EFFECT OF PROCESSING ON NUTRITIONAL AND ANTI-NUTRITIONAL CONTENT OF SELECTED EDIBLE INSECTS

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DECLARATIONS

This thesis is my own original work and has not been presented for a degree or any academic certification in any other university.

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DEDICATION

This thesis is dedicated to my parents**,** my spouse Simon and our children Vicky, Ryan and Neema.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
AOAC	Association of Official Analytical Chemists
FAO	Food and Agricultural Organization
KDHS	Kenya Demographic Health Survey
KIRDI	Kenya Industrial Research and Development Institute
RDA	Recommended Daily Allowance
UNICEF	United Nations Children's Fund
UV-VIS	Ultra Violet Visible
WFP	World Food Programme
WHO	World Health Organization

ABSTRACT

Balanced diet during formative years is essential for the growth of a child's full human potential; otherwise lack of it may result in malnutrition. Inadequate intake of nutrients, onset of diseases in children and poor complementary feeding practices are the primary causes of malnutrition during infancy. In Africa complementary foods, which are based on starchy foods are usually inadequate in proteins and minerals. Entomophagy contributes to reduced malnutrition as well as a healthy population since insects have sufficient amounts of proteins, vitamins and minerals. Insects can be used in enhancing the nutrients in children food formulations. However, processing of insects may affect their nutritional content. The aim of this research was to assess the effect of processing on some nutrients and anti-nutrient in selected edible insects. The termites and lake fly samples were collected from Got Agulu village in Bondo sub-county, Siaya County, while the grasshoppers and locusts were collected from Emaene village in Khwisero Sub-County, Kakamega County. Sun drying was achieved by direct exposure of the samples to sunlight for 3 days, 8 hours a day, while oven drying was achieved by drying the samples in the air- oven at 60^0c for 48 hours. Defatting was done manually by use of a press machine. Dry matter, proteins, minerals and anti-nutrients in fresh, sun dried, oven dried and defatted edible insects (termites, lake-fly, locusts and grasshoppers) were determined using standard methods. The proximate analysis (moisture, ash and protein) was done using AOAC methods while minerals analysis was done using atomic absorption spectrophotometry (AAS). Anti-nutrients analysis was done using a UV-VIS spectrophotometer for phytates and tannins, and titration method for oxalates. The moisture content for the fresh insects ranged from 40.00% - 55.01% and was significantly reduced to less than 10% on drying. The ash content of the fresh insects was between 1.09% and 2.8% and was significantly increased by more than 50% on processing. Fresh edible insects had crude protein ranging from 16.24 g/100g to 19.20 g/100g and processing significantly increased it in the order: defatting > oven-drying > sun-drying. The zinc and iron levels were not significantly reduced on processing. However, calcium content was significantly reduced on drying the samples. The levels of phytates, tannins and oxalates in the fresh edible insects ranged between 0.41±0.01 mg/100g and 15.45±0.89 mg/100g. These levels were however lowered significantly on processing by 2% - 74% with oxalates and phytates having the highest decrease. The findings suggest that processing significantly decreased moisture content to levels that encourage long storage while it increased protein content significantly, thus improving the nutrition content of the edible insects. Processing significantly reduced antinutrients levels and retained zinc and iron indicating that these nutrients are more bioavailable. Oven-drying was found to be the most appropriate in reducing the anti-nutrient levels. Therefore, processed edible insects can be used as alternative source of food thus improving foodsecurity.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Global food insecurity is an immense challenge due to an increasing human population, inadequacies in food production and climate changes (Nwozor and Ake, 2019). The global population has tripled in the past three decades, with present approximations of about 8.6 billion in the next ten decades (Abel *et al*., 2016). Reliance on agricultural production for food security will not therefore be feasible (Tadele, 2017). The increase in population increases food demand particularly proteins from animals (Van Huis, 2016). Furthermore, the soaring rate of infant mortality in third world countries is presently associated with scarcity of animal proteins in their diets (Adler and Newman, 2002).

The immediate consequence of food insecurity is malnutrition, with millions of children currently affected by malnutrition in the world (Alaimo *et al*., 2020). Inadequate intake of nutrients, onset of diseases in children and poor complementary feeding practices are the primary causes of malnutrition during infancy (Masuke *et al*., 2021). Infants become more active as they grow and therefore breast feeding fails to provide the complete nutritional requirements. Complementary feeding plays a vital role in bridging the gaps. In Africa most complementary foods consists of starchy foods (Oladiran and Emmambux, 2022) with inadequate protein and minerals. Malnutrition is characterized by stunted growth during the early years and recent studies have indicated that protein deficiency is high among the Kenyan children (Olack *et al*., 2011).

Animal proteins such as eggs, beef and milk being the main sources of animal proteins for human consumption are rated higher in quality and thus ideal for most people (Bryant, 2022). However these animal proteins are pricey (Sahito *et al*., 2022) and hence sustainable unconventional sources of proteins such as edible insects has been sought (Lange and Nakamura, 2021). Insect consumption, known as entomophagy is a potential solution to the challenge of the continually-increasing food demand (Ebenebe *et al.*, 2017).

Edible insects have been consumed for ages in different regions of the world such as Asia, Australia, South American countries, majority of Asian countries and in Africa (van Huis, 2016). More than 2,000 insects species are consumed globally (Roos and Van Huis, 2017), Africa, Asia and Latin America being the highest consumers (Shockley and Dossey, 2014). This is in spite of the fact that some cultures still link consumption of insects with a sheer endurance ploy in curbing food security challenges (Imathiu, 2020). However the mind-set is gradually shifting in various parts of the world (Kinyuru *et al*., 2018). For instance, in countries such as Nigeria and Uganda edible insects are not only economically important but also offer diets diversity (Ssepuuya, 2016). Elsewhere, there exists an international trade between Zambia and South Africa which involves mopane worms with yearly proceeds of about US$ 85 million (Madibela, 2007). In addition, the trade offers employment opportunities to more than 35,000 persons in a period (Ghazoul, 2006).

Edible insects are a suitable source of proteins and minerals which are vital nutrients required in the human diet (Finke, 2015). Protein levels in edible insects are comparable to those in animal and plant based proteins (Van Huis, 2017). Moreover, consumption of edible insects provides micronutrients such as magnesium, zinc,

calcium and iron into the body. Rumpold and Schluter (2015) reported that edible insects have sufficient magnesium content, specifically insect species from the *Hemiptera* order, grasshoppers, crickets and locusts, with termites having high iron content while grasshoppers and locusts have high zinc content and can be used in zinc supplementation. Hence, a move to consider edible insects as an essential element of the human diet is crucial.

Antinutrient such as phytic acid are abundant in various plants and some insects feed on these plants (Thakur and Kumar, 2017). This increases the concentration of antinutrients in the insects and may decrease nutrient bioavailability which can be lethal when they exceed the permissible limits (Samtiya *et al*., 2020). However, they can easily be detoxified during processing (Samtiya *et al*., 2020). For this reason, reducing their levels in edible insects is an area of concern owing to the need of preventing toxicity and illness caused by these antinutrients (Joye, 2019).

Communities that consume insects have diverse post harvest techniques such as solar-drying, boiling, roasting and pan-frying (Kinyuru *et al*., 2018) in an effort of making them tasty (Mutungi *et al*., 2019). Processing eventually affects the final product in terms of nutrient and antinutrient composition (Meyer-Rochow *et al*., 2021; Lange and Nakamura, 2021). For instance, a study on fresh pupae of *Bombyx mori* in India found that the protein content was 17.1 g/100g protein compared to 56.9–75.2 g/100g protein of sun-dried pupae (Ademola *et al*., 2017). Elsewhere, in a study that assessed the levels of antinutrients in wild harvested beetles, 2800 mg/100g, 278mg/100g and 700 mg/100g were reported for oxalates, tannins and alkaloids respectively. However, the levels were decreased by multiples of 10, 1.6 and 2.3 respectively after boiling the raw

insects for thirty minutes followed by drying (Musundire *et al*., 2016). Studies on the effect of thermal treatment and defatting processes on nutrient and antinutrient content in edible insects are limited. Hence this study was undertaken to determine nutritional and anti-nutritional composition of processed (sun-dried, oven-dried and defatted) lake fly, grasshopper, locusts and termites.

1.2 Problem Statement and Justification

Edible insects are highly nutritious and inclusion of these insects into diets to reduce malnutrition and food insecurity is gaining traction globally. To increase palatability, traditional processing methods such as boiling, sun drying are being used by communities that consume insects. Different processing methods could potentially impact on the nutrient and antinutrient composition of edible insects. However, there is data paucity on the effect of the processing methods on the levels of nutrient and antinutrient in edible insects, thus the aim of this study.

1.3 Null hypothesis

The levels of nutrient and antinutrient in edible insects are not significantly affected by the processing method.

1.4 Objectives

1.4.1 General Objective

To assess the effect of processing on the nutrient and antinutrient content of edible insects.

1.4.2 Specific Objectives

i. To assess the effect of processing (sun-drying, oven-drying and defatting) on moisture, ash and protein levels in lake fly, termites, grasshoppers and locusts.
ii. To determine the effect of processing (sun-drying, oven-drying and defatting) on the levels of minerals (Fe, Zn and Ca) in lake fly, termites, grasshoppers and locusts.
iii. To determine the effect of processing (sun-drying, oven-drying and defatting) on the levels of antinutrient (phytates, oxalates and tannins) in lake fly, termites, grasshoppers and locusts.

1.5 Significance of the study

The data obtained from this study will show how processing affects on nutritional and anti-nutritional content of the edible insects in addition to providing information on whether processed edible insects meet the recommended dietary allowances (RDA) for use as alternative source of protein.

1.6 Scope and limitation of the study

The study focused on four commonly consumed insects in western Kenya (termites, lake fly, locusts and grasshopper). The insects are the commonly consumed insects in the region. The level of the nutrients and antinutrients is affected by age, gender and diet of the insects. However, these factors were not considered in the study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Food insecurity and malnutrition

Food insecurity by definition is a state where people experience food shortage or live in fear of hunger (Munirah *et al*., 2022). Statistics shows that in 2016, global hunger increased affecting over 800 million people while about two billion people faced food insecurity periodically due to poverty and high population growth (WHO, 2017). In Sub-Saharan Africa, more than 307 million people (about 31%) experienced hunger in the year 2016 (FAO and UNICEF, (2019); WFP and WHO, (2017). The transformational vision of the 2030 Agenda for sustainable development is to realize food security, enhanced nourishment, and ensure access to safe healthy and ample food by everyone by 2030 (WHO, 2017).

The global population is likely to double in the next three decades. Increase in human population increases food demand. This implies that reliance on agriculture for food security will not be feasible (Tadele, 2017). Food insecurity in Kenya is evident in various parts such as western Kenya. A report by Kenya Demographic Health Survey-KNBS (2014) revealed that 32% of the population nationally was food insecure with about 39% of the households in Western part of Kenya experiencing severe food insecurity. A report by Korir *et al*., (2022) further indicated that there were high rates of food insecurity and poverty in western Kenya. Hence a boost in food production is necessary since this situation is likely to continue. To address this, consumption of insects has been proposed in contributing to food safety in the world (Van Huis, 2015). One of the consequences of food insecurity is malnutrition. Young children and infants

are predominantly affected since their regular food intake consists mainly of starchy foods (Whitney and Rolfes, 2018). A report by KDHS (2014) stated that malnutrition in young children is still intolerably high with about 27% of infants underdeveloped and 12% underweight. However, entomophagy can improve food security as well as changing the trend of malnutrition particularly in the developing countries (Kelemu *et al.*, 2015; (Alemu *et al*., 2017).

2.2 Entomophagy

Entomophagy is consumption of insects by humans and have been accepted and put into practice by various ethnic groups globally (Pambo *et al.*, 2016). More than 2000 insect species are consumed in the world (Jongema, 2017) with about 3070 cultural groups in the world consuming them as a critical element of their diet (Premalatha, *et al*. 2011) . Majority of the underprivileged people in the world habitually consume insects, particularly in Africa and Asia (Shockley and Dossey, 2014). Edible insects from the C*oleoptera*, *Lepidoptera* and *Orthoptera* orders have the highest edible insect diversity (van Huis, 2013; Jongema, 2017) while the edible insects from other orders such as *diptera*, *Isoptera* and *Hemiptera* account for about 21% (Jongema, 2017). However some communities consider insect consumption as a custom for the underprivileged people and thus regarded as culturally inapt (Shockley and Dossey, 2014; Kinyuru *et al*., 2018). Consequently, numerous international organizations have disregarded entomophagy as a means of ensuring food security (Yen, 2009). In spite of these hurdles, FAO is shifting the global attention towards adopting the practice of entomophagy.

Insect rearing offers considerable fiscal, dietary and environmental benefits for rural communities (Van Huis *et al.*, 2013). Other than being nutritious, insects have; a high reproduction rate, are very efficient in feed conversion, provide nutrients which are readily absorbed compared to plant-based foods (Van Huis, 2016) and minimal land requirement. It also contributes to reduced use of insecticides which reduces economic burden on farmers. Compared to livestock keeping it is more ecologically friendly due to reduced greenhouse gas emissions (Van Huis, 2013). For instance some edible insects such as crickets produce less greenhouse gases, approximately 100 times less than what livestock such as cows produce (Payne, 2018). Also some insect species are collected for other uses, for instance making animal feeds (Makkar *et al*., 2014).

In Kenya entomophagy has been practiced in the western part of the country and this has been an ancient custom in the region (Kinyuru *et al*., 2013). The commonly consumed insects in the region are grasshoppers *(Ruspolia differens sp*), lake fly (*Chaoboridae and Chironomidae*), locusts (*Locustana migratoria* and *Schistocerca gregaria sp*), termites (*Macrotermes bellicosus and Macrotermes subhylanus sp*), and crickets (*Gryllus bimaculatus sp*) (Muyonga *et al*., 2018) .

2.3 Insects overview

2.3.1 Termites

They are tiny insects which mostly feed on fibre (Evans, 2015). They are social insects greatly attracted towards lights. They are divided into colonies comprising of workers, soldiers, winged adults, a queen and a king (Korb and Thorne, 2017). These colonies put up enormous earthen mounds which can be more than 5m far above the ground. Cyclically, the winged adults come out in huge swarms, mate and then establish new

colonies. Methods of harvesting vary depending on season and species (Ayieko *et al*., 2010). In Kenya they are gathered from their natural habitat around Lake Victoria (Kinyuru and Ndung'u, 2020). Some of the termite species commonly consumed in the region are *macrotermes subhylanus* and *macrotermes bellicosus* (Kinyuru *et al*., 2013). Termites are mostly collected during the rainy periods; however they can be made to surface during dry periods (Banjo *et al*., 2006). They are either consumed raw immediately on surfacing, solar-dried or roasted or as a constituent element of foods such as porridge (Anyuor *et al*., 2021).

Termites have a high nutritive value with considerable amounts of minerals and significant levels of protein (Kinyuru *et al*., 2013). In a study on wild harvested termites (*Macrotermes bellicosus)* in Kenya, 39.74 g/100g protein, 4.65% ash, 115.97 mg/100g iron, 10.76 mg/100g zinc and 63.6 mg/100g of calcium was reported (Fombong *et al*., 2017).

Figure 2.1 Termites (*macrotermes bellicosus*)
Source: *https://www.westerpest.com*

A different study on *Macrotermes subhylanus* species in Kenya, 38.42 g/100g protein, 58.72 mg/100g calcium, 53.33 mg/100g iron and 8.1 mg/100g zinc was reported (Kinyuru *et al*., 2013). Further, in a review that evaluated the effect of insect consumption on development in infants, weight gains and increased length was

recorded in infants fed with complementary foods enriched with termites (Konyole *et al*., 2019).

2.3.2 Grasshoppers

They are classified in the *orthoptera* order (Akhtar *et al*., 2012). Utilization of grasshoppers (*Ruspolia differens*) for food is a major component of the feeding norm in some parts of East-Africa (Ng'ang'a *et al*., 2019). *Ruspolia differens* is a non destructive species which is spread across Africa (Fombong *et al*., 2017). It does not cause harm to crops making them distinctive from locusts. It is also called *nsenene* or *senesene* across the region and is the commonly consumed species in East Africa (Mmari *et al*., 2017). To date, this insect species is harvested in their natural habitat during the swarming seasons. They are nocturnal hence harvested at night (Kinyuru *et al*., 2018). These species exhibits color polymorphism, but green and brown morphs are the most dominant (Kinyuru *et al*., 2010). *Ruspolia differens* has a broad diet which includes grains and grasses and thus their nutrient composition is considered high and variable (Van Huis, 2013).

Figure 2.2 Green and brown grasshoppers (*Ruspolia differens*)
Source: *https://www.istockphoto.com*

For instance, a study on wild harvested grasshoppers (*Ruspolia differens)* in Uganda reported high protein values of 43.8 g/100g, 3.1 % ash, 7.6 mg/100g iron, 14.63 mg/100g zinc and 895.67 mg/100g of calcium (Nyangena *et al*., 2020). In a different study in Kenya, protein levels were found to be 47.7 g/100g for the same insect species (Fombong *et al*., 2017). This signifies its remarkable potential to fight nutrient deficiencies in human beings (Ssepuuya *et al.*, 2016).

2.3.3 Locusts

Locusts are consumed by humans globally in several continents, in the countryside and in the cities (Costa-Neto, 2016). In China they are deemed a delicacy which is served on skewers in various marketplaces (Mohamed, 2015). Large-scale farming of the insect intended for consumption is developing in Asia, Japan, China and Korea (Makkar *et al*., 2014). In Sudan, they are consumed and prepared in various ways such as boiling, frying or solar-dried. In Africa, the commonly consumed species are; the desert locust *(Schistocerca gregaria),* the migratory locust *(Locustana migratoria),* the red locust (*Nomadacris septemfasciata)* and the brown locust -*Locustana pardalina* (Mariod, 2020). The migratory locust form swarms consisting of massive numbers which can travel enormous distances, causing substantial harm to crops (Moharana *et al*., 2020). Ever since 2000s, the development of aquaculture in the African continent coupled with exploration of unconventional sources of food led to feeding trials of locusts along with other edible insects since they are regarded for their high protein value and minerals (Mohamed, 2015). A review on nutritional composition of migratory locusts (*Locustana migratoria)* in Africa established that, the raw insect had a protein content of 18.2 g/100g, while the fried insect had 30.0 g/100g protein. Mineral content for the fried migratory locust was 288 mg/100g calcium, 9.6 mg/100g

iron and 8.4 mg/100g zinc (Van Huis *et al*., 2013). Elsewhere, analysis of oven dried *L. migratoria and S. gregaria sp.* reported protein content of 54.16 g/100g and 61.41 g/100g respectively. Mineral content was 129.79 mg/100g and 80.48mg/100g calcium, 12.70 mg/100g and 24.88 mg/100g zinc, 6.59 mg/100g and 7.31 mg/100g iron for *L. migratoria and S. gregaria sp* respectively (Fombong *et al*., 2021). Hence locusts are significant source of food which adds proteins and minerals to diets, particularly during periods of food shortage (Imathiu, 2020).

Figure 2.3 Locust *(Locustana migratoria*)
Source: *https://www.inaturalist.org*

2.3.4 Lake fly

They belong to the *diptera* order constituting *Chaoboridae, Chironomidae* and *Ephimeroptera* and are the commonly consumed insect species around the Lake Victoria (Ayieko *et al*., 2010). They are collectively known as mayfly by the locals basically due to their distinctive appearance in the month of May (Ayieko and Oriaro, 2008). In the Luo dialect, lake fly are referred to as *Sam* (Ayieko *et al*., 2010). The only species collected for human consumption is the *Chaoboridae* and *Chironomidae*

sp (Nyangena, 2021). The Luo community who reside around the Lake Victoria region value the fly as an extraordinary edible insect with numerous financially viable and edifying values (Pambo *et al*., 2016). The lake fly usually patch on particular shrubs or hills close to the lakeshore usually known in Luo as ‘kitambo’. In certain villages, such hills are revered as sacred sites and are guarded shrines for collection of the flies (Ayieko and Oriaro, 2008).

Few studies that have evaluated nutrient composition of lake fly demonstrated that they are excellent sources of proteins and minerals. For instance, a review on nutritional composition of lake fly in Uganda established that, the protein content was 48.6 g/100g, while the mineral content was 166 mg/100g calcium, 78 mg/100g iron and 14.5 mg/100g zinc (Van Huis *et al*., 2013).

Figure 2.4 Lake fly *(Chironomidae sp)*

Source: *https://www.alamy.com/sptock-photo/midges-lake-victoria.html*

2.4 Nutritional value of edible insects

2.4.1 Proteins

Amino acids are the building blocks of proteins (Feger *et al*., 2020). Edible insects boast a significant protein source which can supplement plant-dominated diet, characteristic in developing nations (Siulapwa *et al*., 2014; Imathiu, 2020). In a study that assessed the protein content of silk worm larvae, the content was found to be high and comparable to animal protein (Longvah *et al*., 2011). Elsewhere, it was demonstrated that feeding weaning rats with house cricket was significantly superior to soy protein in every stage of intake (Ehoche *et al.*, 2019). In a different study, consumption of 100g of caterpillars provided about 76% of the Recommended Daily Allowance-RDA of protein intake (Mohamed, 2015) while four silkworm larvae were found to be equivalent in nutrients as a chicken egg (Sheikh *et al*., 2018). According to a report that assessed different studies analyzing different insect, protein content was shown to range between 14 -81% of dry matter (van Huis *et al*., 2013). Thus insects are undeniably excellent sources of proteins and thus including them in diets may help in preventing malnutrition in infants and young children (Das *et al*., 2019; Raheem *et al*., 2019).

Protein content of insects varies strongly with species, geographical location, feed substrates, the stage of metamorphosis and the processing method (Meyer-Rochow *et al*., 2021; Lange and Nakamura, 2021). For example in Nigeria, grasshoppers fed with bran, were found to contain nearly twofold the protein content of those fed on maize (Makkar *et al*., 2014). Adults insects are more often than not superior in protein content than instars (Belluco *et al*., 2013).

2.4.2 Minerals

Some of the essential minerals needed in the body include iron, zinc, calcium and magnesium. Minerals deficiencies in the human body have detrimental health outcomes and nutrition interventions may not always reverse these effects (Stein, 2010). These outcomes damage the immune system, intellectual and physical development (Prasad, 2013; Sauer, 2016)). Edible insects are excellent sources of zinc, calcium, magnesium, iron, sodium and potassium (Mwangi *et al*., 2018) and these minerals are often undersupplied in most diets in developing nations (Akhtar and Isman, 2018).

Insects contain equivalent or higher iron contents than red meat which contains 1.1–3.3 mg/100g iron (Williams, 2007). Mopane caterpillar contains 31–77 mg/100 g iron (Fombong and Kinyuru, 2018) while locusts (*Locustana migratoria)* has iron content of 7.31 mg/100g (Fombong *et al*., 2021). Beef has zinc content of 12.5 mg /100 g, while the palm weevil larva has 26.5 mg /100 g zinc (Adeyeye and Olaleye, 2016). A report by Rumpold and Schluter (2015) on nutrient composition based on dry matter that compiled 236 insect species reported that grasshoppers and locusts have high zinc content and thus can be used in zinc supplementation. Hence insects are irrefutably excellent sources of iron and zinc therefore including insects them in the daily diet may improve zinc and iron status and abet in preventing anemia and maternal deaths in the society (Hlongwane *et al*., 2020).

2.5 Antinutrients in edible insects

These are chemical compounds that bind with essential nutrients in the body thereby making them unavailable for absorption leading to micronutrient deficiency (Sam,

2019). They include phytates, oxalates, flavanoids, tannic acids, phenols, hydrocyanic acids among others (Ekop *et al*., 2010; Tadele, 2015). Their mode of action involves precipitation of proteins and reducing solubility of minerals thus interfering with their bioavailability in the body (Kunatsa *et al*., 2020). They are mainly found in plants especially legumes (Matilla *et al*., 2018). Some insects feed on plants which contain antinutrients and this increases the concentration of antinutrients in the insects (Kunatsa *et al*., 2020) while retaining considerable levels of the antinutrients in their bodies (Samtiya *et al*., 2020).

2.5.1 Tannins

They are phenolic compounds commonly found in plant leaves, fruits and bark (Tanase *et al*., 2019). When ingested they can produce detrimental outcomes if present in significantly high quantities (Joye, 2019). This is because they have the capability of forming tannin-protein complexes leading to precipitation of proteins thereby making them unavailable for assimilation (Derix, 2017). Previous studies have shown that the level of tannins in edible insects lies within the acceptable range. For instance, a study of winged termites obtained in Nigeria reported that the tannin content was in the range of 0.024 to 0.051mg/100g (Idowu *et al*., 2019). These values were supposedly safe since they were below the permissible limit of tannins which is between 150–200 mg/100 g of dry weight (Alimentarius, 2003).

2.5.2 Oxalates

Oxalic acid which is present in the cell sap of most plants combines with minerals to form oxalates (Franceschi and Loewus, 2020). It forms complexes with minerals such as calcium, zinc and magnesium making them unavailable for absorption (Nyonje,

2014). Calcium oxalate is stored in the kidney in crystalline form which could lead to severe health problems such as kidney stones (Kunatsa *et al*., 2020). Patients with such health problems should not exceed oxalate intake of 40–50 mg /day of dry weight (Kumar *et al*., 2020). According to Alimentarius (2003), the permissible limit of oxalate is between 250 mg/100g - 500mg/100g. Antinutrients analyses of various edible insects have reported levels of oxalate that are below the lethal dose (Ekop *et al*., 2010; Kunatsa *et al*., 2020).

2.5.3 Phytates

Phytic acid is abundant in various plants but when chelated to a mineral it is referred to as phytate (Nissar *et al*., 2017). Phosphorus in most plant tissues is stored in form of phytic acid (Samtiya *et al*., 2020). In nature phytic acid concentrates in legumes, peanuts, cereals, and oilseeds (Samtiya *et al*., 2020; Feizollahi *et al*., 2021). Most edible insects feed on the plants and this enhances the concentration of the antinutrient in the insects (Kunatsa *et al*., 2020). Phytic acid binds with minerals such as zinc, iron and calcium leading to formation of complexes thus reducing their bioavailability. These may lead to micronutrient deficiencies in human beings (Zhang *et al*., 2022). A study on oven-dried *O. monoceros* larvae by Fadipe *et al*., (2019) showed that the levels of phytates were 178mg/100g and were within acceptable levels. A different study on dried *C. forda* larvae in southern Nigeria reported 1.1 mg/100g of dry weight for phytates (Yohanna, 2021). These values are below 250–500 mg/100 g (Alimentarius, 2003) which is the permissible limit for phytate hence these insects are safe for consumption with respect to phytate toxicity (Idowu *et al*., 2019).

2.6 Effect of processing on nutrients and antinutrients

2.6.1 Effects of processing on nutritional value

Customarily, edible insects are consumed raw or processed (Mmari *et al*., 2017). Several studies have assessed the effects of processing on nutritional value of edible insects. A study by Shadung *et al*., (2012) on oven-dried Sternocera *orissa* beetle species reported an increase in minerals and protein content by two fold. Another study in Kenya on grasshopper *Ruspolia differens* species and winged termites (*Macrotermes subhylanus* species) revealed that sun drying enhanced the protein content of the insects (Kinyuru, 2014).

Studies on other edible insects such as fresh pupae of *Bombyx mori* in India found that the protein content was 17.1 g/100g protein compared to 56.9–75.2 g/100g protein of dried pupae (Ademola *et al*., 2017). Defatted *Bombyx mori* pupae reported a protein content of 57.4 g/100g compared to non-defatted pupae that reported a protein content of 48.3 g/100g (Nyangena, 2021). In Botswana, Madibela *et al*., (2009) compared protein content of processed and degutted belina larvae and found that the degutted samples had a higher protein content of 56.7–57.9 g/100g compared to the non-degutted belina larvae that had a protein content of 50.5–53.7 g/100g. These studies showed that processing methods enhances the levels of protein in edible insects. However, there is wide variability of the protein composition upon using different processing methods (Lange and Nakamura, 2021)

2.6.2 Effects of processing on antinutritional content

The levels of majority of antinutrients can be decreased by means of undemanding treatments such as heating, roasting, blanching or freezing (Kumar *et al*., 2020) with heat treatment being the most efficient method (Samtiya *et al*., 2020). Different studies have assessed the antinutritional properties of edible insects. In a study that assessed the levels of antinutrients in wild harvested beetles in Zimbabwe, 2800 mg/100g, 278mg/100g and 700 mg/100g were reported for oxalates, tannins and alkaloids respectively. However, the levels were decreased by multiples of 10, 1.6 and 2.3 respectively after boiling the raw insects for thirty minutes followed by drying (Musundire *et al*., 2016). Elsewhere in Sudan, on evaluating antinutritional factors on tree locust, it was found that fry-sun dried tree locust contained significantly higher tannin content (9.0 mg/100g) than boiled-sun dried locusts (5.8 mg/100g) possibly because of leaching of tannins into the boiling water (El Hassan *et al*., 2008). These studies demonstrated that different processing methods reduce considerably the levels of antinutrient in edible insects.

2.7 Analytical Techniques for determination of metals

2.7.1 Atomic Absorption Spectroscopy (AAS)

Energy absorption by atoms in the gaseous state in the ground state forms the basis of AAS. In AAS, the amount of radiation absorbed is proportional to the density of the atoms in the flame. Hollow cathode lamp is the source of radiation to be absorbed. It has a lining made of the metallic element of interest. A wavelength selector, monochromator selects from the spectral lines released from the lamp. Each lamp emits the spectrum of that metal lining in the hollow cathode. A standard curve is

drawn using standards of the sample. Determination of the concentration of the sample is done using Beer's Lambert law as shown below:

Equation 2.1

$$A = \log\frac{Io}{I} = \varepsilon cl$$

Where,

A = Absorbance

Io = Incident radiation

I = attenuated radiation

ε = Molar Extinction coefficient (L/mol/cm)

c = Concentration (mol/dm^3)

I = Length of the cell used

2.7.2 Atomic Absorption Spectrometer

The instrumentation of AAS comprises of a hollow cathode lamp, nebulizers, atomizers, detector and amplifier. The hollow cathode lamp is made up of the cathode (made up of metallic element to be determined) and tungsten as the anode. They are enclosed in a glass tube with a quartz window and filled with rare gas such as neon at decreased pressure. When high voltage is applied, the gaseous atoms are ionized at the anode. The positively charged ion migrates at a speed towards the cathode and bombards it causing some metal to sputter and vaporize. Continued collision by gaseous ions with high energy excites the metal vapor to higher energy levels. When

the metal vapor relaxes, it emits radiation of specific wavelength characteristic of the element of interest. The emission passes through the sample and the quantity of radiation absorbed is proportional to the sample concentration (Gary, 1994). The sample is introduced into the flame and is converted into an aerosol droplet. In the flame the sample is changed into gaseous atoms which then absorbs incident light from the lamp proportional to the metal atoms concentration in the flame (Skoog and Leary, 1992). The flame temperatures are regulated to warrant sample atomization without ionization of the metal of interest. The detectors are fitted with photosensitive cathodes materials capable of amplifying signals from the monochromator. Once the photons strike the photosensitive material, electrons are ejected and amplified for read out.

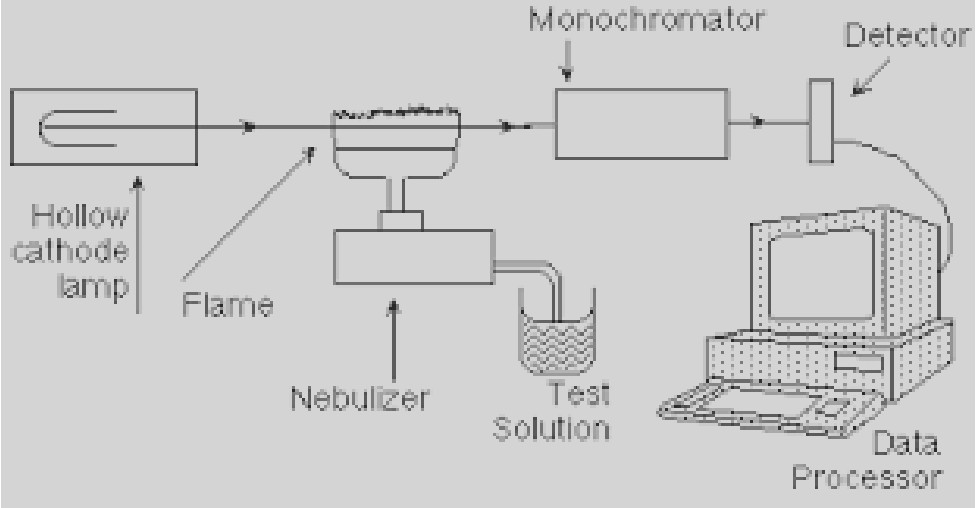

Figure 2.5: Schematic diagram of Atomic Absorption Spectrometer

2.8 Analytical techniques for determination of antinutrients

2.8.1 UV/Visible Spectrophotometry

UV/Vis spectrophotometry is usually used for the quantitative determination of substances which absorb ultra violet light. It is based on Beer Lamberts Law which states that absorbance is directly proportional to the concentration of the substance. This is illustrated below:

Equation 2.2

$A = Ecl$

Where,

A = Absorbance

Є = Molar Extinction coefficient

c = Concentration

l = Length of the cell used

During determination of the concentration of a compound, wavelength of utmost absorption for the substance is selected. The absorbances of the standard solutions are measured for several concentrations of the solution to give standard curve usually a straight line if the law is obeyed. This curve is used in determination of the concentration of the compound of interest (Skoog and Leary, 1992).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

The study involved processing of fresh samples to give sun dried, oven dried and defatted edible insects followed by determination of dry matter, proteins, minerals and antinutrients using standard methods. The analysis was carried out in triplicates.

3.2 Instrumentation

Muffle furnace (Nerberthem, Germany) was used for ash determination and air oven (Gellenkamp, UK) for moisture determination. Atomic absorption spectrophotometer (AAS) make (Shimadzu, AA-6200) was used to analyze micro nutrients (iron, zinc and calcium) Tannins and phytates were analyzed using ultraviolet-visible (UV-VIS) spectrophotometer make - Analytik Jena, Germany model - SPEKOL 1500.

3.3 Chemicals and Reagents

All reagents and standards used in this study were of analytical grade from Kobian limited (Nairobi, Kenya) an outlet of Sigma- Aldrich Company (USA). They consisted of nitric acid, hydrogen peroxide, boric acid, sodium hydroxide, methyl red and bromocresol indicators, lithium sulphate, selenium, sulphuric acid, trichloroacetic acid, potassium manganate (VII), sodium tungstate, phosphoric acid, phosphomolybdic acid, methanol, iron (III) chloride, sodium carbonate and commercial standards (zinc, iron and calcium commercial standards, tannic acid and iron (III) nitrate). Deionised water was used in all the experiments.

3.4 Sample collection and processing

The termites (*Macrotermes bellicosus*) and lake fly (*chaoboridae*) were obtained from Got Agulu village in Bondo sub-county, Siaya County while the grasshoppers (*Ruspolia differens*) and locusts (*Locustana migratoria*) were obtained from Emaene village in Khwisero Sub County, Kakamega County. The two regions were considered because the practice of entomophagy is already established in the region. A sample weight of 4 kg for each fresh insect species was obtained from vendors in the local market. The insects were put in plastic bags and kept in cool boxes containing dry ice and transported to the Kenyatta University for laboratory analysis within twelve hours of collection. For termites and grasshoppers 4kg of each insect sample was separated into four equal portions; one portion was used for the analysis of the fresh insect, while two portions were sun-dried and oven-dried. The fourth portion was dried (sun-drying and oven-drying) and then defatted at Kenya Industrial Research and Development Institute - KIRDI using a press machine. 3 kg of the locusts was sub-divided into three equal portions. One portion was used for the analysis of the fresh insect, while the other two portions were sun-dried and oven-dried. Lake-fly were already dry at the instance of collection since they lose moisture quickly.

Sun-drying was attained by spreading 1 kg of each fresh insect sample on an aluminium foil and exposing them to the sun for 3 days, 8 hours in a day. Oven drying was achieved by spreading 1 kg of each fresh insect sample on an aluminium foil which was then inserted in an air-oven at 60°C for 48 hours. For defatting, 0.5 kg of sun-dried termites and grasshoppers were separately placed in a bag which was placed in the press machine where oil was expelled from the insects mechanically by continuous application of pressure on the machine. The procedure was repeated using 0.5 kg of

oven-dried termites and grasshoppers. All the fresh and processed insect samples were grinded well using mechanical blender and transferred into airtight containers with proper labeling and stored in a freezer at -21^{o}C.

3.5 Laboratory Analysis

3.5.1 Method validation

Method validation was achieved by calibration method. The AAS instrument was calibrated using freshly prepared working standards in the range of 1 ppm-4 ppm for zinc, 2 ppm-8 ppm for iron and 2 ppm-10 ppm for calcium. The working standard solutions were aspirated into the flame with wavelengths set at 259.9 nm for iron, 213.9 nm for zinc, 422.7 nm for calcium and their absorbances were used to plot standard calibration curves. The regression equations of the curves were determined, and co-efficient of determination (r^2) values were computed Standard solutions were run before and after sample solutions for better precision. A similar procedure was repeated to calibrate the UV-VIS instrument. Working standards in the range of 20 ppm-100 ppm for phytates and 0 ppm-10 ppm for tannins were prepared. The absorbances were read at 700 nm and 480 nm for tannins and phytates respectively.

3.5.2 Proximate analysis

3.5.2.1 Moisture determination

Moisture content (MC) determination followed the method described by AOAC (2005). A 2 g sample was weighed into dry crucibles. The crucibles were placed in a hot air oven at 105°C for 3 hours. The crucibles were covered to avoid loss of sample by spattering during the heating process. The dried samples were cooled and weighed

in a desiccator until a steady weight was attained. Analysis was done in triplicate and the moisture content was calculated as:

Equation 3.1

$$\%\mathrm{MC} = \frac{(\text{weight of cruciblefresh sample}) - (\text{weight of crucible} + \text{dry sample})}{\text{weight of sample taken}} \times 100$$

3.5.2.2. Ash determination

Crude ash content was determined as described by AOAC (2005). A 2 g sample was put into dried crucibles in triplicate which were placed in a muffle furnace followed by incineration at 600°C for 5 hours. The ash was cooled in a desiccator followed by weighing. The ash content was calculated as follows:

Equation 3.2

$$\%\ \mathrm{Ash} = \frac{\text{weight of incinerated sample}}{\text{Weight of sample taken}} \times 100$$

3.5.2.3 Determination of crude protein

Protein content determination followed the micro-kjedahl method as described AOAC (2005). A 1 g sample was put in a digestion flask. 4.4 mL of a digestion reagent prepared as (hydrogen peroxide 350 mL, Lithium sulphate 14 g, selenium 0.42 g, sulphuric acid 420 mL mixed and topped up to 1000 mL) was added. The sample was soaked overnight and then digested until it was colorless. The digested mixture was cooled followed by dilution with 75 mL distilled water. 10 mL of the digested mixture was neutralized by adding 10 mL of 40% NaOH. NH_3 (g) produced was distilled into a 4% H_3BO_3 solution where the indicators methyl red and bromocresol had been added. The distillate was titrated with 0.005N sulphuric acid. A reagent blank was also

run and analysis was done in triplicate. Nitrogen content in the sample was determined by:

Equation 3.3

$$\% \text{ nitrogen} = (V_1 - V_2) \times N \times D \times 0.014 \times \frac{100}{V} \times \frac{100}{S}$$

Where:

V_1 = Titre for sample (mL)

V_2 = Titre for blank (mL)

N = Concentration of sulphuric acid solution (mol/L)

D = dilution factor

0.014 = constant value.

v = Volume of diluted digest taken for distillation (10 mL)

s = weight of the sample taken (1 g)

Crude protein was obtained by multiplying % nitrogen with a conversion factor of 6.25 which is the protein conversion factor for meat and fish.

Equation 3.4

% protein =% nitrogen × 6.25

3.5.3 Determination of iron, zinc and calcium

Mineral content determination followed the method described by Fadigas *et al*., (2010). A 2 g sample was weighed and placed into 250 mL digestion flask, 5 mL of 50% conc. HNO_3 was added and the mixture heated on a hot plate at 80°C for 30 min. The mixture was allowed to cool to room temperature. 30% H_2O_2 was added to the mixture drop wise until the digest was clear. Filtration was done and the filtrate transferred into a 100 mL volumetric flask where distilled water was added to the mark. The diluted sample extract was then transferred into plastic bottles which were then

sealed and labeled properly. Analysis of iron, zinc and calcium was done in triplicate under similar conditions of the blank and standard solutions. The samples and standards were aspirated in an AAS instrument for analysis.

Table 3.1 AAS instrument operating conditions

Parameter	**Calcium**	**Zinc**	**Iron**
Wavelength	422.7 nm	213.9 nm	259.9 nm
Slit width	0.5 nm	0.6 nm	0.2 nm
Current	3 mA	10 mA	10 mA
Flow rate	2.2 l/min	2.2 l/min	2.4 l/min
Flame	air – acetylene	air – acetylene	air – acetylene

3.5.4 Preparation of standards and reagents

3.5.4.1 Iron, zinc and calcium standards

The commercial standard solutions (1000 ppm) of iron, zinc and calcium were used in determining their concentration. 10 mL of stock solution of iron standard was placed in a 100mL volumetric flask and topping up to the mark using distilled water. Working standards of 2ppm, 4ppm, 6ppm and 8ppm were made by appropriate dilution of the stock solution. This procedure was repeated for zinc and calcium. Standard and samples were run using AAS at a wavelength of 422.7 nm, 213.9 nm and 259.9 nm for calcium, zinc and iron respectively. Standard calibration curves for iron, zinc and calcium were plotted and the concentration of minerals in the samples was determined from the standard curve using the regression equations.

3.5.4.2 Tannins

10 mL of tannic acid stock solution (1000 ppm) was placed in a 100mL volumetric flask and topped up to the mark using distilled water. Working standards of 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm were made by diluting the stock solution appropriately. They were treated as the sample extract. A UV-VIS spectrophotometer was used to obtain the absorbances of the sample and standards at 700 nm. A standard calibration curve was plotted from which the concentration of the tannins in the sample was determined.

3.5.4.3 Phytates

10 mL of Iron (III) Nitrate stock solution (1000 ppm) was placed in a 100 mL volumetric flask and topping to the mark using distilled water. Working standards in the range of 20 ppm-100 ppm were made by appropriate serial dilution. A UV-VIS spectrophotometer was used to obtain the absorbances of the sample and standards at 480 nm. The absorbances were used to plot standard calibration curve**.**

3.5.4.4 Folins -Denis reagent

20 g of phosphomolybdic acid, 100 g of sodium tungstate and 50 mL of phosphoric acid were refluxed in 600 mL of water in a 1000 mL volumetric flask for 3 hours. The mixture was allowed to cool followed by dilution to the mark using distilled water.

3.5.5 Determination of Tannins

Tannin content determination followed the method described by Olatoye and Arueya (2019). A 2 g sample was put into 100 mL beaker followed by addition of 30 mL of CH_3OH (50%) and the contents of the beaker allowed to stand in a water bath for about

1hr at 78ºC for homogenization with stirring to prevent formation of lumps. The mixture was filtered into a 250 mL volumetric flask with rinsing using 50% methanol. A 1.0 mL portion of the extract was placed in a 50 mL volumetric flask where 2.5 mL Folins - Denis reagent, 20 mL distilled water and 10 mL of sodium carbonate (17%) was added, mixed thoroughly and then left to stand for about 30 min awaiting a blue-green color to appear. Absorbance was read at 700 nm using a UV-VIS spectrophotometer. Quantification of the tannins was done using a standard calibration curve of tannic acid which was treated in a similar way as the samples.

3.5.6 Determination of oxalates

Oxalate content in the sample was determined as described by Makinde and Akinoso (2013). A sample of 1 g was put into a conical flask, 75 mL of 3M sulphuric acid was added and the contents of the conical flask stirred then filtered. Hot (82-88^{0}C) $KMnO_4$ (0.05 M) was titrated against 25 mL of the filtrate to a point where a light pink color was obtained and lasted for about 25 seconds. Analysis was done in triplicate and the oxalate content calculated by taking 1 mL of 0.05 M KMnO4 as equivalent to 2.25 mg oxalate using the formula:

Equation 3.5

$$\frac{T \times \ \mathrm{Md} \times \mathrm{Mo} \times 100}{W} = \mathrm{mg}/100\mathrm{g}\ \text{Oxalate}$$

Where,

T = titre of $KMnO_4$ (mL)

Md is the number of moles of potassium permanganate that reacted

Mo is the number of moles of oxalate reacted

W is the weight of the sample.

3.5.7 Determination of Phytates

Phytate content was determined according to the method described by Agbaire, (2011). For extraction, 20 mL of 3% trichloroacetic acid was added to 5 g of the sample. Filtration was done and the filtrate transferred to a boiling water bath followed by addition of 3 mL of $FeCl_3$ solution. The phytates present in the sample were precipitated as ferric phytate. 30 mL of 1.5N NaOH was added and the precipitate was converted to insoluble ferric hydroxide and soluble sodium phytate. Hot 3.2N HNO_3 was added to dissolve the precipitate and the absorbance read at 480 nm without delay. Analysis was done in triplicate and the results were expressed as Fe $(NO_3)_3$ equivalent with the assumption that Iron: Phosphorus molecular ratio is 4:6.

3.6 Data Analysis

The raw data obtained was tabulated on Microsoft Excel spreadsheet program and reported as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed for comparison of means of the different concentrations followed by Tukey's post hoc test for comparison of concentrations. The analyzed data was presented in tables.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This section presents the results and discussion of proximate composition (moisture, ash and protein), method validation of the AAS and UV-VIS methods used in the study, mineral and the antinutrient content in the edible insects. The analysis was conducted on fresh and processed insect samples.

4.2 Method validation

The linear regression data for the minerals and antinutrients is as shown in Table 4.1. The coefficient values are close to 1 as it ranges from 0.9939 to 0.9985 which indicates 99.39% - 99.85% correlation between absorbance and concentration. A coefficient above 0.99 signifies a good linear relationship (United Nations Office on Drugs, Crime, Laboratory and Scientific Section (2009). Hence, the calibration curves obtained can certainly be used to determine the concentration of the analyte in the samples.

Table 4.1 Linear regression data for the calibration of minerals and anti-nutrients

Parameter	Equation of the calibration curve	Coefficient of Determination ($r^{2)}$
Zinc	y=0.0316x-0.0066	0.9951
Iron	y=0.0094x+0.0054	0.9971
Calcium	y=0.0346x-0.0148	0.9985
Phytates	y=0.0029x+0.1511	0.9939
Tannins	y=0.0127x+0.0536	0.9974

Appendix B shows the calibration curves for the minerals and antinutrients.

4.3 Proximate Analysis

The proximate analysis of the fresh and the processed termites, grasshoppers, locusts and lake fly is presented in Table 4.2

Table 4.2 Proximate composition of fresh and processed insect

Insect	Moisture (%)	% Ash	Protein (g/100g)
Grasshoppers			
fresh grasshoppers	55.01±2.35^{a}	2.8±0.38^{d}	16.24±0.28^{e}
sun-dried grasshoppers	7.05±0.05^{b}	3.8±0.13^{b}	42.31 ±0.20^{d}
oven-dried grasshoppers	4.00±0.51^{c}	4.01±0.24^{a}	45.53±0.24^{b}
defatted sun-dried grasshoppers	6.68±0.64^{b}	3.05±0.09^{c}	44.11±0.26^{c}
defatted oven-dried grasshoppers	3.42±0.88^{c}	2.29±0.09^{e}	46.02±0.25^{a}
Termites			
fresh termites	40.00±0.12^{a}	2.23±0.32^{c}	19.20±0.24^{e}
sun-dried termites	3.70±0.03^{c}	2.96±0.18^{b}	33.00 ±0.07^{d}
oven-dried termites	1.50±0.05^{e}	4.31±0.40^{a}	35.20±0.40^{c}
defatted sun-dried termites	4.02±0.18^{b}	1.35±0.05^{d}	37.22±0.10^{b}
defatted oven-dried termites	3.28±0.14^{d}	0.86±0.08^{e}	39.01±0.10^{a}
Locust			
fresh locust	44.98±0.41^{a}	1.09±0.10^{c}	17.77±0.79^{c}
sun-dried locust	2.35±0.26^{b}	1.59±0.06^{b}	45.15±0.93^{b}
oven-dried locust	1.38±0.28^{c}	3.35±0.06^{a}	48.77±0.98^{a}
Lake fly			
sun-dried lake fly	15.29±0.36	5.88±2.89	49.58±2.63

NB: Values represent mean ± standard deviation (n=3). Values with different superscripts in the same column per each insect are significantly different ($P \leq 0.05$).

The moisture content for the fresh grasshoppers, termites and locusts was 55.01%, 40% and 44.98% respectively. This was however lower than 73.66% - 82.39% moisture levels reported by Fombong *et al*., (2021) for fresh edible insects. The differences in moisture content in the fresh insects may be attributed to species difference, age and habitat of the insects (Ekop *et al*., 2010).

Processing significantly reduced the moisture content to less than 10% on drying ($P < 0.001$). Defatting the dried samples did not change the moisture content significantly in grasshoppers (between sun-dried samples and defatted sun-dried samples ($p=0.3186$); between oven-dried and defatted oven-dried samples ($p=0.3743$). The oven-dried grasshoppers had moisture content of 4.00% which was consistent with the reported values on oven dried grasshoppers by Rumpold and Schluter (2013) that gave mean moisture content of 4.42%. Processed termites had a moisture content ranging from 1.5% to 4.02% which was comparable to 1.7% reported by Kinyuru *et al*., (2015) for the dried termite consumed in western Kenya. The oven-dried locusts had moisture content of 1.38 %, which compared well with the values within the range of 1.46% to 1.97% reported for oven-dried *L.migratoria* and *S. gregaria* species (Mohamed, 2015). A review on sun-dried lake fly in Uganda gave a moisture content of 15.7 % (Van Huis *et al*., 2013) which was consistent with the current study findings with a moisture content of 15.29%. Oven-drying attained lower moisture levels compared to sun-drying owing to the higher temperatures in the oven in contrast to lower temperatures in the sun.

The ash content of the fresh insects was 2.8%, 2.23% and 1.09% for grasshoppers, termites and locusts respectively and was significantly increased by more than 50% on processing ($P < 0.001$) (Table 4.2) with oven-dried samples having the highest ash

content. Defatting process caused a decrease in ash content in the sun-dried and oven-dried samples. Grasshoppers had ash content similar to 3.1 % reported for *Ruspolia differens sp* in Uganda (Nyangena *et al*., 2020). Oven dried termites reported ash content of 4.31% which was comparable to 3.7% indicated for dried winged termites of *Nasutitermes spp*. reported by Kinyuru (2014) and close to 4.65% reported for oven-dried *Macrotermes bellicosus* by Fombong *et al*., (2017). Locusts reported ash content ranging from 1.09% to 3.35% which collaborated well with the values 5.5±4.0% as reported by Mohamed (2015) for different locust species. Sun-dried lake fly were found to have high ash content of 5.88±2.89%. The findings agreed with 4.1 % reported by Van Huis *et al*., (2013) on dipterans. Oven dried insects had higher ash content than sun-dried insects. Generally ash content in most edible insects have been reported to be low compared to vertebrates. This is because insects lack a mineralized skeleton (Weru *et al*., 2021).

The protein content for the grasshoppers, termites, locusts and lake fly ranged from 16.24±0.88g/100g to 46.01±0.65g/100g, 19.2±0.24g/100g to 39.01±0.10g/100g, 17.77±0.79 g/100g to 48.77±0.98 g/100g and 46.95 g/100g to 52.21 g/100g respectively and was significantly increased on processing ($P < 0.001$) (Table 4.2). The value for the fresh grasshoppers was comparable to the values within the range 12.1g/100g to 26.8g/100g reported on different fresh grasshopper species (Usman and Yusuf, 2021; Ebenebe *et al*., 2017) while for the fresh termites was higher than 14.2g/100g reported by Shockley and Dossey (2014) but comparable to 20.4 - 22.1g/100g reported for different species of the insect (Banjo *et al*., 2006). Oven-dried termites had protein levels close to 38.42 g/100g reported for *Macrotermes subhylanus sp* in Kenya by Kinyuru *et al*., (2013). The oven-dried locust reported high protein levels close to 54.16 g/100g reported by Fombong *et al*., (2021) for the same insect

species. The lake fly posted relatively higher protein levels of 49.58±2.63 g/100g compared to the other insects. This was similar to 48.6 g/100g reported elsewhere for the same insect species (Van Huis *et al*., 2013).

Oven-dried insects had higher protein content than sun-dried insects. This was in agreement with a study by Aniebo and Owen (2010) where oven-dried maggots were found to contain 51 g/100g while sun-dried maggots contained 48 g/100g protein. The fact that processing increases the protein content is in line with a previous study on black crickets which reported increase in the protein yield of the insect after processing (Aniebo and Owen, 2010). In another study on *Sternocera orissa* beetle species, oven-drying the insect increased the protein content by twofold (Shadung *et al.*, 2012).

The wide variability of the protein composition in the current study has been linked to use of different processing method (Meyer-Rochow *et al*., 2021; Lange and Nakamura, 2021) which may also account for the disparity between findings of this study and reports of other researchers. Processes such as sun-drying, oven-drying and defatting may lead to a considerable loss of elements for instance moisture and fat, consequently making the other nutritional elements to concentrate (Parniakov *et al*., 2022). Further, heat treatment denatures the structure of tannins-protein complexes leading to an increase in protein bioavailability (Derix, 2017).

Generally, the protein levels for the insects analyzed were relatively high compared to many convectional foods as reported by Wyness *et al*., (2011). According to WHO (2007) the recommended daily allowance for protein is 10 g per 100 g of body weight. The limit is far exceeded by protein content in grasshopper, locusts, lake fly and termites. Consequently, these edible insects may be deemed as an excellent protein

source viable for supplementation in protein deficient diets (Das *et al*., 2019; Raheem *et al*., 2019). The four insects generally posted high protein content compared to red meat as given by Williams (2007) who reported 23.2% for beef, 24.8% for veal and 21.5% for mutton. Insects can therefore offer a reasonably priced protein source to counter malnutrition in infants and young children (Das *et al*., 2019; Raheem *et al*., 2019).

4.4 Mineral content of the edible insects

Table 4.3 Mineral content (mg/100g) of fresh and processed insect (Dry weight)

Insect	Calcium	Iron	Zinc
Termites			
fresh termites	128.50±3.15[a]	8.82±0.80[a]	34.58±8.35[a]
sun-dried termites	66.96±2.35[b]	8.67±0.63[a]	22.93±6.40[a]
oven-dried termites	56.17±0.71[c]	7.80±1.59[a]	27.60±9.36[a]
defatted sun-dried termites	64.17±1.14[b]	7.99±1.92[a]	32.50±4.41[a]
defatted oven-dried termites	56.28±0.71[c]	8.71±0.89[a]	24.98±1.24[a]
Grasshoppers			
fresh grasshoppers	61.05±2.97[a]	8.60±3.36[a]	19.14±0.95[a]
sun-dried grasshoppers	54.42±0.36[b]	6.33±2.77[a]	22.06±2.02[a]
oven-dried grasshoppers	43.19±0.56[c]	4.36±0.76[a]	14.76±2.13[a]
defatted oven-dried grasshoppers	43.51±2.15[c]	6.43±1.40[a]	22.23±6.58[a]
defatted sun-dried grasshoppers	54.08±1.34[b]	8.89±1.65[a]	27.43±1.08[a]
Locust			
fresh locust	128.56±9.60[a]	8.48±0.31 [a]	21.81±5.01[a]
sun-dried locust	72.25±2.90[b]	7.72±1.03[a]	22.01±1.94[a]
oven-dried locust	52.00±0.52[c]	8.15±1.53[a]	22.17±2.16[a]
Lake fly			
Sun-dried lake fly	74.91±0.24	12.14±2.90	34.37±1.26

NB: Values represent mean ± standard deviation (n=3). Values with different superscripts in the same column per each insect are significantly different ($P \leq 0.05$).

The results for mineral analysis of the fresh and the processed termites, grasshoppers, locusts and lake fly is presented in Table 4.3

Calcium content was 128.50 mg/100g, 61.05 mg/100g and 128.56 mg/100g for fresh termites, grasshoppers and locusts respectively and was significantly reduced on drying (Table 4.3) ($P < 0.001$). Defatting did not lower the content, as also reported by Madibela *et al*., (2007). Calcium content was highest in fresh insects and lowest in oven dried samples.

The calcium content for the termites ranged from 128.50±3.15 mg/100g to 56.17±0.71 mg/100g which was lower than 132mg/100g reported by Rumpold and Schluter (2013), and within levels of 58.72 mg/100g of dry weight reported by Kinyuru *et al*., (2013). Banjo *et al*. (2006) reported 42.16 mg/100g calcium content for oven dried grasshoppers, which was close to 43.19±0.56 mg/100g detected in the oven dried samples. Cacium level in locusts ranged from 52 mg/100g to 128.56 mg/100g which was similar to 129.79 mg/100g reported for oven-dried *Locustana migratoria sp* (Fombong *et al*., 2021). The decrease in calcium during processing depends on quantity initially present, its chemical structure, its distribution in the insect and its interaction with proteins and antinutrients which may reduce its bioavailability (Dima *et al*., 2020). Further, use of heat treatment could initiate heat –induced reactions such as maillard browning reactions which happens between reducing sugars and amino acids or proteins. These reactions may lead to formation of compounds that bind calcium more tightly thus decreasing their bioavailability (Delgado-Andrade *et al*.,

2006). Such factors could account for the decrease in calcium content in the dried insect samples.

Calcium content in most edible insects have been reported to be low compared to the RDA of 700mg/100g-1300mg/100g (WHO and FAO, 2005) for all age groups (Ghosh *et al*., 2017). This is because insects lack a mineralized skeleton (Weru *et al*., 2021). Nevertheless, so long as they are consumed, they add significant extra calcium to the main foods. This implies that alternative ways of complementing traditional diets with calcium in most third world countries are vital (Neumann *et al*., 2014).

The zinc content in the insects samples was not significantly different even after processing (Table 4.3) (p=0.234 for termites, p=0.100 for grasshoppers, p= 0.226 for locusts). Zinc content for the termites ranged from 22.93±6.40 mg/100g to 34.58±8.35 mg/100g and was lower than 10.76 mg/100g reported by Fombong *et al*., (2017) but compared well with the value 21.79 mg/100g for sun-dried termites reported by Anyuor *et al*., (2021). Grasshoppers had zinc content ranging from 14.76±2.13 mg/100g to 27.43±1.08 mg/100g which closely resembled values within the range 18.64 mg/100g –21.79 mg/100g reported by Paul *et al*., (2016) on sun-dried grasshoppers and 14.63 mg/100g reported for *Ruspolia differens species* in Uganda (Nyangena *et al*., 2020). Locust had a content of 21.81±5.01mg/100g - 22.17±2.16 mg/100g which was consistent with results reported elsewhere for the same insect species (Fombong *et al*., 2021). Sun-dried lake fly had a zinc content of 34.369 ± 1.264 mg/100g.

Zinc deficiency is another public health problem. Health consequences consist of delayed development, poor appetite and high vulnerability to diseases (Prasad, 2013; Sauer, 2016). The zinc content found in the edible insects exceeded the RDA for Zn

that is between 4.9 and 7.0 mg /100g (WHO and FAO, 2005). Therefore these insects could be utilized in combating zinc deficiency health problems.

The iron content in the insect samples was not significantly different even after processing (Table 4.3) (p=0.800 for termites, p=0.150 for grasshoppers, p= 0.312 for locusts). Iron content for the termites ranged between 7.80±1.59 mg/100g and 8.82±0.81mg/100g which was lower than 53.33 mg/100g reported by Kinyuru *et al*., (2013) and 24.6 mg/100g reported by Kinyuru (2014) on oven dried termites. Grasshoppers had iron content ranging from 4.36±0.76 mg/100g to 8.89±1.65 mg/100g similar to 7.6 mg/100g reported elsewhere (Nyangena *et al*., 2020). The iron content in locusts was in the range of 7.72±0.63 mg/100g to 8.48±0.44mg/100g which was similar to 7.31 mg/100g reported for different species of the insect (Fombong *et al*., 2021). Lake fly posted the highest iron content of 12.14±2.90 mg/100g which was however lower than 78 mg/100g reported by Van Huis *et al*., (2013).

Iron deficiency is more widespread in developing nations particularly in children (Rezk *et al*., 2015). It was listed as the global most prevalent dietary disorder (WHO, 2016). It can lead to anemia and an increase in maternal deaths (Charles, 2012). Though it's preventable, it contributes to over 20 % of all maternal fatalities (Khaskheli *et al*., 2016). Levels of iron in the insects analyzed were remarkably high and were satisfactorily significant to meet the RDA for iron that is between 7.5 mg/100g and 59 mg/100g (WHO and FAO, 2005). It was generally higher than the iron content of 1.1–3.3 mg/100 g found in red meats (Williams, 2007). Therefore, these insects could be utilized in combating iron deficiency health problems.

The finding that iron and zinc levels are not influenced by processing is in line with previous studies done on black crickets that established that processing does not affect

the micronutrient component of the insect (Irungu *et al*., 2018). It is of nutritional significance as it implies processed edible insects could be incorporated in foodstuffs without reducing their mineral value (Madibela *et al*., 2007). Hence these insects can be considered as useful in efforts to mitigate the threat of micronutrient deficiencies in the society (Mutungi *et al*., 2017).

4.5 Antinutrient content of the edible insects

The results for antinutrient analysis of the fresh and the processed termites, grasshoppers, locusts and lake fly is presented in Table 4.4

The fresh edible insects reported tannins content of 1.69±0.01 mg/100g, 0.93±0.01 mg/100g 3.10±0.38 mg/100g for termites, grasshopper and locust respectively. These levels were however significantly reduced by processing treatments by between 2% - 74% ($P < 0.001$). The values for the tannins in oven dried termites in the current study compared well with values from previous studies on oven dried *Macrotermes faciger* and *Macrotermes nigeriensis* termite species that gave tannin content ranging from 0.47 to 170 mg/100g (Omotoso, 2015; Oibiokpa *et al*., 2017; Kunatsa *et al*., 2020). They were however higher than values within the range of 0.024 mg/100g to 0.051mg/100g reported for winged termites in Nigeria (Idowu *et al*., 2019). A review on edible insects in sub-Saharan Africa established that the tannin content of various oven dried grasshopper species ranged between 0.72-4.35 mg/100g (Omotoso and Adesola, 2018). These were however higher than the current study findings that posted a content of 0.57±0.01 mg/100g.

Table 4.4: Antinutrient content (mg/100g) of fresh and processed insect (Dry weight)

Sample	Tannins	Phytates	Oxalates
Termites			
fresh termites	1.69±0.01[a]	0.67±0.01[a]	7.88±0.02[a]
sun-dried termites	0.95±0.01[b]	0.66±0.01[b]	4.91±0.00[b]
oven-dried termites	0.72±0.01[c]	0.24±0.01[c]	4.27±0.02[c]
defatted sun-dried termites	0.77±0.01[c]	0.29±0.01[c]	6.57±0.01[ad]
defatted oven-dried termites	0.76±0.01[d]	0.31±0.01[d]	5.97±0.01[bd]
Grasshoppers			
fresh grasshoppers	0.93±0.01[a]	0.41±0.01[a]	14.03±0.03[a]
sun-dried grasshoppers	0.60±0.01[b]	0.35±0.01[b]	5.65±0.08[bc]
oven-dried grasshoppers	0.57±0.01[c]	0.18±0.01[c]	3.83±0.05[b]
defatted oven-dried grasshoppers	0.83±0.01[ac]	0.39±0.01[d]	6.43±0.06[c]
Defatted sun-dried grasshoppers	0.86±0.01[ad]	0.37±0.01[d]	6.75±0.05[c]
Locust			
fresh locust	3.10±0.38[a]	0.53±0.01[a]	15.45±0.89[a]
sun-dried locust	0.92±0.20[b]	0.31±0.01[b]	9.68±1.59[b]
oven-dried locust	0.80±0.15[c]	0.24±0.01[c]	5.73±0.05[c]
Lake fly			
Sun-dried lake fly	1.78±0.05	3.29 ±0.02	47.74±1.07

NB: Values represent mean ± standard deviation (n=3). Values with different superscripts in the same column per each insect are significantly different ($P \leq 0.05$).

Tannin content was 0.80±0.15 mg/100g and 1.78±0.05 mg/100g for oven dried locusts and lake fly respectively which was lower than concentrations reported elsewhere by Hassan *et al*., (2009) and also below the permissible limits for tannins. These variations could be dependent the processing method (Essack and Mellem, 2017).

The presence of tannins inhibits the action of trypsin and chymotrypsin hence reducing the protein digestibility. They form insoluble complexes thereby interfering with the bioavailability of proteins (Derix, 2017). The decrease in tannins after processing treatments could be as a result of destruction of protein-tannin complexes at elevated temperatures (Essack and Mellem, 2017). Since the levels of tannins were below the permissible limits of 150-200 mg/100g (Alimentarius, 2003) in all the insects analyzed, they may not have an effect on the digestibility of proteins obtained from these insects

The oxalate content of the fresh edible insects was 7.88±0.02 mg/100g, 14.03±0.03 mg/100g and 15.45±0.89 mg/100g for termites, grasshopper and locust respectively. These levels were however significantly reduced by processing treatments ($P < 0.001$). Oven-dried grasshoppers had oxalate content of 3.83±0.05 mg/100g which was lower than 8.28 mg/100g reported for oven dried *Zonocerus variegatus* grasshopper species (Omotoso and Adesola, 2018). Oven-dried termites posted oxalates values of 4.27±0.02 mg/100g. This was higher than 0.270 mg/100g reported for oven dried termites in Nigeria (Idowu *et al*., 2019). Sun-dried lake fly reported the highest oxalate content of 47.74±1.07 mg/100g.

Oxalate was found to be exceptionally higher in all the insects compared to other antinutrients as reported by Ekop *et al*., (2010). This could imply that oxalate is the predominant antinutrient in edible insects. The permissible limit of oxalate is 250 mg/100g-500 mg/100g (Alimentarius, 2003). The findings from this study were however far below this limit. Its decrease with processing is in line with earlier studies which showed that oxalate is easily destroyed during food processing using heat

treatment (Mutungi *et al*., 2019). This is because oxalates are naturally thermally unstable and heat treatment denatures their structure (Ram *et al.*, 2020). Findings by Kasimala *et al.*, (2018) further revealed that elevated temperatures decreased oxalate levels present in vegetables.

Phytate content was between 0.18±0.01 mg/100g - 0.41±0.01 mg/100g, 0.24±0.01 mg/100g - 0.67±0.01 mg/100g, 0.24±0.01 mg/100g - 0.53±0.01 mg/100g and 3.29 ±0.02mg/100g for the grasshoppers, termites, locusts and lake fly respectively. Analysis of variance showed a significant difference between the fresh and the processed insect samples ($p<0.001$). Omotoso and Adesola (2018) reported that the phytate content of oven dried grasshoppers and termites were within the range of 2.81 mg/100g to 26.49 mg/100g and 0.09 mg/100g to 15.21 mg/100g respectively. These values are in line with the current study findings.

Phytates levels can effectively be decreased by heating. This is because phytates are thermally labile and heat treatment denatures their structure (Samtiya *et al*., 2020). Findings by Ojha *et al*., 2020 further revealed that phytic acid level in grains was significantly reduced after subjecting them to heat. The permissible limit for phytate is 250–500 mg/100 g (Alimentarius, 2003). The current study findings reported figures far below this limit hence these insects are safe for consumption with respect to phytate toxicity.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This section presents conclusions and recommendations made from the results regarding moisture, ash, protein, mineral and antinutrient levels in selected processed edible insects.

5.2 Conclusions

i. Moisture content reduced significantly to less than 10% on processing, while ash content increased significantly. Processing methods were found to increase the protein content significantly in all insects in the order: defatting > oven-drying > sun-drying. The increase was lower for termite samples than for grasshoppers and locusts. Defatting and oven-drying are promising processing methods of increasing the protein composition of edible insects.

ii. Processing does not affect the zinc and iron levels significantly but reduces calcium content in the insects significantly. Oven drying method led to a significantly higher loss in calcium content than sun drying in comparison to the fresh samples.

iii. Processing was also established to decrease the antinutrient levels in the insects significantly. Oven drying method led to a significantly higher decrease in antinutrient levels than sun drying in comparison to fresh samples.

5.3 Recommendations

5.3.1 Recommendations from the findings

I. Since the processing methods studied retained most nutrient content in the edible insects, these methods can be recommended in preparation of the edible insects and their utilization in fighting malnutrition in the society.

II. The study recommends use of oven drying as the most effective method for reducing anti-nutrients.

5.3.2 Recommendations for further studies

I. Only four species of insects were considered in this study. Hence evaluation of other types of edible insects in their promotion as conventional food source is necessary.

II. There is need to evaluate other forms of processing such as roasting and pan-frying and their effect on nutritional and antinutritional value of the final product evaluated.

III. Determination of levels of nutrient and antinutrient in edible insects at various stages of development.

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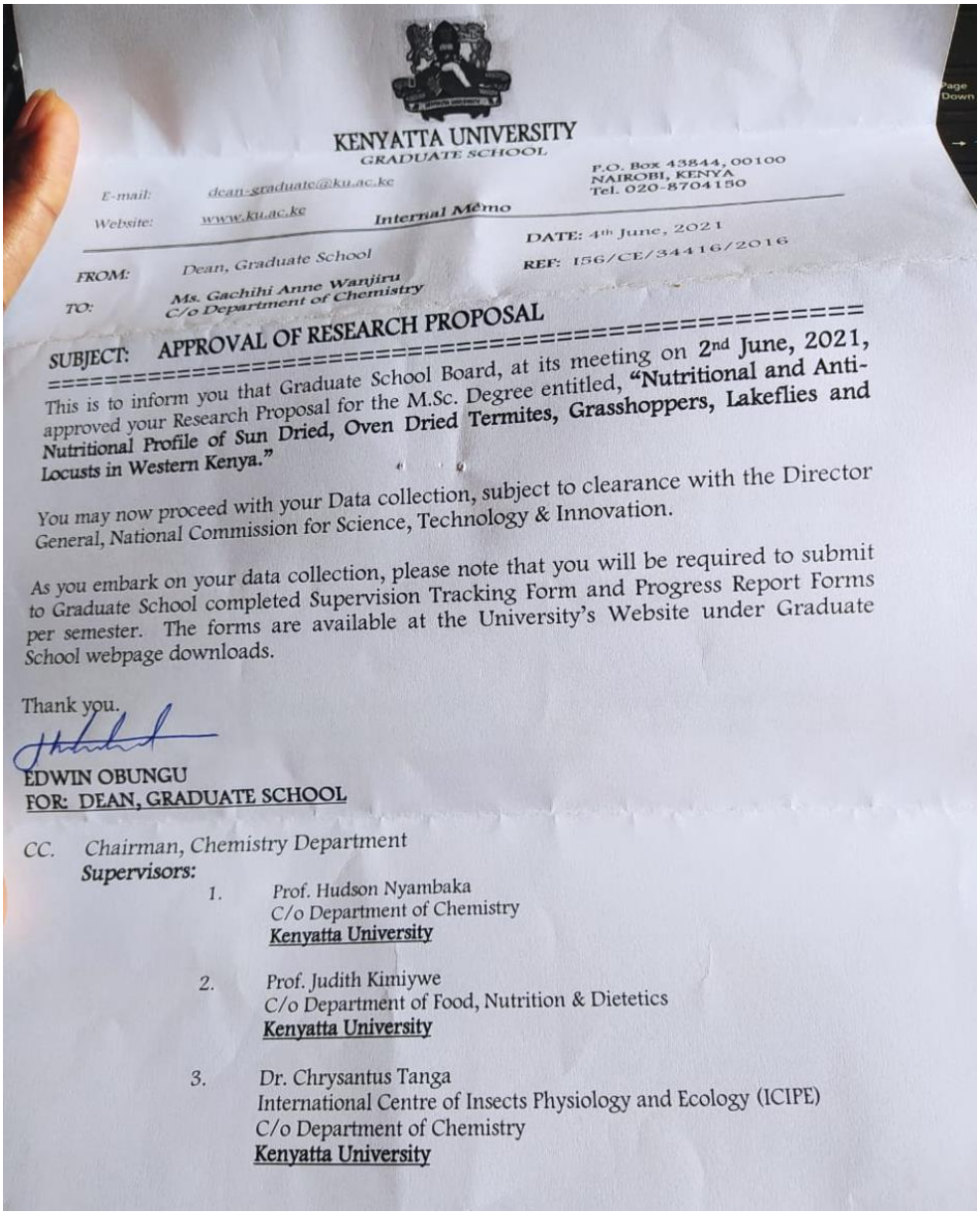
APPENDIX D

RESEARCH APPROVAL BY GRADUATE SCHOOL BOARD



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Internal Memo

FROM: Dean, Graduate School
TO: Ms. Gachihi Anne Wanjiru
C/o Department of Chemistry

DATE: 4th June, 2021
REF: I56/CE/34416/2016

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 2nd June, 2021, approved your Research Proposal for the M.Sc. Degree entitled, **"Nutritional and Anti-Nutritional Profile of Sun Dried, Oven Dried Termites, Grasshoppers, Lakeflies and Locusts in Western Kenya."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Form and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

EDWIN OBUNGU
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Chemistry Department
Supervisors:

1. Prof. Hudson Nyambaka
C/o Department of Chemistry
Kenyatta University

2. Prof. Judith Kimiywe
C/o Department of Food, Nutrition & Dietetics
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3. Dr. Chrysantus Tanga
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