherichia spp*	Fecal matter (Gozde and Emek, 2019)
Big Yellow colonies	MHA	N/A	+	Cocci cells in clusters	*Staphylococcus spp.*	Human handling (Wirtanen & Salo, 2016)
-Pink Colony	MAC	Lactose Fermentation	-	Short single Rods	*Escherichia spp*	Fecal matter (Gozde & Emek, 2019)
Cream White colony	SDA	N/A	N/A	Unicellular Yeast cells	Yeast	Environment (Costa, *et al*, 2010)
Filamentous mold	SDA	Non septate, ribbon-like with wide angle branching hyphae with conidia at apex			Zygomycetes *spp.*	

-, Negative; +, Positive

Cream white colonies on SDA and single celled under wet prep microscopic examination (table 11) is presumptuously identified as yeast cells. A negative germ tube confirmatory biochemical test ruled out *Candida albicans* because no germ tubes grew after inoculation in serum for 24 hours. This study however confirms the occurrence of yeast cells other than *Candida albican* in edible termites traded in open air markets through the macromophological colony identification and microscopic examination. A lactophenol blue stained filamentous mold isolated from SDA showed non-septate, ribbon-like with angle branching hyphae with conidia on the apex. This was presumptive of Zygomycetes species.

Table 10: Biochemical identification of Microorganisms

Colony appearance on media	Gram rxn	Catalase	Coagulase	Germ tube	Triple Sugar Iron				Sulphide Indole Motility			Identity of isolates
					Butt	Slant	Gas	H_2S	H_2S	Motility	Indole	
Small White Colony	-	N/A	N/A	N/A	A	A	+	-	-	+	+	*E.coli*
Big Yellow Colony	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	*S. aureus*
Pink Colony	-	N/A	N/A	N/A	N/A	N/A	+	-	-	+	+	*E. coli*
Cream white Colony	N/A	N/A	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	*Candida sp.* Other than *albicans*

-, Negative; +, Positive; A, Acidic; H_2S, Hydrogen Sulphide

Results from the qualitative analysis of the open air marketed edible termites showed that both bacteria and fungi are involved in the contamination of traded samples. Table 11 and 12 shows the microscopic and biochemical characteristics of the isolated bacteria and fungi. These show that *E.coli*, *Staphylococcus aureus,* Yeasts other than *Candida albican* and *Zygomycetes spp*. are contaminants of wild harvested and open air traded edible termites.

CHAPTER FIVE:

5.0 DISCUSSION

This study revealed that the majority of edible termite traders were female (69.2%). Mutopo, *et al* (2014) recognize that resource poor African women tend to be breadwinners of their homes, as such, they engage in small scale trades on open air markets while balancing with family care. In this study, only 7.7% of traders had received some form of training in food safety and hygiene. The World Health Organization (WHO) encourages that food handlers should receive basic training on the principles of food safety (WHO, 1998). Muinde and Kuria, (2005) revealed that food traders often have poor levels of education, are untrained in food hygiene, technology and work under unsanitary conditions. Other studies have shown that food safety knowledge and attitudes differ amongst traders based on their educational levels (Ncube, *et al*., (2020); Octavian, *et al.* (2020)). With that, it is important to enhance knowledge for a change in attitude, behaviors and practices in food safety and hygiene (Lestantyo, *et al*, 2017).

In this study, most traders revealed a knowledge of basic food safety principles, however, they lacked correct practice. For instance, 88.5% of edible termite traders of Chisokone market were aware that washing hands can prevent contamination of food whereas only 11% washed their hands with soap always. This result is consistent with Aslam, *et al*, (2021) who found that while 74% of the handlers reported correctly regarding personal hygiene, only 26% had proper practice of hand washing techniques. Amegah K (2020) found that good hygiene practice at critical times was 99% lower in food handlers with a negative attitude as compared to food handlers with positive attitudes towards hand hygiene. Proper hand hygiene is highly effective in limiting the transmission of foodborne diseases and a range of other disease conditions (Amegah, k, 2020). 61.5% of traders did not think that gloves could prevent contamination of the food handler contrary to Aslam, *et al*, (2021) who found that the majority of food handlers believed that face masks, gloves, and head caps avoid contamination during food processing. This may be attributed to the difference in food safety training received amongst the groups.

Traders at the market could only relate practices such as drying termites, reducing moisture and temperatures in storage areas to shelf life extension of their termites and not reduction of microbial growth on the food stuff. As such, most (73%) traders did not know whether typhoid and other diarrheal diseases are caused by bacteria. These inconsistencies may be attributed to the lack of training and the low formal education levels of the traders.

In practice, most (96%) of the traders of edible termites trade in other food stuff. These food stuffs include other insects, grains, and fish among others. The WHO in their five keys of delivering safe food warns that mixing of raw food is a food safety risk practice (WHO, 2006) . Nonetheless, the traders (68%) did not wash their hands or change gloves after handling other foods before handling termites contrary to the 5 keys to delivering safe food of the World Health Organization. In their study, Aslam, Malik, and Kausar (2021) found that 60% of food handlers separated utensils among different food stuffs to avoid cross contamination. Consistently so, this study revealed that 64% of traders did not mix storage and display containers of edible termites with other foods. Only a cumulative 36% of traders interchanged the storage and display containers a practice posing a similar risk of cross contamination.

In this study, only 12% of the edible termite traders were found to always wash their hands with soap and water when handling their termites. According to Angelillo, et al., (2000) to prevent food contamination and the further spread of food borne illnesses, all foodservice and food handlers should be aware that personal hygiene such as effective hand washing and use of gloves is vital. Other than poor hand washing practices, the edible termite traders of Chisokone market (69%) did not use gloves to protect their food stuffs from contamination. Further, 81% of the traders never use masks in their trade of edible termites in the open air markets. This practice has become of high risk especially now in the face of diseases such as the corona virus diseases 2019 (COVID19). During these pandemic times, the United States Food and Drug Administration (FDA) have released a fact sheet detailing how workers in the food and agriculture sector can use respirators, disposable facemasks or cloth face coverings to prevent the spread of the virus (FDA, 2020).

Table 8 shows that all mean TVC and Enterobacteriaceae loads were ranging between 6.87 and 9.287 $\log_{10}$ cfu/g for TVC and 6.64 and 8.537 $\log_{10}$ cfu/g for Enterobacteriaceae. There lacks a specific microbiological criteria for insects used for human consumption. However, process hygiene criteria for minced meat described by the European Union Regulation (EC) No. 1441/2007 can be used for edible insects (Nganga, *et al*, 2018). Against this standard, loads of this study were higher than the range recommended (5.7 and 6.7 $\log_{10}$ cfu/g) for minced meat (Rudy Caparros, *et al*. 2017). Generally, microbial limit of TVC should be less than 7 $\log_{10}$ cfu.g (Ramashia, *et al.* 2020). These results were consistent with Nyangena *et al* (2020) who found counts ranging between 7.0 – 9.1 $\log_{10}$ cfu/g in *Hermetia. Illucens, Ruspolia differens, Acheta domesticus* and *Spodoptera littoralis*. A study assessing the microbiological load of edible insects found in Belgium showed that traditionally smoked termites and caterpillar from

Africa had high TVCs of 7.48 log_{10} cfu/g and 7.63 log_{10} cfu/g respectively. These high loads indicate that wild collected edible termites sold on african open air markets are highly susciptible to contamination, consistent with Murefu, *et al* (2019) concern that microbial loads were the major edible insect food safety concern especially for wild harvested insects in Africa. These high loads result from unhygienic handling, which includes contact with contaminated environments, improperly cleaned hands or handling equipment (Mmari, *et al*, 2017) and transport material by the traders. The TVC and Enterobacteriaceae loads can be reduced to undetectable amounts once exposed to a heating stage such as blanching or deep frying (Nganga, *et al*, 2018).

The presence of *E.coli*, a gut microorganism was contrary to the results by Ng'anga, et al., (2018) who showed an absence of *E.coli* in *Ruspolia differens*. This difference could probably be due to difference in sampling environments and the varying feedstuff of termites and grasshoppers as SHC and FASFC (2014) recommends. Though this study shows mere presence of the bacteria, *E.coli* has a recommended most probable number of <230/100 g in minced meat. The presense of this bacteria, suggests fecal contamination of the sample along the value chain (Gozde and Emek, 2019) by either interaction with contaminated environments or handling by contaminated hands or implements with fecal matter. *E. coli* is a gastrointestinal bacteria and is highly sensitive to heat (Klunder, *et al*, 2012). The detection of *E. coli* in the sample implies recontamination along the value chain since the initial frying process should have been sufficient to eliminate all enteric *E. coli* of the edible termites as indicated by Grabowsky and Klein, (2017).

S. aureus' presence may be attributed to human handling. This is in line with a study by Wirtanen and Salo, (2016) which showed that *S.aureus* in dairy products originated directly from food handlers or via use of utensils contaminated beforehand by humans. *S.aureus* has been noted as one of the major food contaminant and is ubiquitous (Braide and Nwaoguikpe, 2011). A study comparing microbiological loads of 3 insects found that *S aureus* was higher in termites (2.2562 log_{10} cfu/g) than in mopane worms (1.9948 log_{10}cfu/g) and stink bugs (1.7707 log_{10}cfu/g) (Ramashia, *et al.* 2020). This bacterium may cause spoilage of food and may lead to food borne illnesses. *S. aureus* is an enterotoxin when allowed to incubate in the food stuff and may make the consumption of food hazardous even though it may appear normal (Braide and Nwaoguikpe, 2011). Ng'anga, et al., (2018) have however shown that a process of deep frying can considerably remove vegetative forms of *S.aureus*, but may not necessarily remove enterotoxins because they are heat stable (Prescott, Harle, and Klein, 2002).

Yeast and mold are known to poses relatively versatile environmental requirements and improper handling of food may expose them to contamination with these microbes (Ramashia, *et al.* 2020). Therefore, the presence of mold (*Zygomycetes*) and yeasts (Not *C.albicans*) in this study entail contamination of the termites from their surrounding environment within their value chain (Costa *et al*, 2010) due to improper handling. This is consistent with the assertion by Ramashia, *et al.* (2020) that some vendors sold their insects were they could be easily contaminated with microorganisms and small particles like stones and unwanted material which may carry yeasts and mold. Yeast and mold counts of less than 5 log_{10} cfu/g on dried insects are recommended (Ramashia, *et al.* 2020), however, mere presence is an indicator of contamination. A study therefore that enumerates the yeast and mold in termites can be conducted to understand whether termites in Serenje market occur at higher than recommended rates.

5.1 Study Limitations

1. Objective one survey was conducted in a single setting with a small sample size thereby limiting the generalizability of the study.
2. Considering that objective one and two were studied in different settings, findings of objective one in Chisokone market cannot be used to inform the microbial loads observed in Serenje district market.
3. The study does not quantify specific microorganisms and only shows occurrence of *S. aureus, E.coli,* Yeasts and Molds due to lack of selective media for the stated organisms.

CHAPTER SIX:

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

This thesis aimed at assessing microbial load of edible termites (*Macrotermes spp*.) and trader food safety knowledge and practices in open air markets for food safety. This study informs the conclusion that edible termites sold in Serenje District had higher totalviable counts and Enterobactericeae than recommended. The occurrence of *E. coli, S. aureus* and yeasts and molds lead to the conclusion that the termites had been exposed to faecal contamination, unhygienic handling and they were most likely exposed to unclean environments. This study further concludes that Serenje market areas are a source of significant levels of contamination in edible termites, these contaminations could be attributed to increased number of handlers in the market.

This study also concludes that traders's personal hygiene practice of hand washing before handling food items is limited in Chisokone market. The study further concludes that customer demands for better food safety and hygiene practices from traders improves their compliance. The study concludes that edible termite traders of Chisokone market have a relatively high knowledge of cross contamination of food except the knowledge of microbial losd reduction.

6.2 Recommendations

6.2.1 Recommendations from this study

From the findings of this study, we recommend that Food safety and hygiene education on processing and handling practices that reduce microbial loads at collection sites, transportation and display points are important especially because termites are sold as ready to eat foods in African open air markets. The study further recommends that consumers of edible termites must consider reheating edible termites before consumption in order to kill recontaminating microbes. Legislation on open defecation, use of toilets and promotion of handwashing hygiene can effectively reduce the risk of contamination with gastrointestinal microflora from faecal matter.

6.2.2 Recommendations for further study

We recommend that further studies that assess the microbial reduction potential of edible termite processing activities and suggest better processing and handling practices that reduce microbial loads at collection sites, transportation and display are needed.

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8.0 APPENDICES

Appendix 1: Consents and Survey Tools

Consent letter

RESEACH TITLE: MICROBIAL LOAD ASSESSMENT OF EDIBLE TERMITES. *Macrotermes* spp.: TRADED IN OPEN AIR MARKETS FOR FOOD SAFETY

Principal Investigator

Mumbula Inyambo

MSc Student,

Plant, Animal and Food Sciences

Jaramogi Oginga Odinga University of Science and Technology

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inyambomumbula@gmail.com

Dear participant,

You are randomly selected to participate in this study as a respondent. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information.

The purpose of this study is to identify edible termites' trader food safety and hygiene practices. Results of this study will better inform legislators, traders and consumers on the best practices that ensure food safety. Participants' identity shall be kept confidential and anonymous. You are therefore asked not to write any identifying information on your questionnaire (s).

If you have questions at any time about this study, or you experience adverse effects as the result of participating in this study, you may contact the researcher whose contact information is provided above. If you have questions regarding your rights as a research participant, or if problems arise which you do not feel you can discuss with the Primary Investigator, please contact the; Student supervisors Professor Regina Nyunja at +254721465969 or reginanyunja@yahoo.com and Dr. Donald Chungu at +260966155583 or donald.chungu@gmail.com or the Institutional Review Board at 057-2501804 or email erc@jooust.ac.ke.

Thank you.

Consent

I have read and I understand the provided information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study.

Participant's signature ________________________________ Date ________________

Investigator's signature ______________________________ Date ________________

Sample questionnaire

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY
SCHOOL OF AGRICULTURAL AND FOOD SCIENCE
DEPARTMENT OF PLANT AND FOOD SCIENCES

TOPIC: Microbial load assessment of edible termites. *Macrotermes spp.*: traded in open air markets for food safety.

Questionnaire #:		**Date Filled**	/ /	**Time Filled**	

For the respondent:

1. Responses provided herein are confidential and respondents identity is kept anonymous
2. Respondents reserve the right to withdraw at any point from the research
3. Responses provided herein are used only for Academic purposes
4. Respondents may request for findings of the research

Section A: Demographics

1. Sex of respondent
   (a) Male ☐ (b) Female ☐
2. Age at Last Birthday
   (a) 15 to 20 ☐ (b) 21 to 30 ☐ (c) 31 to 40 ☐ (d) Above 41 ☐
   years
3. Marital Status
   (a) Single ☐ (b) Married ☐
4. Level of Education
   (a) None ☐ (b) Primary ☐ (c) Secondary ☐ (d) Tertiary ☐
5. Number of years as Edible termite trader
   (a) 0 ☐ (b) 1 to 5 years ☐ (c) 6 to 10 years ☐ (d) above 10 years ☐

Section B: Knowledge on food Safety and Hygiene

6. I have received training on food safety and Hygiene issues.
   (a) Yes (b) No
7. Washing hands can prevent microbial contamination of food.
   (a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐
8. Covering the food can prevent cross contamination of food

(a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐

9. Food poisoning is the illness resulting from eating food containing biological or chemical toxins
   (a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐

10. Food borne diseases can result only from chemical contamination

    (a) TRUE ☐ (b) FALSE ☐ (c) I don't know ☐

11. Growth of most bacteria can be controlled by reducing temperature
    (a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐

12. Typhoid and diarrheal diseases common bacterial illnesses?
    (a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐

13. People, Insects, and mixing different food staffs can cause transfer of bacteria
    (a) TRUE ☐ (b) FALSE ☐ (c) I don't Know ☐

14. Cross contamination is the transfer of harmful microorganism from?
    (a) Food to Food ☐ (b) Person to food ☐ (c) contact surfaces to food ☐ (d) All of them ☐

15. What is the purpose of wearing gloves during handling of food?
    (a) To protect food handlers from contamination ☐ (b) To protect food from Contamination ☐

16. Why should the Insects be dried before storage?
    (a) To increase shelf life ☐ (b) to prevent microbial growth ☐ (c) both A and B ☐ (d) none ☐

17. Why should your storage room have low moisture content?
    (a) To prevent microbial growth ☐ (b) to make it look clean ☐ (c) I don't know ☐

Section C: Legislation

18. Insect sale requires to register with local authorities before commencing my business
    (a) Yes ☐ (b) No ☐ (c) I don't know ☐

19. We have designated places where waste is damped
    (a) Yes ☐ (b) No ☐ (c) I don't know ☐

20. Waste is regularly collected and is not left to heap up in the market place

    (a) Yes ☐ (b) No ☐ (c) I don't know ☐

21. The local authorities are regular checkers of sanitary conditions of sale points
    (a) Yes ☐ (b) No ☐ (c) I don't know ☐

22. There are fines related to unsanitary conditions around a sale point
    (a) Yes ☐ (b) No ☐ (c) I don't know ☐

Section D: Food safety and Hygiene Practices

23. Do you sell any other food stuff other than Edible termites?

(a) Yes ☐ (b) No ☐

If you answered 'no' to question 23 above, please jump to question 27

24. What other food stuff do you sale?
(a) Other insects ☐ (b) Grains ☐) Vegetables ☐ Fish (e) ☐ er specify ☐

25. Do you wash hands or change gloves after handling other foods before handling the termites?
(a) Yes ☐ (b) sometimes ☐ (c) No ☐

26. Do you exchange storage and display containers between the edible termites and other foods?
(a) Yes ☐ (b) sometimes ☐ (c) No ☐

27. How often do you wash your hands with soap and water when handling your insects?
(a) Always ☐ (b) sometimes ☐ (c) My hands are already clean ☐ (d) Never ☐

28. How often do you wear gloves when handling your insects?
(a) Always ☐ (b) sometimes ☐ (c) when customer demands ☐ (d) Never ☐

29. How often do you use a mask when handling your insects?

(a) Always ☐ (b) sometimes ☐ (c) when customer demands ☐ (d) Never ☐

30. How often do you clean your implements before sale

(a) Always ☐ (b) Sometimes ☐ (c) when I see dirt ☐ (d) Never ☐

31. How often do you clean your storage and sale area?
(a) Always ☐ (b) Sometimes ☐ (c) when demanded by law ☐ (d) Never ☐

32. What packaging material do you use to sell your insects?
(a) Plastic bags ☐ (b) Printed/plain paper ☐ (c) Customers provide there on ☐

(d) Other ☐ specify__________________

33. What preservation techniques do you use for your insects?
(a) Drying only ☐ (b) smoking only ☐ (c) Boiling then dryi ☐ (d) Frying then drying ☐
(e) other ☐

If other, specify_______________________

Appendix 2: Ethical Clearances

JOOUST ERC Authorization Letter

JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH
JOOUST-ETHICS REVIEW OFFICE

Tel. 057-2501804
Email: erc@jooust.ac.ke
Website: www.jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E2

16th December, 2019

Mumbula Inyambo
SAFS
JOOUST

Dear Ms. Inyambo,

RE: APPROVAL TO CONDUCT RESEARCH TITLED *"MICROBIAL LOAD ASSESSMENT OF EDIBLE TERMITES MACROTERME SPP TRADED IN OPEN-AIR MARKET FOR FOOD SECURITY"*

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **7/15/ERC/12/19-4.** The approval period is from 16th December, 2019 – 15th December, 2020.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Anga wa
Chairman, JOOUST ERC

Copy to: Deputy Vice-Chancellor, RIO Director, BPS Dean, SAFS

TDRC ERC Letter

TROPICAL DISEASES RESEARCH CENTRE
Tel/Fax +260212 615444
P O Box 71769
tdrc.ethics@gmail.com
NDOLA, ZAMBIA

TDRC ETHICS REVIEW COMMITTEE
IRB REGISTRATION NUMBER : 00002911
FWA NUMBER: 00003729

TRC/C4/01/2020

20th January 2020
Mumbula Inyambo
Jaramogi Oginga Odinga
University Of Science and Technology
Bondo, Kenya

Dear Mr. Inyambo,

RE: ETHICAL APPROVAL OF STUDY PROTOCOL

Reference is made to the protocol entitled "**Microbial Load Assessment of Edible Termites (Macroterme Spp) Traded in Open Air Markets for Food Safety**"

On behalf of the Chairperson of the TDRC Research Ethics Committee (REC), I wish to inform you that the Committee reviewed your protocol and ethical approval was granted based on the following conditions.

No personal identifiers should be used in your study data and the data you will collect shall be used only for academic purposes.

You are now required to submit your protocol to the National Health Research Authority for final approval following the link: **https://www.nhra.org.zm** and submit a final report to the REC Secretariat at the end of the study.

Should there be any protocol modifications or amendments, you are required to notify the ERC and submit protocol amendments for approval.

This approval is valid for the period **20th January 2020** to **20th January 2021**

The Committee wishes you success in the execution of the study.
Yours faithfully,
TROPICAL DISEASES RESEARCH CENTRE

Edna Mwale. Simbayi
SECRETARY – TDRC Ethics Review Committee
cc: Chairperson – TDRC Ethics Review Committee

TROPICAL DISEASES RESEARCH CENTRE
ETHICS REVIEW COMMITTEE
20 JAN 2019
P.O. BOX 71769
NDOLA, ZAMBIA