INFLUENCE OF POST-HARVEST TECHNIQUES ON NUTRITIONAL AND MICROBIAL QUALITY OF SELECTED EDIBLE INSECTS

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Influence of Post-Harvest Techniques on Nutritional and Microbial Quality of Selected Edible Insects

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university.

Signature…… Date…….

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This thesis has been submitted for examination with our approval as University supervisors

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DEDICATION

This work is dedicated in its entirety to the Tendeka family of Nyaronge village, Gesima ward, Nyamira County, as an embodiment of excellence and dedication. May this work be an inspiration to the younger generation to soar higher.

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ABSTRACT

Edible insects have traditionally been a dietary source in many African and Asian rural populations. Their potential use as food has increased globally due to their high nutritional content. Microbiological quality and safety concerns regarding edible insects however calls for simple processing methods which are capable of decontaminating this food type while maintaining their nutritional profiles. The aim of this study was to evaluate the impact of traditional post-harvest processing techniques: boiling, toasting, solar drying, oven drying, boiling + solar drying, boiling + oven drying, toasting + solar drying, and toasting + oven drying on the nutritional composition and microbiological quality of adult house cricket (*Acheta domesticus*), adult grasshopper (*Ruspolia differens*), black soldier fly (*Hermetia illucens*) pre-pupae and cotton leaf worm (*Spodoptera littoralis*) 5th instar larvae. All the insect species were collected from the insect rearing and containment unit of the International Centre for Insect Physiology and Ecology (ICIPE) in Kenya, except for *R. differens* which was harvested from the wild in Uganda. The chemical and microbiological analysis were performed in all the processed edible insect samples, with their raw counterparts as the control. The experiments were replicated three times and the data analysed using Stata SE version 12. Raw edible insects had crude protein and crude fat values ranging from 36.3-52.3% and 17.4-29.6%. Processing significantly increased the crude protein ($P < 0.001$) by 1.2-22% in the order: toasting > boiling > oven-drying > solar-drying, with the exception of solar-dried *A. domesticus* ($P = 0.144$), solar-dried and oven-dried *R. differens* ($P = 0.95$; $P = 1.00$) whose crude protein increments were insignificant. Conversely, processing significantly decreased ($P < 0.001$) the crude fat by 0.8-51% following the same order. However, solar-dried edible insects had the least crude fat reduction, which was insignificant (*H. illucens*: $P = 0.051$; *A. domesticus*: $P = 1.000$; *R. differens*: $P = 0.148$; *S. littoralis* $P = 1.000$). Both *H. illucens* and *S. littoralis* had more saturated fatty acids than *A. domesticus* and *R. differens*. Processing significantly increased ($P < 0.001$) the saturated fatty acids 1-1.6 times, while unsaturated fatty acids significantly decreased ($P < 0.001$) by 0.6-0.9 factors in all the edible insect samples, with toasting and toasting + drying having the highest impact. Toasted + oven-dried insect samples had the least desirable amount of linoleic acid (2.96-25.13%) and polyunsaturated fatty acid/saturated fatty acid (PUFA/SAFA) ratio (0.04-0.49). *Hermetia illucens* had the most stable omega-6/omega-3 (n-6/n-3) ratio (1-1.01) during processing, compared to all the other edible insects in this study. The iodine value was significantly decreased ($P < 0.01$) by 0.2-1 factors, while the peroxide and saponification values were significantly increased ($P < 0.01$) by 1.1-25.4 and 0.9-3.9 factors respectively in all the processed edible insect samples. Solar and oven drying significantly increased ($P < 0.01$) the cholesterol content of all the edible insect species by 2.3-15.9%, while the other processes significantly decreased ($P < 0.001$) the cholesterol value. Processes that involved boiling (boiling and boiling + drying) led to a 2.4 to 80.2% decrease in the mineral content of all the edible insect species, with boiled insect samples having the highest decrease. However, processes such as toasting, drying and toasting + drying significantly increased the insect's mineral content ($P < 0.01$). Combined processing (boiling/toasting + drying) had the most

negative impact on all the vitamins and amino acids evaluated. Raw insect samples had high microbial counts. For instance, the total viable counts (TVC), yeasts and moulds counts (YMC) and *Enterobacteriaceae* counts ranged from 7-9.1 Log cfu/g, 6.4-8.2 Log cfu/g and 5.3-7.9 Log cfu/g respectively. Moreover, these samples tested positive for *Salmonella* spp, hence unfit for consumption. Boiling and toasting of all edible insects samples reduced microbial TVC by 4-5 log cycles, *Staphylococcus aureus* by 4.5-6.4 log cycles and totally eliminated *Enterobacteriaceae*, faecal coliforms, Lactose positive enteric (Lac+) bacteria, *Salmonella* spp, and YMC. Oven drying significantly lowered the edible insects TVC ($P < 0.001$) and YMC ($P < 0.001$) by 1-2 and 2-4 log cycles respectively, while solar drying had no significant effect ($P > 0.05$) on these parameters. Combined processing maintained the microbial quality of the boiled/toasted edible insects. These results suggest that the edible insects in this study are highly nutritious but harbour both spoilage and pathogenic microorganisms, which could be detrimental upon ingestion. Actionable hazard control mechanisms like boiling and toasting, which are considered basic and traditional, could be used to decontaminate edible insects and improve their microbial safety.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The continual increase in the world's population without an increase in food production poses a great threat to food security now and to the coming generations (van Huis et al., 2013). A general decline in agricultural production throughout the years has led to a decrease in production of edible proteins, especially in developing countries, hence widespread chronic malnutrition (Elemo, Elemo, Makinde, & Erukainure, 2011). In order to satisfy the protein demand currently and in the future, there is a need to find alternative sources that are affordable, so as to supplement animal meat whose prices are also on the rise (Dinar, Hassan, Mendelsohn, & Benhin, 2011; Elemo et al., 2011). Edible insects can offer such an alternative.

Edible insects have been part of the human diet in many parts of the world including; Central and South America, most Asian countries, Australia and some countries in Africa for many ages (van Huis, 2003). Some cultures however, associate entomophagy (insect consumption) with a mere survival tactic to curb food insecurity in times of droughts and famine or during war (Allotey & Mpuchane 2003; Kinyuru et al., 2018). Although this attitude is slowly changing in many parts of the world (Imathiu, 2020; Verbeke, 2015), the full potential of insects as a source of food is yet to be realised as both their nutritional and economic value is still neglected in many countries (DeFoliart, 1992), including Kenya. However, in countries such as Zimbabwe (DeFoliart, 1999; Kozanayi & Frost, 2002), Nigeria (Agbidye, Ofuya, & Akindele, 2009), Uganda (Agea, Biryomumaisho, Buyinza, & Nabanoga, 2008; Ssepuuya, Aringo, Mukisa, & Nakimbugwe, 2016), Malawi (Munthali & Mughogho, 1992) and Botswana (Zitzmann, 1999), insects not only provide diversity within diets but are also important economically. For example, there are inter-country trades among Botswana, Zambia and South Africa involving mopane worms (*Imbrasia belina*) (Baiyegunhi, Oppong, &

Senyolo, 2016; Madibela, Seitiso, Thema, & Letso, 2007) whose annual sale is estimated at US$ 85 million and offers employment to over 30,000 people in a season (Ghazoul, 2006). In addition, the sale of locusts and grasshoppers have been reported to yield more revenue than some traditional cereal grain such as millet among farmers within the Sahelian region (van Huis, 2003).

Communities that practice entomophagy have different post-harvest techniques in an attempt to make the edible insects palatable (Mutungi et al., 2019). Some insects are collected from the wild, while others are domesticated. Among such insects are grasshoppers, black soldier flies, crickets, termites, cotton leaf worms and lake flies (Kinyuru et al., 2018; Makkar, Tran, Heuzé, & Ankers, 2014; Sayed, Ibrahim, Hatab, Zhu, & Rumpold, 2019). Edible insects have been found to be good sources of proteins, lipids, minerals and vitamins (Finke, 2015b, 2015a). They also contain higher content of essential amino acids and unsaturated fatty acids compared to animal meat (Barker, Fitzpatrick, & Dierenfeld, 1998; DeFoliart, 1991). Therefore, a shift to considering insects as an integral part of the human diet as a source of proteins is needed. Moreover, diversification among insect food products is needed to ensure that consumers benefit from a wide array of acceptable edible insects.

The rich nutritional profile that insects have provides a good environment for growth and proliferation of many spoilage micro-organisms (Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). In addition to microbial spoilage, most edible insects are also highly susceptible to lipid oxidation (Assielou, Due, Koffi, & Kouame, 2015). These are the major causes of quality loss and reduced shelf life in edible insect food type. These post-harvest losses along the insect-food chain commence at harvest and progress through processing, distribution and even storage (Belluco et al., 2013; Mpuchane et al., 2000; Mujuru, Kwiri, Nyambi, Winini, & Moyo, 2014). In East Africa, affordable traditional processing techniques, such as sun-drying, toasting, boiling, roasting and deep frying are practiced so as to improve the general palatability and storability of edible insects (Kinyuru et al., 2018; Mutungi et al., 2019). These processes are done alone or in combination with size reduction methods such as separation of body parts or

crushing (Glew, Sena, Pastuszyn, Millson, & Vanderjagt, 1999; van Huis et al., 2013). Processing ultimately influences the end product in terms of chemical and microbiological quality (Klunder et al., 2012; Manditsera, Luning, Fogliano, & Lakemond, 2019). There could also be contamination of the processed edible insects through soil, air and packaging material, hence rendering them potentially unfit for human consumption (Banjo, Lawal, & Adeyemi, 2006; Kamau et al., 2018a). Storage conditions can also make edible insects susceptible to spoilage. For example, dry environments offer less susceptibility to spoilage due to less humidity compared to moist environments, whose high humidity would lead to proliferation of micro-organisms (Banjo, Lawal, & Adeyemi, 2006).

There is a need therefore to present scientific information on the chemical and microbial quality of edible insects during post-harvest handling for both nutritional and safety purposes. This can also help develop a more evidence-based legislative framework and regulatory guidelines as far as insect-based food, in various stages of the food value chain is concerned (Belluco et al., 2013; Zhou, 2004).

1.2 Problem Statement

An increase in the world population coupled with a rise in urbanization has led to an upsurge in the demand for animal meat, amidst a decline in agricultural products. Malnutrition (protein energy malnutrition and micronutrient deficiency) is a common problem that most developing countries in Africa and Asia experience, contributing to about 94% of global children underweight problems (Leung et al., 2013; FAO WFP, 2013). Severe malnutrition has been reported to be responsible for around 82,000 deaths annually within Kenya, with a significant percentage being from urban slums, where food and nutrition insecurity is rampant (UNICEF, 2010). Similarly, micronutrient deficiency is prevalent among children under the age of 5 years, but it is rarely noticed hence the name hidden hunger. Such deficiencies are brought about by inadequate animal products within diets (Hanson et al., 2015; Miller et al., 2006). With the cost of conventional animal protein being quite high, there is need for various interventions so

as to mitigate this problem. Edible insects, being widely accepted and consumed, can be an alternate protein and micronutrient source. In order to increase palatability, traditional processing methods such as boiling, toasting, frying and sun drying are being used by communities that consume insects. However, there is limited scientific information on how the many traditional post-harvest handling practices affect the overall nutritional and microbial quality of this food type. Any future prospects of industrialisation and commercialisation of edible insects, with the sole purpose of alleviating malnutrition and improving food security will be difficult, as there are no known standard ways of handling specific edible insects in order to reap from their rich nutritional profiles, while considering their microbiological safety.

1.3 Justification

Eradication or reduction of food and nutrition insecurity in developing countries such as Kenya, will help achieve one of the UN's Sustainable Development Goals (zero hunger). It will also help the country achieve one of its crucial pillars (equity and poverty elimination) towards achieving vision 2030. Entomophagy is one of the ways to ensure food diversification and offer sustainable diets to boost food and nutrition security (Illgner & Nel, 2000). In Kenya, insects are widely eaten in the western region (Ayieko & Oriaro, 2008), a practice that is highly supported by the government, as it not only offers employment, but also boosts food and nutrition security (Omolo, 2010; Republic of Kenya, 2007).

In order to increase nutrition security, particularly in regions with a high prevalence of under nutrition and malnutrition, there is need to produce quality protein throughout the year. Edible insects have been shown to be a good source of such essential nutrients, which could ultimately lead to improved health (van Huis, 2003). The advent of mini-livestock (rearing of insects) could ensure production of edible insects throughout the year, thereby increasing accessibility of this already seasonal food type (Halloran et al. 2017). In Kenya for instance, cricket farming is being embraced as a source of food and feed security especially in humanitarian/refugee camp settings (Kamau, Kibuku, &

Kinyuru, 2021). Further, in support of mini-livestock, the Kenya Bureau of Standards (KEBS) has come up with regulations governing safe insect farming, processing and labelling in order to ensure safety upon ingestion. To increase palatability of edible insects, traditional processing methods such as drying in the sun, toasting, boiling, roasting, frying or a combination of these processes are being used especially in rural settings (Mutungi et al., 2019). Some of these processes have been shown to significantly reduce microbial hazards, while others have been shown to be inadequate (Klunder et al., 2012). The combined effect of such processes on the microbial quality of edible insects while maintaining their nutrient profile is not well documented and thus the aim of this study. Such knowledge would be an entry point in the development of technological improvements in edible insects' hazard control plans, while securing livelihoods through improved nutrition security.

1.4 Objectives

1.4.1 Main Objective

i. To determine the influence of post-harvest handling techniques on nutritional and microbial quality of selected edible insects.

1.4.2 Specific Objectives

i. To map the post-harvest handling and processing techniques of edible insects among insect collectors in Western Kenya.
ii. To determine the nutrient composition of *Hermetia illucens*, *Acheta domesticus*, *Ruspolia differens* and *Spodoptera littoralis*.
iii. To evaluate the influence of boiling, toasting, solar drying and oven drying on the nutrient composition and chemical stability of the edible insects.
iv. To assess the influence of boiling, toasting, solar drying and oven drying on the microbiological quality of the edible insects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Entomophagy

Entomophagy, the practice of consuming insects, is widely accepted globally with more than two billion people habitually consuming insects (van Huis et al., 2013). It is estimated that over 2000 insect species are consumed worldwide, with the highest diversity being from the Coleoptera, Lepidoptera and Orthoptera orders (Jongema, 2017; van Huis, 2003, 2013). Edible insects from the Diptera, Isoptera, Hymenoptera and Hemiptera orders have been reported to collectively account for about 22% of the total edible insect diversity by van Huis (2003). Other authors like Ohiokpehai, Bulawayo, Mpotokwane, Sekwati, & Bertinuson (1996) and Ramos-Elorduy (1997) reported some examples of the major insect orders consumed, together with examples of insect species and the number of species consumed as summarized in Table 2.1. Some societies however consider entomophagy a taboo (Allotey & Mpuchane, 2003), or a practice for the poor and therefore considered culturally inappropriate (DeFoliart, 1999; Kinyuru et al., 2018). As a result, many donor agencies and other international organisations have neglected to fully embrace edible insects as a means of food security and food sustainability amid the high rise of meat prices (DeFoliart, 1999; Yen, 2009). Regardless of such cultural barriers, FAO is drawing the world's interest towards adoption of edible insects as food for both protein and fat. Consequently, this will drive entomophagy from being a practice for the poor to a recommended healthy eating habit, as edible insects have been shown to have both nutritional and health benefits (Imathiu, 2020; Payne, Scarborough, Rayner, & Nonaka, 2016). However, the nutritional profile of edible insects has however been shown to be dependent on the edible insect species (Rumpold & Schlüter, 2013), age (Kipkoech et al., 2017), habitat, collection site and swarming seasons (Ssepuuya, Smets, Nakimbugwe, Van Der Borght, & Claes, 2019), sex (Kulma et al., 2019), diet (Chia et al., 2020; Ewald et al., 2020) and post-harvest handling techniques (Dobermann et al., 2019; Escamilla-Rosales et al., 2019). Apart from human

consumption, a variety of insect species are also harvested for other purposes such as feed formulations (Makkar et al., 2014).

Table 2.1: Examples of edible insects in their scientific orders

Insect order	Examples	Number of species
Coleoptera	Beetles	336
Hymenoptera	Ants and bees	309
Orthoptera	Grasshoppers and crickets	235
Lepidoptera	Butterflies and moths	228
Hemiptera	True bugs	91
Homoptera	Cicada and leafhopper	73
Isoptera	Termites	39
Diptera	Flies and mosquitoes	33
Odonata	Dragonflies	20
Ephemeroptera	Mayflies	17
Trichoptera	Caddishflies	5
Neuroptera	Dobsonflies	4
Anoplura	Lice	3

Source: Ohiokpehai et al. (1996) and Ramos-Elorduy (1997).

In Africa, studies on edible insects have been conducted way before the 20th century (DeFoliart, 2002). The numbers of insect species consumed in Africa are many since a single community may be consuming more than one species (Takeda, 1990). For example, the Ngandu community in the Democratic Republic of Congo (DRC) was reported by Takeda (1990) to be consuming about 21 insect species. In Angola, Namibia and Zambia, the Mbunda people have been reported to consume at least 31 insect species (Silow, 1976). Some communities such as the Gbaya people of the DRC have been reported to get their dietary protein, as high as 15%, from 96 different species of edible insects (Roulon-Doko, 1998). In East Africa, several edible insect types such

as lake flies (Ephemeroptera and Diptera), crickets (G*ryllus* spp), grasshoppers (R*uspolia* spp) and termites (*Macrotermes* spp) are consumed by a number of communities (Ayieko, Ndong'a, & Tamale, 2010; Muyonga et al., 2018; Okia et al., 2017).

2.1.1 Ruspolia differens (Grasshoppers)

Grasshoppers are insects that belong to the Orthoptera order. In general, more than 80 insect species of this order are eaten in different parts of the world. Grasshoppers undergo a 12 month life cycle, whose metamorphosis is incomplete. Therefore, there are only 3 stages of life (egg, nymph and adult) (Legendre, Robillard, Song, Whiting, & Desutter-Grandcolas, 2010). The young nymphs are wingless, but wings develop and become longer with each molting stage until the adult emerges with fully developed wings (Legendre et al., 2010). They have chewing mouthparts and most of them feed on grass, plant leaves, flowers and cereals such as maize and millet. Grasshoppers of the genus *Ruspolia* are difficult to distinguish externally as they have no diagnostic features and so, molecular evidence is important to determine the specific species (Gwynne & Morris, 2002; Legendre et al., 2010).

The most common grasshoppers that are used as food are the locusts, short-horned and long-horned grasshoppers (Ramos-Elorduy, 1997; van Huis et al., 2013). In Japan, China and Mexico, some grasshopper species that attack rice and alfalfa fields are also harvested for food (van Huis et al., 2013). In East Africa, the grasshopper species, *Ruspolia differens* and *Ruspolia nitidula* are the most popular and common as they are the most widely available (Mmari, Kinyuru, Laswai, & Okoth, 2017; Ssepuuya, Mukisa, & Nakimbugwe, 2017). Grasshoppers are collected from the wild very early in the morning, when they are less active due to the low temperatures or at night by use of artificial light to lure them (Kinyuru et al., 2018). Commercial farming of grasshoppers for both food and feed is a market that is developing especially in Mexico, Japan, Korea and China (van Huis et al., 2013). According to Wang, Zhai, Zhang, Zhang, & Chen

(2007) and Kinyuru, Kenji, Muhoho, & Ayieko (2010), grasshoppers are a good source of protein and fat with ranges varying from 35.3-65.4%, and 4.22-48.2% respectively.

2.1.2 Acheta domesticus (Crickets)

Crickets are insects of the order Orthoptera. They are large, black or brown in colour and tend to be nocturnal (Legendre et al., 2010). Male crickets chirp, making sounds used to attract female crickets and to raise alarm when disturbed (Gwynne & Morris, 2002). There are almost 900 different cricket species but the most common cricket species consumed and utilized in Africa are field crickets (*Gryllus bimaculatus*) and house crickets (*Acheta domesticus*) (Ayieko, Ogola, & Ayieko, 2016). Just like grasshoppers, crickets undergo an incomplete metamorphosis. The young cricket nymphs are wingless, but wings develop with each instar, until they are fully developed when in the adult stage (Legendre et al., 2010). They too are known to be an excellent source of protein and other nutrients. For instance Barker et al. (1998) reported a 22.8% fat content while Kipkoech, Kinyuru, Imathiu, & Roos (2017) reported a maximum fat content of 25% and a maximum protein content of 60.4% in reared crickets (*A. domesticus*).

2.1.3 Spodoptera littoralis (Cotton leaf worm)

The cotton leaf worm is a moth that belongs to the Lepidoptera order. This insect is a dangerous pest, whose larval stages inflict detrimental damages to many crops such as cotton plants, vegetables, orchard and ornamental trees (Abdelgaleil, 2010; Pineda et al., 2007). It is commonly found in North and Central Africa, Middle Eastern countries and Southern Spain. In Africa, Egypt is the most affected country due to the presence of vast fields of cotton plants (Hamouda & Dahi, 2008). *Spodoptera littoralis* undergoes a complete metamorphosis. The female moth lays between 1000 and 2000 eggs in masses of between 100 and 300 eggs on the lower side of the leaf of the host plant (Miyahara, Wakikado & Tanaka, 1971). The eggs then take about 4 days to hatch when the temperatures are warm, but can take longer when the temperatures are lower. The larval stage of *S. littoralis* goes through 6 instars in 15 to 23 days. Their colour varies from

black-grey to dark-green. The pupal stage takes about 13 days before maturing fully (Miyahara *et al*., 1971). The use of this insect as feed has been reported by (Sayed et al., 2019), who concluded that they have a potential as an alternative to soy in the feed industry. The use of *S. littoralis* as food is not extensively documented although researchers like Krishnan and Kodrík (2006) and Vercruysse, Smagghe, Beckers, & Camp (2009) reported that they could boost the antioxidant capacity of human bodies upon ingestion. This insect has also been shown to have high protein content (51.2%), fat content (33.1%) and mineral content (Sayed et al., 2019).

2.1.3 Hermetia illucens (Black soldier fly)

The black soldier fly belongs to the Diptera order. It undergoes complete metamorphosis and an adult *Hermetia illucens* has similar physical features as a wasp (*Vespula vulgaris*). However, unlike the wasp (*V. vulgaris*), *H. illucens* has only one pair of wings and does not sting, therefore, harmless and poses no danger/threat to humans (Sheppard, Tomberlin, Joyce, Kiser, & Sumner, 2002). *Hermetia illucens* have been shown to reduce the mass and nutrients of organic waste, as they feed on decaying matter of both animal and plant waste material, hence leading to improved farm hygiene in terms of pest fly populations and pollution (Mallin & Cahoon, 2003; Nguyen, Tomberlin, & Vanlaerhoven, 2015). Apart from offering the nutrition that these insects need, the dumpsters offer a moist environment in which they meet their reproductive needs and a site for laying eggs (Sheppard et al., 2002). During their life cycle, the larval stage is the most destructive as it feeds insatiably, so as to accumulate enough fat for use in the pupal and adult stages (Newton, Sheppard, Watson, Burtle, & Dove, 2005). Self-harvesting of the pre-pupae is done as they crawl from the organic waste in search of a potential dry area for pupation (Newton et al., 2005). At this stage, they are an excellent source of proteins, fat and minerals (Sprangers et al., 2017), which could be utilized for both food and feed (Makkar et al., 2014; Wang & Shelomi, 2017).

2.2 Benefits of incorporating insects in human diet

Specialists are foreseeing a rapid approach towards famine and a global food crisis owing to the exponential human population growth (van Huis, 2003) and climate change (Trenberth, 2011). As a result, alternative sources of nutritious foods are needed. Insects can contribute greatly towards supplementing the human diet in the process of abating the global food crisis. In addition, edible insects have been part of traditional foods in many ethnic groups in Africa, Australia, South America and Asia and should therefore be considered safe for consumption on a global scale (van Huis, 2003). Furthermore, to some vegetarians, insects are more acceptable and as such they could act as a source of animal nutrients such as essential amino acids (Katayama, Yamashita, Wada, & Mitsuhashi, 2005). The only limitation of incorporating edible insects in human diets is that their availability is highly unpredictable in terms of locality and seasonality (Banjo, Lawal, & Songonuga, 2006). However, when proper preservation methods are used, the insects' shelf life is lengthened (Mutungi et al., 2019). Moreover, some insect species could be reared at domestic and industrial level hence making them more available for consumption (Ayieko et al., 2016; Oonincx & de Boer, 2012).

The nutritional profile of insects could be another reason for their incorporation in human diets as they are generally considered to be excellent sources of protein, fat and minerals (Rumpold & Schlüter, 2013; van Huis et al., 2013). For instance, a study done in Thailand revealed that the protein and fat content of raw edible insects within that region ranged from 9-28 and 1.5-20.5 g/100g edible portion respectively (Yhoung-aree, 2010). Another study done in Mexico revealed that 25 species of edible grasshoppers had protein and fat content ranging from 44-77% and 4-34% respectively. They also reported high amounts of magnesium (0.35-0.94 g/100g) in all the grasshopper species (Ramos-Elorduy, Moreno, & Camacho, 2012). Wang et al. (2007) reported the crude protein, fat and chitin of grasshoppers (*Acrida cinerea*) as 654.2 g/kg, 83.0 g/kg and 87.3 g/kg respectively. The authors also reported significant amounts of essential amino acids such as methionine, cystein and lysine. The black soldier fly (*H. illucens*) larva was found to contain protein, fat, fibre and ash content of 3.8%, 47.0%, 32.6%, 6.7% and

8.6% respectively, while the housefly (*Musca domesticus*) had protein, fat and ash of 61.4%, 9.3% and 11.9% respectively according to Finke (2010). A caterpillar species (*Usta terpsichore*) found in Angola and the palm weevil (*Rhynchophorus phoenicis*) found in Nigeria are rich sources of thiamine and riboflavin according to Oliveira, Passos de Carvalho, Bruno de Sousa, & Simão (1976) and Banjo, Lawal, & Songonuga (2006) respectively. However, when Kodondi, Leclercq, & Gaudin-Harding (1987) analysed three caterpillar species found in Zaire after traditionally processing them by smoking and drying, they reported high riboflavin and niacin values but low thiamine and pyridoxine values. The nutritional profile of edible insects has been shown to be varied depending on the insect species, age, feed substrate and processing techniques (Fombong, Van Der Borght, & Broeck, 2017; Kinyuru et al., 2013; Kipkoech et al., 2017; Lehtovaara et al., 2017).

For maximum benefits to the body, the insects' fatty acids should have a proper balance between the different fatty acid groups (saturated fatty acids (SAFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA)). An ideal food has a balance ratio of 3:4:3 between SFA, MUFA and PUFA (Mann, 1993). Further, a PUFA/SAFA ratio greater than 0.8 has been associated with low risk of coronary heart diseases while a ratio lower than 0.3 has been associated with atherogenesis (Mann, 1993). Therefore, a PUFA/SAFA ratio balance of 0.45 has been recommended for healthy diets (Pereira, Ferrarese-Filho, Matsushita, & de Souza, 2003). A study done by Pereira et al. (2003) revealed that the silkworm (*Bombyx mori*) had a SAFA, MUFA and PUFA content of 32.52%, 35.43% and 32.05% respectively and a PUFA/SAFA ratio of 0.99. According to the Food and Nutrition Board, Institute of Medicine (2002), the Recommended Dietary Allowance (RDA) for protein is about 0.8g per kg body weight of an adult and 1.05g per kg body weight in children under 5 years per day. Authors like Finke (2002) and Johar (2014) concluded that edible insects can be comparable if not superior to traditional meat protein sources after comparing the nutritive value of crickets and beef (Table 2), hence being a good source of dietary protein. With the increase in red meat related illnesses in man such as atheriosclerosis problems (Mann,

1993), insects can be a good alternative to providing the body with the same nutrients that red meat provides. Therefore, edible insects, being inexpensive, underutilized industrially and rich in nutrients, having high quality protein and essential amino acids (Finke, 2015b; Siulapwa, Mwambungu, Lungu, & Sichilima, 2012), should be considered for incorporation in foods and food applications.

Table 2.2: Comparison between the nutritive value of cricket and beef

Parameter	Cricket (100 grams)	Beef (100 grams)
Protein	25	26
Fibre	6.8	0
Iron	45% of DV	14% of DV
Calcium	15% of DV	1% of DV

DV = daily value

Source: Finke (2002); Johar (2014)

2.3 Environmental and economic impact of edible insects.

Insects can thrive in any climatic and environmental condition. Studies suggest that changes in atmospheric temperature and moisture due to global warming, a resultant effect of climate change, has over time favored the reproduction of insects, hence an increase in their population (Dunn & Crutchfield, 2008; Heegaard, Lotter, & Birks, 2006). Warm temperatures have been shown to influence insects' reproduction and development, by shortening their pre-oviposition duration and by offering favorable conditions for breeding and reproduction, hence, increasing their population (Dunn & Crutchfield, 2008; Kang & Banga, 2013). Ayieko et al. (2010) reported that residents along Lake Victoria experienced abundance and frequency of swarming of alate termites (Isoptera) and lake flies (Ephemeroptera and Diptera), within the past ten years, a phenomena that they attributed to the increasing regional atmospheric temperature. On the other hand, the emergence and population of edible insects such as grasshoppers that

depend on onset of rainfall has been unpredictable due to irregular rainfall patterns (Ayieko, 2014). Elsewhere, aquatic edible insects have been reported to be vanishing due to dried up lake beds in Mexico (Ramos-Elorduy, 2006), owing to climate change.

Most, if not all of the edible insects are collected from the wild (Mutungi et al., 2019). However, some edible insects like silkworms (*Bombyx mori*) (DeFoliart, 1995) and yellow mealworms (*Tenebrio molitor*) (Selaledi, Mbajiorgu, & Mabelebele, 2020) are being reared in China for their utilization as food and feed. The advent of rearing edible insects such as black soldier flies (*H. illucens*) (Zarantoniello et al. 2020), crickets (*Gryllus* spp) (Halloran et al. 2017), grasshoppers (*Tetrix subulata* and *Ruspolia differens*) (Forsman 2011; Ssepuuya et al. 2018) and others is being seen worldwide. In addition, there is increasing awareness of the contribution of livestock to the greenhouse effect (Henning, 2011; Silanikove & Darcan, 2015), as opposed to the contribution from reared insects. In fact, Moran & Wall (2011) estimates that the livestock sector contributes about 18% of anthropogenic greenhouse gases (nitrous oxide and methane) and a further 4.4% of global Carbon (II) oxide emissions. The authors further concluded that any expansion in livestock production will lead to associated deforestation, additional emission of greenhouse gases, eutrophication and nutrient imbalances within the ecosystem. On the other hand, other studies have affirmed that insect farming requires very little resources in terms of land, feed, energy and water compared to other traditional animal protein (Gahukar, 2016; Miglietta, De Leo, Ruberti, & Massari, 2015; Oonincx & de Boer, 2012; Oonincx et al., 2010). For instance, according to Oonincx and de Boer (2012), rearing one kilogram of crickets will require about 15 square meters while beef would require about 200 square meters. In places where water conservation is a priority, insect farming can be efficient, as one kilogram of insects uses less than one litre of water compared to 22,000 litres used in one kilogram production of beef cattle (Collavo et al., 2005). Insects feed on food remains such as stems, peels, rinds, husks, and other agricultural and forest wastes, which are considered organic pollutants of the environment (van Huis et al., 2013) whereas livestock's feed comprises of fish meal,

soybeans, cotton seed cakes and sunflower, which are also used as human food, hence increasing competition for these food types (Mutungi et al., 2019).

In addition to rearing edible insects for human consumption, some edible insects can be reared for more than one purpose. For instance, the mealworm (*T. molitor*) can also be used to recycle organic by-products to useful fertilizer, hence playing a useful role in increasing nutrient uptake and water use efficiency within plants. This in turn leads to increased yields (van Huis, 2013; van Huis et al., 2013). Insects can also be used directly as animal feed or be used in the formulation of nutritious feed (Makkar et al., 2014; Zarantoniello et al., 2020). In East Africa, insect farming initiatives have been put in place, with some countries like Kenya developing regulatory procedures to ensure a proper mainstreaming of the production process (KEBS, 2017). Mass rearing of insects will therefore lead to a lighter carbon footprint compared to mass rearing of animal sources of protein (Oonincx et al., 2010; van Huis, 2003) hence eventually help curb global climate change.

Apart from environmental conservation and providing diversity within diets, edible insects are important economically among communities in Africa. Harvesting and marketing of edible insects is mostly done by women and children and the income used for basic household expenditure such as food, farming inputs and education (Ayieko & Oriaro, 2008; Hope, Frost, Gardiner, & Ghazoul, 2009; van Huis, 2016). In Zimbabwe for instance, women and children are actively involved in mopane caterpillar (*I. belina*) collection, degutting, roasting or drying and marketing (DeFoliart, 1999; Kozanayi & Frost, 2002). The sale of insects in Malawi, Botswana, Uganda, Nigeria and Kenya contribute to an improved economic status of the local communities (Agbidye et al., 2009; Agea et al., 2008; Ayieko & Nyambuga, 2009; Munthali & Mughogho, 1992; Zitzmann, 1999). In addition, there is inter-country trade in Zambia, Botswana and South Africa involving mopane caterpillar (Baiyegunhi et al., 2016; Madibela et al., 2007) and this trade leads to an estimated annual sale of US$ 85 million and an employment rate of over 30,000 people in each season in South Africa (Ghazoul, 2006).

The sale of grasshoppers and locusts have been found to potentially yield more revenue for farmers than millet, within the Sahelian region (van Huis, 2003).

2.4 Post-harvest handling and utilization of edible insects

Edible insects are often processed in order to improve their palatability (Kinyuru et al., 2018; Mutungi et al., 2019). Such processes are dependent on the insect type, taste and consumer preference. For instance, some edible insects like termites could be eaten raw, while others are roasted, fried or even boiled before consumption (Yen, 2010; Christensen et al., 2006). Other post-harvest processes with which edible insects undergo are; removal of legs, beheading and dewinging before they are steamed, sun dried, solar dried, smoked, baked or ground and processed into pastes or chutneys (Aguilar-Miranda, Lopez, Escamilla-Santana, & Barba de la Rosa, 2002). On most occasions, edible insects such as beetles and crickets are roasted, while grasshoppers, locusts and bamboo worms are fried (Aguilar-Miranda et al., 2002). In Kenya, drying in the sun is the most common way of drying edible insects, not only for palatability but also for preservation (Kinyuru, Kenji, Njoroge, & Ayieko, 2009). Some of the common traditional processing techniques used in different countries are shown in Table 3. Improved processing techniques such as solar drying, oven drying, freeze drying, microwave processing and modified atmosphere packaging have been developed and explored in an attempt to increase insects' palatability and shelf life (Fombong et al., 2017; Kamau et al., 2018b; Stoops et al., 2017; Vandeweyer, Lenaerts, Callens, & Van Campenhout, 2017). When specific insect recipes are followed correctly, insect-based foods are highly acceptable among rural areas, with their favorite species reaching urban markets and restaurants (Mitsuhashi, 2010; Mutungi et al., 2019).

Table 2.3: Traditional processing methods of different edible insect species consumed in different countries

Insect species	Stage consumed	Processing method	Country consumed	Reference
Anaphe panda (wild silkmoth)	Larvae	Dry fried or roasted	Congo, Tanzania	(DeFoliart, 1995)
Batocera lineolate (longhorn beetle)	Larvae	Roasted or toasted	Japan	(Mitsuhashi, 1997)
Chaoborus edulis (glassworm)	Adult	Ground and sun dried	Uganda	(van Huis, 2003)
Cirina forda (emperor moth)	Larvae	Boiled then fried in karite butter	Mali, Burkina faso	(van Huis, 2003)
Oryctes monocerus (coconut beetle)	Larvae	Washed and fried	Nigeria	(Banjo, Lawal, & Adeyemi, 2006)
Acheta domesticus (house cricket)	Adult	Toasted and/or dried	Kenya	(Ayieko et al., 2016)
Ruspolia differens (longhorn grasshopper)	Adult	Dewinged, toasted and/ or dried	Kenya	(Kinyuru et al., 2009)
Macrotermes subhylanus (winged termites)	Adult	Dewinged, toasted and/ or dried	Kenya	(Kinyuru et al., 2009)
Leucopolis irrorata (toy beetle)	Larvae	Crilled over charcoal	Philippines	(Adalla & Cervancia, 2010)
Anomala spp (leaf chafer)	Adult	Roasted or boiled	India	(Chakravorty, Ghosh, & Meyer-Rochow, 2011)

The use of edible insects as additives or ingredients in foods, has led to the development of many delicious dishes in restaurants, with a completely different look from a fresh insect, making it attractive (Nonaka, 2009; Yen, 2010). However, there are contradicting opinions on the use of the insects as food ingredients. Western societies, who are not traditionally insect eaters, prefer the use of insects as additives while insect eating communities such as some Asian communities prefer visible, whole insects or their body parts on the food (Nonaka, 2009). As a result, communities from Europe and North America, incorporate the insects as ingredients and eat them for their nutritional value while those of Asian countries and South America not only enjoy eating them whole, but also enjoy collecting them (Nonaka, 2009; Mitsuhashi, 2010). Transformation of the insect-based foods may eventually lead to insect product diversification and industrialisation (Mitsuhashi, 2010). Product diversification has been reported in some studies such as Aguilar-Miranda et al. (2002), who added mealworms (*T. molitor*) powder to maize flour, making it more nutritious, as the resultant flour had an increase in the total protein and total fat content by 2% and 1% respectively. They also noted an increase in essential amino acids when compared to the original maize flour. In another study, Ayieko, Oriaro, & Nyambuga (2010) also incorporated termites and mayflies in the production of muffins, crackers, sausages and meatloaves. Elsewhere, Homann, Ayieko, Konyole, & Roos (2017) incorporated 10% cricket flour in biscuit and reported that they had higher protein content, vitamin B12, zinc, iron and iodine compared to milk biscuits. Insect-based pre-cooked complementary foods, through processes such as extrusion, have also been developed, in an attempt to alleviate food and nutrition insecurity, particularly, malnutrition among children under five, in developing countries (Kinyuru et al., 2015; Konyole et al., 2019).

2.5 Post-harvest spoilage of edible insects

2.5.1 Microbial contamination and spoilage of edible insects

Insects are rich in nutrients which offer a favorable environment for microbial growth and multiplication. In addition, most edible insects have a high moisture content of between 60% and 75% (Finke, 2012), which increases their water activity and thus promotes microbial growth (Glew et al., 1999). Post-harvest handling processes such as sun drying, ash drying, roasting and boiling can also influence microbial growth and proliferation within the edible insect food value chain (Belluco et al., 2013; Glew et al., 1999; Mujuru et al., 2014). For instance, although sun drying and ash drying reduce the insect's water activity, these processes could potentially lead to contamination or recontamination with micro-organisms due to the exposure of the insects to soil, ash, insect predators and other environmental contaminants (Allotey & Mpuchane, 2003; Mujuru et al., 2014). In Nigeria, Banjo, Lawal, & Adeyemi (2006) studied the microbial fauna associated with the larvae of a Rhinoceros beetle (*Oryctes monocerus*) and reported the presence of pathogenic bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*) and Lactose positive enteric (Lac+) bacteria (*Escherichia coli* and *Klebsiella aerogenes*). Earlier, Mpuchane, Taligoola, & Gashe (1996) reported fungi in mopane worm (*I. belina*) of the genera *Aspergillus, Penicillium, Cladosporium, Fusarium* and others. Micro-organisms of the genera *Aspergillus, Penicillium, Fusarium, Proteus, Pseudomonas, Staphylococcus, Escherichia* and *Bacillus* were isolated in processed emperor moths (*Bunaea alcinoe*) by Braide et al. (2011) and concluded that the insects could have been inadequately processed or contamination occurred post-processing. Elsewhere, Mujuru et al. (2014) showed the importance of hygiene and sanitation in the reduction of micro-organisms after reporting less microbial contamination on mopani worm (*Gonimbrasia belina*) handled with gloves compared to those handled with bare hands. Therefore, poor processing, poor sanitation and inadequate handling procedures especially during insect retailing could lead to contamination or recontamination with micro-organisms (Braide et al., 2011).

High temperature treatment of edible insects before consumption has been shown to reduce or eliminate most micro-organisms. According to Klunder et al. (2012), roasting (> 100°C) and blanching (about 100°C) of whole mealworms (*T. molitor*) and crickets (*A. domesticus*) for 5 minutes can sufficiently reduce the total bacterial count from greater than 7 Log colony forming units (cfu)/g to less than 3 Log cfu/g. The authors also demonstrated that the same processes could reduce bacterial spores by about 2 Logs. They further noted that roasting alone was not a sufficient method of eliminating *Enterobacteriaceae* and therefore recommended a short blanching step before the roasting process. When decontaminated insects are improperly stored, there could be a further growth of spoilage and pathogenic microorganisms. For instance, Banjo, Lawal, & Adeyemi (2006) reported more bacteria and fungi in processed rhinoceros beetle larvae (*O. monocerus*) that were stored at room temperature compared to those that were stored under refrigeration conditions. Elsewhere, a similar observation was done by Klunder et al. (2012), who reported a fairly stable bacterial level on boiled crickets (*A. domesticus*) kept under refrigeration for 16 days, as opposed to those that were stored under ambient temperature for the same duration of time. In regions where refrigeration is not an option, proper drying was revealed to be an effective preservation method as Klunder et al. (2012) reported a fairly stable bacterial count in dried crickets during 16 days of storage. Some of these micro-organisms for instance the mycotoxigenic fungi, release toxins such as aflatoxins, ochratoxins and fumonisin, which render the edible insect products unfit for both human and animal consumption (Banjo, Lawal, & Adeyemi, 2006). In general, to boost the shelf life of edible insects, there necessitates optimal conditions for their preservation and storage, in terms of post-harvest handling, processing, packaging and storage, so as to ensure a low microbial spoilage rate.

2.5.2 Chemical contamination and spoilage of edible insects

Contamination of edible insects with contaminants such as insecticides and heavy metals should be considered, as most insects are harvested from the wild (Mutungi et al., 2019). Most insects, including edible ones are considered as agricultural pests and to eradicate them, farmers could use pesticides in their fields. These pesticides could be present at

the time the insects are collected for consumption. For example, Saeed, Abu Dagga, & Saraf (1993) reported high levels of residual organophosphates (malathion and sumithion) and some organochlorides (Aldrin and benzene hexachloride (BHC)) in locusts captured for consumption in Kuwait. Elsewhere, Charlton et al. (2015) evaluated the chemical safety of insects reared on different waste substrates such as poultry manure, brewery solid waste and pig offal, for feed use in Ghana and Mali. They reported the presence of contaminants such as pesticides, veterinary residues, heavy metals, polychlorinated biphenyls and dioxins.

Heavy metals such as nickel (Ni), cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) have been reported to be present in edible insects, which could potentially lead to health problems upon prolonged ingestion. For example, Handley et al. (2007) investigated a lead poisoning outbreak in Monterey, California and concluded that the elevated Pb levels in children and pregnant women was due to consumption of contaminated dried chapulines (grasshoppers) that were imported from Oaxaca, Mexico. Reared mealworm larvae (*T. molitor*) were also reported by Vijver, Jager, Posthuma, & Peijnenburg, (2003) to contain high levels of Pb and Cd, when they were fed on feed substrates collected from soils containing these metals. In Nigeria, Banjo, Lawal, Fasunwon, & Alimi (2010) assessed contamination with heavy metals in edible insects such as the palm weevil (*R. phoenicis*), crickets (*Brachytypes* spp), termites (*Macrotermes* spp), African silkworm (*Anaphe* spp) and others and found that they contained heavy metals such as Ni, Cd, Zn and Pb. In another study, Idowu, Ademolu, & Bamidele (2014) analysed heavy metals contamination in mound termites (*Macrotermes bellicosus*) that were collected from an industrial estate, a dump site and a farmland and their research showed that these termites have a low tendency to accumulate heavy metals from soil, irrespective of the soil's contamination level. The effect of insect processing on these chemical contaminants is not well known and calls for more research.

Apart from chemical contaminants, the high unsaturated fatty acids content in edible insects make them more susceptible to deteriorative lipid reactions. These reactions can be divided into two major groups according to O'Connor et al. (1995): Enzymatic

reactions, which is related to hydrolytic and oxidative pathways or non-oxidative isomerisation of carbon-carbon double bonds and non-enzymatic reactions, which is related to oxidative pathways and isomerisation and occurs slowly at ambient pH and temperature values. Insect handling and processing, prior to consumption, can lead to changes in the natural organisation of the lipids, which in turn renders them more susceptible to deteriorative reactions (Choe & Min, 2006; Dobermann, Field, & Michaelson, 2019). Oxidation is attributed with the destruction of natural anti-oxidants during processing and presence of oxygen, hence leading to insect spoilage by formation of objectionable compounds such as peroxides, aldehydes, ketones and others (Choe & Min, 2006; Guillén & Cabo, 2002). In addition, the presence of poly-unsaturated fatty acids (linoleic, linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids) give the insects a high oxidative power during processing (Guillén & Cabo, 2002), hence leading to hydrolysis and oxidation (Kamau et al., 2018a). Insect freezing is a basic handling technique that is practiced, with the aim of reducing the insects' metabolism. However, enzymatic breakdown continues slowly by the action of phenol oxidase/phenolase, leading to formation of an off-flavoured brown or black insect product, which is unfit for consumption (van Huis, 2013).

CHAPTER THREE

MATERIALS AND METHODS

The flowchart below (Figure 3.1) shows a summary of the methodology carried out on the raw and processed edible insect samples.

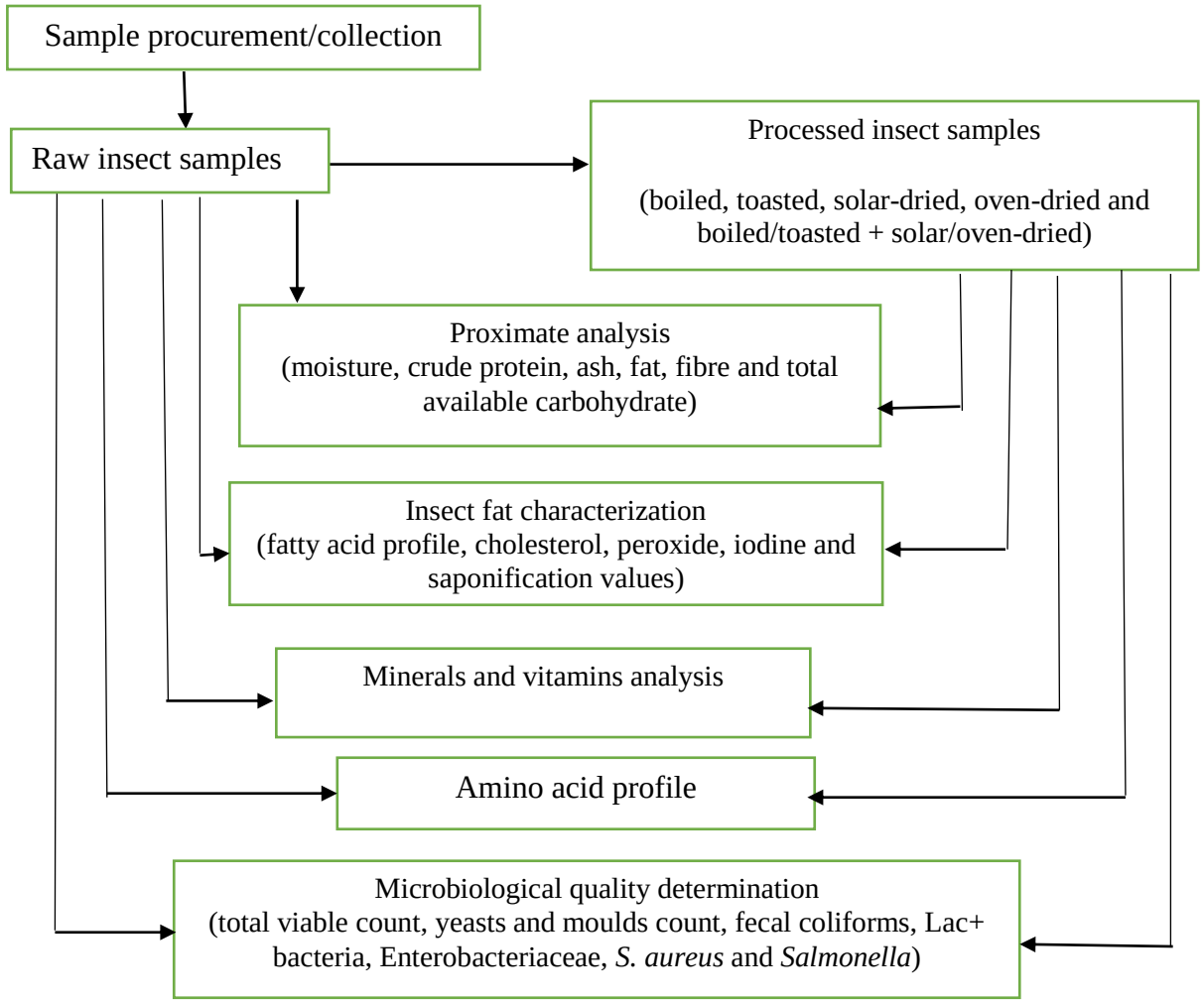

Figure 3.1: Summary of the processes and methods carried out in this study

3.1 Mapping post-harvest practices among insect collectors

Primary data on the post-harvest practices among insect collectors was obtained from the western region of Kenya as communities residing in this region (Luhya and Luo) traditionally consume edible insects. Urban centers/emerging urban centers: Kakamega, Lwanda, Ahero and Bondo were randomly selected, as they contained both urban and rural populations, hence could have little or no manifestation of change within traditional dietary practices. A total of 136 adults, with each town having 34 adult respondents, were interviewed face-to-face using guiding questions (Appendix I) by three trained assistants. All the respondents were randomly selected in strategic places like the local markets and specific homes, which were identified by locals to have adults with a vast knowledge on edible insects within their respective communities. Within the local market, individuals observed selling or buying edible insects were randomly approached and interviewed. Where the targeted respondent was uninterested in participating in the interview, the next randomly selected buyer or seller was chosen. Some of the information sought for included: the commonly consumed edible insects within their community, the post-harvest handling techniques locally used as well as the insects' utilization in terms of food and feed within their household. All the interviews done were within the project budget and time constraints.

3.2 Sample collection and preservation

Raw 5kg of individual edible insect species were collected from the insect rearing and containment unit of the International Centre for Insect Physiology and Ecology (*icipe*) Nairobi, Kenya, that was maintained at a temperature range of 28-30°C. Adult house crickets (*Acheta domesticus*) were reared on a mixture of brewer's waste and kales, black soldier fly (*Hermetia illucens*) pre-pupae were reared on a mixture of brewer's and kitchen waste and African cotton leaf worm (*Spodoptera litorallis)* 5^{th} instar larvae were reared on black nightshade leaves. Five kilograms of adult grasshoppers (*Ruspolia differens*) were collected from the wild in Kampala Central, Nakawa and Nakidye divisions, Uganda and transported overnight in cool boxes to Jomo Kenyatta University of Agriculture and

Technology (JKUAT), Food biochemistry laboratory for analysis. All the raw edible insect samples were washed with chilled tap water (4°C) before any processing and/or analysis. Raw and processed insect samples meant for microbiological analysis were analysed within 24 hours of collection or processing, during which period they were packed in polythene zip-lock bags and stored in a refrigerator maintained at 4°C. Raw and processed insect samples meant for chemical analysis were packed in polythene zip-lock bags and stored in a deep freezer at -21°C. Sample collection and preservation was replicated three times.

3.3 Experimental design

A factorial design of two factors: insect species and processing method was used. There were four levels of insect species: *H. illucens, A. domesticus, R. differens* and *S. litorallis*; and nine levels of processing method: raw insects/control (washed in chilled water), boiling, toasting, solar drying, oven drying, boiling + solar drying, boiling + oven drying, toasting + solar drying, and toasting + oven drying. The experimental analysis was carried out in triplicates.

3.4 Post-harvest processing

3.4.1 Boiling

A 500g sample of raw insects was placed on a wire-mesh kitchen sieve and submerged in a boiling water bath at 96°C for 5 minutes (Klunder et al., 2012). The sieve was lifted from the boiling water and the contents allowed to drain for one minute. The boiled insect sample was transferred to an aluminum foil and left to cool to room temperature (22-25°C) for 20 minutes. The sample was then subdivided into two portions of an approximate ratio of 1:2. They were packed in polythene zip-lock bags and the smaller portion stored in a refrigerator at 4°C, while the other portion stored in a deep freezer at -21°C awaiting microbiological and chemical analysis respectively.

3.4.2 Toasting

A clean, dry, stainless pan was placed over an open flame and heated to about 150°C after which 500g of raw insect sample was placed on it without addition of cooking oil and toasted for 5 minutes with regular turning using a wooden cooking stick to avoid sticking and burning (Kinyuru et al., 2009). The toasted insects were then transferred to an aluminum foil and left for 20 minutes to equilibrate to room temperature (22-25°C). The sample was then subdivided into two portions of an approximate ratio of 1:2. They were packed in polythene zip-lock bags and the smaller portion stored in a refrigerator at 4°C, while the other portion stored in a deep freezer at -21°C awaiting microbiological and chemical analysis respectively.

3.4.3 Solar drying

Solar drying was carried out using a locally fabricated cabinet solar dryer. Raw insect samples (500g) were placed in a solar dryer, and left to dry to constant weight for 2-3 days. The solar dryer consisted of a clear plastic (polythene) sheet stretched over a wooden box (0.6 m wide x 1.2 m long x 0.2 m high) which was placed longitudinally on a slanting metal frame constructed to a height of 1 m off the ground on the air inlet end and 1.2 m on the air exit end. The inside of the box was lined with a black polythene sheet, and the air entry and exit ends were drilled with closely spaced holes of 1 cm diameter (Kamau et al., 2018b). The solar-dried insect samples were then subdivided into two unequal portions (1:2) and packed in polythene zip-lock bags. The smaller portion was stored in a refrigerator at 4°C, while the other portion was stored in a deep freezer at -21°C awaiting microbiological and chemical analysis respectively.

3.4.4 Oven drying

A 500 g sample of raw insect was placed in an air-oven dryer (TD–384KN model Tokyo Thermo Tec) maintained at 60°C and dried to constant weight in 2-3 days. The oven-dried insect samples were removed from the drying chambers and left to cool for 20 min at

room temperature (22-25°C) after which they were subdivided into two unequal portions (1:2) and packed in polythene zip-lock bags. The smaller portion was stored in a refrigerator at 4°C, while the other portion was stored in a deep freezer at -21°C awaiting microbiological and chemical analysis respectively.

3.4.5 Combined processes (boiling/toasting and drying)

Separate 500g of raw insect samples were boiled or toasted as described in section 3.4.1 and 3.4.2 respectively. These were then dried either in the solar-dryer or in the oven-dryer as described in section 3.4.3 and 3.4.4 respectively. Each of the final dried samples were subdivided into two unequal portions (1:2) and packed in polythene zip-lock bags. The smaller portion was stored in a refrigerator at 4°C, while the other portion was stored in a deep freezer at -21°C awaiting microbiological and chemical analysis respectively.

3.5 Proximate analysis of raw and processed insect samples

3.5.1 Determination of moisture content

Hot air drying method (925.10-32.1.03) was used, whereby moisture dishes were washed and placed in a cabinet dryer at 105°C for one hour. They were then placed in a desiccator to cool and their initial weight recorded (W_1). Five grams of insect samples were weighed into the moisture dish and weight recorded (W_2). The dishes were then placed in a cabinet dryer at 105°C for 3 hours. The dishes were then cooled and final weight recorded (W_3). The moisture content was then calculated as shown below (AOAC, 2000).

Moisture content (%) = $(W_3 - W_1)/ (W_2 - W_1) \times 100$

3.5.2 Determination of crude protein

The semi-micro Kjeldahl method (920.87-32.1.22) was used, whereby 1g of the insect sample was weighed into a digestion flask together with a catalyst (5g of potassium

sulphate and 0.5g copper (II) sulphate and 15 ml concentrated sulphuric acid). The mixture was heated in a fume hood till the digest turned blue, signifying the end of the digestion process. The digest was cooled, transferred to a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion was also prepared. A 10 ml of diluted digest was then transferred into the distilling flask and washed with about 2 ml distilled water. A 15 ml of 40% sodium hydroxide was added and washed with 2 ml of distilled water. Distillation was done to about 60 ml distillate. The distillate was then titrated using 0.02 mol/L hydrochloric acid to the end point (AOAC, 2000).

Crude protein was then calculated as described below;

Nitrogen% = $(V_1 – V_2)$ x N x f x 0.014 x100/v x 100/s

Where: V_1 = Titer for sample (ml)

V_2 = Titer for blank (ml)

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

f = Factor of hydrochloric acid solution.

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

s = weight of the sample taken (g)

Protein % = nitrogen x protein factor (6.25).

3.5.3 Determination of crude fibre

The Henneberg-Stohmann method (920.86-32.1.15) was used, whereby 2g of insect sample was weighed into a 500 ml conical flask and 200 ml of boiling 1.25% sulphuric acid added and boiled for 30 minutes under a reflux condenser. The digest was then filtered with a Pyrex glass filter and the residue washed with boiling water to completely

remove the acid. 200 ml of boiling 1.25% sodium hydroxide was added to the washed residue and boiled under reflux for 30 minutes. Filtration was then done using the same glass filter as used before. The residue was rinsed with boiling water followed by 1% hydrochloric acid. The residue was dried at 105°C for 1 hour in a porcelain dish, cooled and weight recorded (W_1). Incineration in a muffle furnace at 550˚C for 3 hour was carried out followed by cooling in desiccators and weight recorded (W_2). Crude fibre was calculated as shown below (AOAC, 2000).

Crude fibre % = ((W_1-W_2)/ weight of sample) x 100.

3.5.4 Determination of crude fat

Extraction of crude fat was done using the Soxhlet extraction method (920.85-32.1.13). A 5g insect sample was weighed into the extraction thimble and the initial weights of the extraction flasks taken. Fat extraction was carried out using petroleum spirit in apparatus for 16 hours. The extraction solvents were evaporated and the extracted fat dried in an oven for 15 minutes before the final weights of the flasks with the extracted fat was taken. Crude fat was calculated as shown below (AOAC, 2000).

$$\text{Crude fat (\%)} = \left(\frac{\text{Weight of fat extracted}_{(g)}}{\text{Weight of sample}_{(g)}}\right) \times 100$$

3.5.5 Determination of crude ash

Crude ash was determined by incineration in a muffle furnace (method 923.03-32.1.05). A 5g insect sample was weighed into pre-conditioned crucibles. The samples were then charred by flame to eliminate organic material before being incinerated at 550°C in a muffle furnace to the point of white ash. The residues were cooled in desiccators and the weights recorded. Crude ash was then calculated as shown below (AOAC, 2000).

$$\text{Crude ash (\%)} = \left(\frac{\text{Weight of ash}_{(g)}}{\text{Weight of sample}_{(g)}}\right) \times 100$$

3.5.6 Determination of total available carbohydrate

This was done by the difference method whereby the other proximate components (total moisture, crude protein, ash, fat, fibre) were subtracted from 100%.

3.6 Extraction and characterization of the edible insects' oil

The samples oil extraction was carried out by solvent extraction method (Bligh & Dyer, 1959) whereby 50 ml of chloroform-methanol (2:1 v/v) solvent containing 10 mg/L of butylated hydroxy toluene was added to a 5 g insect sample. The mixture (1:10 w/v) was homogenized in a waring blender for 2 minutes before centrifuging at 1500 rpm for 5 minutes to give an aqueous top phase and an organic bottom phase. The bottom organic phase was drawn out using a Pasteur pipette and the solvent separated from the lipid fraction by evaporation on a rotary evaporator (rotary evaporator RE100B) at 40°C. Chemical characterization of the resultant oil was then done.

3.6.1 Determination of fatty acid profile of the edible insects' oil

The fatty acid profile was determined by the gas chromatography (GC) method with the insect oils being derivatized into methyl esters based on the method described by Jeon et al. (2016). Oil aliquot (0.25g) was weighed into a conical flask, 6 ml of 0.5 mol/L methanol sodium hydroxide added and heated in a water bath at 80°C for 10 minutes. The mixture was then cooled on ice for 3 minutes after which 7 ml of 14% boron trifluoride methanol was added. The mixture was heated at 80°C for 2 minutes and allowed to cool on ice for 3 minutes before adding 5 ml of n-hexane. The resultant mixture was heated for 1 minute before transferring the top layer (methyl esters) into a vial for analysis. Methyl esters (1 µl) was injected into a GC (Shimadzu, GC-2010A series; Shimadzu, Tokyo, Japan) that was equipped with a capillary column (BPX70 – 30 m x 0.32 mm x 0.25 µm) maintained at 210°C and a flame ionization detector whose detection temperature was maintained at 260°C. Helium, whose flow rate was set at 1.10 ml/minute, was used as the carrier gas. Fatty acid peaks were identified by comparing

the retention times with those of known standards and individual peaks expressed as a percentage of the total peak area. The total saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 fatty acids (n-6) and omega-3 fatty acids (n-3) were also computed.

3.6.2 Determination of peroxide value of the edible insects' oil

The peroxide value (PV) was determined by titration method (AOAC, 2000). A 2.5g of insect oil aliquot was measured into an Erlenmeyer flask and 25 ml glacial acetic acid: chloroform mixture (3:2 v/v) added, followed by 1 ml of freshly prepared saturated potassium iodide solution and allowed to stand in the dark for 30 minutes at room temperature (25°C). A 30 ml water was added to the solution and the mixture titrated against 0.01 mol/L sodium thiosulphate to the end point using starch solution as the indicator. A blank was prepared under the same conditions and PV (mEq O_2/Kg) calculated using the equation:

$$PV = \left((V_S - V_B) \times N \times 1000\right)/W$$

Where: V_S = Titer required for the oil sample (ml)

V_B = Titer required for the blank (ml)

N = Concentration of sodium thiosulphate (mol/L)

W = Sample weight (g).

3.6.3Determination of iodine value of the edible insects' oil

The iodine value (IV) was determined by titration method (AOAC, 2000). A 2g of insect oil aliquot was weighed into an Erlenmeyer flask and dissolved in 10 ml carbon tetrachloride. A 25 ml Wijs' solution was then added and allowed to stand in the dark for 1 hour at room temperature (25°C). A 20 ml of 10% potassium iodide solution and 100 ml of distilled water were added and mixed. The mixture was titrated against 0.1 mol/L

sodium thiosulphate to the end point, using starch solution as the indicator. A blank was prepared under the same conditions and IV (g I_2/100g) calculated using the equation:

$$IV = \left((V_B - V_S) \times M \times 12.69\right)/W$$

Where: V_B = Titer of blank (ml)

V_S = Titer of sample (ml)

M = Concentration of sodium thiosulphate (mol/L)

12.69 = Iodine equivalence factor

W = Sample weight (g).

3.6.3 Determination of saponification value of the edible insects' oil

The saponification value (SV) was determined by titration method (AOAC, 2000). A 2g insect oil aliquot was weighed into an Erlenmeyer flask, 0.5 mol/L alcoholic potassium hydroxide (25 ml) added and the mixture boiled under a reflux condenser for 30 minutes. Titration was then done with 0.5 mol/L hydrochloric acid using phenolphthalein as the indicator. A blank was prepared under the same conditions and SV (mg KOH/g) calculated using the equation:

$$SV = \left((V_B - V_S) \times N \times 28.05\right)/W$$

Where: V_B = Titer of the blank (ml)

V_S = Titer of the sample (ml)

N = Concentration of hydrochloric acid (mol/L)

28.05 = Equivalent molecular weight of 0.5 mol/L potassium hydroxide solution

W = Sample weight (g).

3.7 Determination of total cholesterol of raw and processed insect samples

Direct saponification method was used for cholesterol determination in the raw and processed insect samples as described by Stajić, Živković, Perunović, Šobajić, & Vranić (2011) whereby 100mg of each edible insect sample was added to 2 ml of 0.5 mol/L potassium hydroxide in methanol in a tube, mixed and vortexed for 30 seconds. The mixture was saponified at 80°C for an hour after which cooling was done before the addition of 2 ml distilled water saturated with sodium chloride. Vortexing was then carried out for 30 seconds before the addition of 3 ml diethyl ether: hexane (1:1v/v) and centrifugation at 300 rpm for 10 minutes. The supernatant was transferred to a clean tube and the diethyl ether: hexane extraction step repeated twice. All the three extracts were combined and evaporated to dryness under a stream of nitrogen. The dry extract was then dissolved in 1000 µl of the mobile phase, acetonitrile: isopropanol (50:50) that was used for High Performance Liquid Chromatography (HPLC) analysis and filtered. A 10 µl of the filtered sample mixture was injected into a reverse phase C-18 column HPLC whose mobile phase was acetonitrile: isopropanol (50:50) at room temperature (25°C). The column eluate was monitored with a photodiode-array detector and quantification of cholesterol done by external standardization in a linear concentration range from 25 mg/100g to 125 mg/100g.

3.8 Determination of mineral content of raw and processed insect samples

Five grams of insect sample was weighed in a crucible and transferred to a hot plate in the fume hood chamber where it was charred to clear all carbonaceous material before transferring them to the muffle furnace. The charred material was incinerated at 550°C until it was reduced to white ashes. The ashes were cooled and 15 ml of 6 mol/L hydrochloric acid added to sample in the crucible before transferring the content to a 100 ml volumetric flask and topping up with distilled water. Mineral analysis was then carried out using atomic absorption flame emission spectrophotometer (AAS) (Model A A-6200, Shimadzu, Corp., Kyoto, Japan) (Paul et al. 2014).

3.9 Determination of fat soluble vitamins of raw and processed insect samples

3.9.1 Determination of retinol content

To a 50 ml glass stoppered centrifuge tube, 2.5g of ground insect sample was added. A 10 ml of absolute ethanol containing 0.1% (wt/vol) ascorbic acid was then added followed by 2 ml of 50% (wt/vol) potassium hydroxide. The tubes were stoppered, agitated using a shaker and placed in a water bath at 80°C for 20 minutes with periodic agitation to effect complete digestion of fat. Cooling was then done before addition of 10 ml hexane containing 0.01% (wt/vol) butylated hydroxyltoluene. The tubes were stoppered and vigorous agitation done with a vortex for 1 minute. The content was allowed to stand for 2 minutes before vortexing again for 1 minute. Five ml of cold water at 1°C was then added to the tube and inverted 10 times before centrifugation at 1000 rpm for 10 minutes. Ten millilitres of the supernatant was pipetted into a tube and solvent evaporated at 40°C using a rotary evaporator. The residue was then dissolved in 1 ml methanol. The standard solution was prepared in a similar manner but with the following modifications: to 1 ml of the standard solution, 0.1 ml peanut oil was added before saponification to avoid oxidation. Five ml of the supernatant was pipetted and residue dissolved in 5 ml methanol. Twenty microlitres of the prepared sample and standard were then injected into a HPLC whose mobile phase was methanol: water (95:5), a flow rate of 0.8ml/minute and a UV detector at 325nm (Zahar & Smith, 1990).

3.9.2 Determination of vitamin E

A 20g of previously extracted fat was weighed into a 50 ml volumetric flask and 15 ml of hexane added before shaking vigorously for 15 minutes. The content was filtered by a cotton wool before micro-filtration using a 0.45 µm syringe filter. A stock solution was prepared by weighing 100mg of standard vitamin E and dissolving it in 100 ml of hexane. The standard was then diluted to a working standard range of 10-100ppm. Twenty microlitres of the prepared sample and standard were injected into normal phase

silica 60 HPLC whose mobile phase was hexane: isopropanol (98:2), a flow rate of 105ml/minute at room temperature (25°C) (Diack & Saska, 1994).

3.10 Determination of water soluble vitamins of raw and processed insect samples

The sample was prepared using solid-phase extraction (SPE) whereby 20g of deionized water was added into a 5g sample and homogenized at medium speed for 1 minute before centrifuging for 10 minutes at 14000 rpm. The stationary phase was flushed with 10 ml methanol and 10 ml water to adjust the pH to 4.2. Ten millilitres of the homogenized and centrifuged sample was loaded and eluted with 5 ml water (pH 4.2) followed by 10 ml methanol at a flow rate of 1 ml/minute. The eluent was collected in a bottle and evaporated to dryness in a rotary dryer. The residue was then dissolved in the mobile phase and filtered through a 0.45 μm pore size micro-filter. Twenty microlitres of the sample solution was then injected into a reverse phase C-18 column HPLC, whose mobile phase was 0.1 mol/L potassium dihydrogen phosphate (pH 7): methanol (90:10) and a flow rate of 0.7 ml/minute at room temperature (25°C). The column eluate was monitored with a photodiode-array detector and identification and quantification of the vitamins achieved by comparing their retention times with those of respective standards of known concentrations (Ekinci & Kadakal, 2005).

3.11 Determination of amino acid composition of raw and processed insect samples

Essential and non-essential amino acids were analysed using ion exchange chromatography (IEC) whereby 2g of insect sample was defatted using chloroform and then hydrolyzed using 6M-HCl before injecting the hydrolysate into the technicon sequential multisample amino acid analyser (Technicon Instruments Corporation, Dublin, Ireland) for the separation and characterization of amino acids (Ertingshaausen, Adler, & Reichler, 1969). Due to budget constraints (US$ 120 per insect sample), amino acid analysis was performed on selected edible insect samples (raw and boiled + dried).

3.12 Assessment of microbiological quality of raw and processed insect samples

3.12.1 Preparation of sample homogenates

Raw and processed insect samples were pulverized separately in a mini-blender that was pre-rinsed with 70% ethanol. A 10g pulverized sample was then transferred into a conical flask containing 90 ml of sterile peptone water as the diluent. The mixture was homogenized using a stomacher for 2 minutes. Aliquots (1 ml) of the homogenate was aseptically diluted through a series of tubes containing 9 ml sterile diluents up to 10^{-7}.

3.12.2 Enumeration of total viable count (TVC)

Aliquots of 0.1 ml of individual dilutions were inoculated through the pour plate method using prepared plate count agar (PCA) and incubated at 35°C for 48 hours. Plates with less than 300 colonies were counted and the number of bacterial colonies expressed as Log colony forming units per gram (Log cfu/g) of the sample using the equation described by International Dairy Federation method (IDF), (1996) as shown below.

$$\text{Log } C = \sum x/n_1 + (0.1n_2)^x d$$

Where; C = Count cfu/g

x = Total number of colonies in all plates

n_1 = Number of plates from initial dilution where counts were made

n_2 = Number of plates from second dilution from where counting was done

d = Initial dilution of counting

3.12.3 Enumeration of total yeasts and moulds (YMC)

Aliquots of 0.1 ml of individual dilutions were inoculated using the spread plate method on prepared potato dextrose agar (PDA) containing 10% tartaric acid and incubated at

25°C for 72 hours. Plates with less than 300 colonies were counted and the YMC number expressed as Log cfu/g of insect sample using the equation described in section 3.12.2 above (IDF, 1996).

3.12.4 Enumeration of Lactose positive enteric (Lac+) bacteria

Aliquots of 0.1 ml of individual dilutions were inoculated through the spread plate method on prepared MacConkey agar and incubated at 37°C for 24 hours. Pink-red Lac+ bacteria colonies were counted and numbers expressed as Log cfu/g of sample (IDF, 1996).

3.12.5 Enumeration of Enterobacteriaceae

Aliquots of 0.1 ml of individual dilutions were inoculated through the pour plate method using prepared violet red bile glucose (VRBG) agar with an overlay of the same medium and incubated at 37°C for 24 hours. Purple-pink, surrounded by a halo *Enterobacteriaceae* colonies were counted and numbers expressed as Log cfu/g of sample (IDF, 1996).

3.12.6 Enumeration of Staphylococcus aureus

Aliquots of 0.1 ml of individual sample dilutions were inoculated via spread plate method on prepared baird parker agar containing 5% egg yolk tellurite emulsion and incubated at 35°C for 48 hours. After a coagulase confirmatory test, black shiny with clear halos *Staphylococcus aureus* colonies were counted and numbers expressed as Log cfu/g of sample (IDF, 1996).

3.12.7 Enumeration of faecal coliforms

Five grams of raw and processed samples were individually added into sterile peptone water and mixed. Fifty millilitres of the water-sample mixture was passed through a 0.45 nm micro-membrane filter before aseptically placing the membrane onto a Petri dish

containing m-Endo agar LES. The inoculum was incubated at 35°C for 24 hours before counting and expressing faecal coliform colonies as Log cfu/g of sample (IDF, 1996).

3.12.8 Detection of Salmonella species

Twenty five grams of raw and processed insect samples were individually enriched with 225 ml nutrient broth and incubated at 35°C for 24 hours. Selective enrichment was then done by transferring 25 ml of the enriched homogenate to 225 ml of tetrathionate broth and incubated at 37°C for 24 hours. A loopful of the tetrathionate broth culture was streaked on Salmonella-Shigella agar and colourless with black centres *Salmonella* spp colonies identified. They were further confirmed using the triple sugar iron (TSI) test, whereby colonies were inoculated (stab butt and the slants streaked) into slants of the TSI agar at 35°C for 24 hours. *Salmonella* spp gave pink to orange (alkaline) slants, yellow (acid) butt, with black precipitate from hydrogen sulphide production (IDF, 1996).

3.13 Statistical analyses

Qualitative data was coded and descriptive statistics computed using Statistical Package for Social Sciences (SPSS) version 21, while quantitative data was analysed using Stata SE version 12, where it was subjected to descriptive statistics to determine means and standard errors of means. A two-way analysis of variance (ANOVA) to determine the interaction effects between the two independent factors (insect species and processing method) was also done. Where interactions were significant, a one-way ANOVA was performed to determine the significance of these differences between the two factors. Means were separated using Bonferroni adjustment at 95% confidence level.

CHAPTER FOUR

RESULTS

4.1 Consumption and post-harvest handling practices of edible insects in western Kenya

Respondents within the Luo and the Luhya communities whose reported age was between 18 and 72 years old identified a number of edible insects consumed (Figure 4.1) within their region. Termites/white ants (Isoptera) (100%) had the highest consumption distribution, as all the respondents acknowledged eating them. This was closely followed by grasshoppers/locusts (Orthoptera) (89.0%) and black ants (Hymenoptera) (77.9%). Dung beetle larvae (Coleoptera) (63.2%) were common among the Luhya community, while lakeflies (Diptera) (57.4%) were common among the Luo community. Other edible insects identified were crickets (Orthoptera) (52.9%), armyworms (Lepidoptera) (30.8%) and earthworms (Opisthopora) (22.8%).

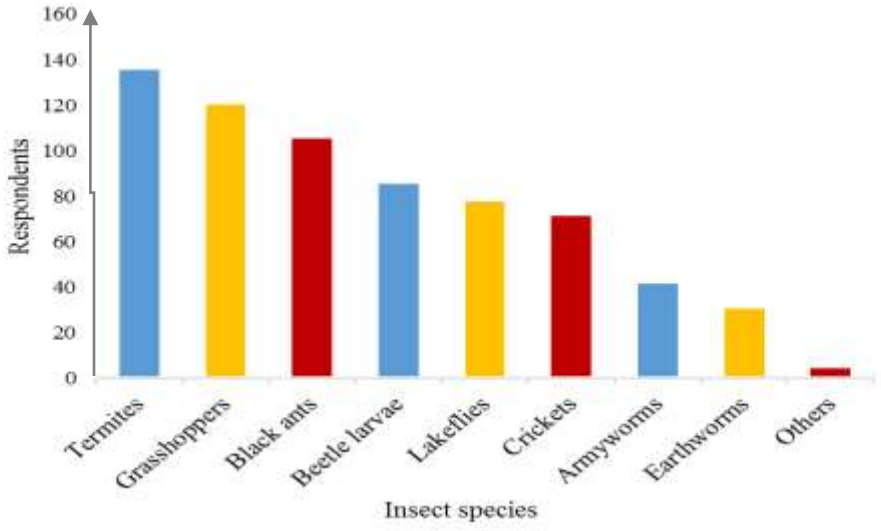

Figure 4.1: Commonly consumed insects in the Western region of Kenya

All the respondents acknowledged that women and children were the most involved with the collection and handling of edible insects, although men would occasionally be involved in setting up night traps for insects such as grasshoppers (*Ruspolia* spp). In addition, in the case of edible insects like termites (*Macrotermes* spp and *Pseudacanthotermes* spp) which they identified could be eaten raw, men could also be seen collecting and eating them from ant hills or swarming areas. In Bondo, some residents revealed to have benefited from knowledge passed onto them by the Jaramogi Oginga Odinga University of Science and Technology (JOOUST) regarding insect rearing, but had personally not fully ventured into mini-livestock.

According to the respondents, the most common methods of handling the insects prior to cooking were washing (100%), de-winging (88.9%), degutting (83.1%), de-heading (76.5) and removal of appendages (66.2%). They also identified a number of preparation methods, which they claimed to be dependent on the insect species, prior to consumption such as sun drying (100%), toasting (98%), frying (96.3%), roasting (92.6%) and boiling (80.1%) being the most common cooking methods (Figure 4.2). According to the respondents, toasting involved placing raw edible insects on a hot pan to fry in their own oil, while frying involved heating some vegetable oil/fat in a pan before placing the insects in the pan. The time for preparation was reported to vary with respect to the edible insect with termites taking the least time of up to 5 minutes, while dung beetle larvae took the most time of not less than 15 minutes. Sun drying was also identified as the most preferred method of increasing the edible insects' shelf life among all the respondents and it involved spreading edible insects on a tray or woven bag and leaving them in the sun to dry. The average storage period for most sun-dried edible insects was between 7-14 days depending on the insect species. Salting (67.6%) and smoking (63.9%) were also used as methods of preservation. Seven respondents (5.1%) reported refrigeration as a means that they used in preserving edible insects. A majority of the respondents (72.8%) reported that grinding of edible insects was done and the powder incorporated in other flours such as millet used in weaning infants. Most of the respondents (94%) reported that they would buy edible insects from local vendors once

their swarming seasons are over. All the respondents reported that they were able to tell spoiled edible insects by smelling. The respondents who had domestic animals including dogs, cats and chicken revealed that they would occasionally feed the animals with edible insects, but their priority was leaning towards the edible insects' utilization as food.

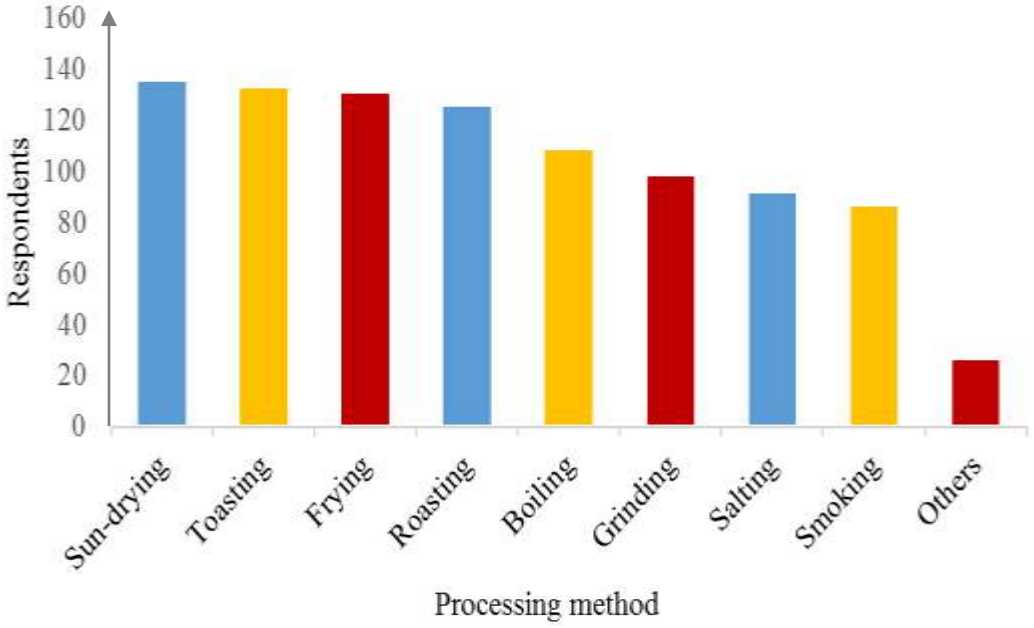

Figure 4.2: Common post-harvest techniques used prior to edible insect consumption

4.2 Effect of processing methods on the proximate composition of *Hermetia illucens*, *Acheta domesticus*, *Ruspolia differens* and *Spodoptera littoralis*

The proximate composition of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* is presented in Table 4.1. The initial moisture content (MC), crude protein (CP), crude fat (CF), crude ash (CA), crude fibre (CFR) and available carbohydrate (AC) of the edible insects in this study ranged between 64.5-74.0%, 36.3-57.5%, 17.4-29.6%, 3.1-7.6%, 6.4-8.6% and 17.7-30.1% respectively. The MC of all the processed edible insects was dependent on the interaction of species and processing method (F =3184.5; df = 19; $P < 0.001$), with significant differences being observed in processing method as a main effect (F = 628.7; df = 4; $P < 0.001$) but not in edible insect type as a main effect (F =1.69; df =3; P = 0.18). Drying (oven and solar) decreased the MC significantly ($P < 0.001$) to less than 10.1% in all edible insects species, with oven-dried *S. littoralis* having the highest moisture loss at 90.3%. Boiling increased the MC of all the edible insects significantly ($P < 0.001$), with boiled *S. littoralis* having the highest MC increment (17.8%). On the other hand, when the edible insects were toasted, there was a significant MC reduction, with toasted *R. differens* having the least moisture loss at 29.5%.

The CP was a function of the interaction of insect species and processing method (F = 398.3; df = 19; $P < 0.001$) with significant differences in the main effects as well (processing method: F = 69.4; df = 4; $P < 0.001$ and insect species: F = 598.9; df = 3; $P < 0.001$). All the processes increased CP significantly ($P < 0.001$), with the exception of solar-dried *A. domesticus* (P = 0.144) and solar and oven-dried *R. differens* (P = 0.95; P = 1.00) whose increments in CP were insignificant. Toasting had the greatest effect on CP increment, with toasted *R. differens* having the highest CP increase of 22.1%.

The CF of the processed insect products varied with processing method (F = 79.3; df = 4; $P < 0.001$) and species (F = 168.9; df = 3; $P < 0.001$), and the interaction effect of insect species and processing method was significant (F = 648.0; df = 19; $P < 0.001$). Processing led to a 0.8-51.7% decrease in CF in all the edible insect species with solar-dried edible insects having the least reduction, which was insignificant (*H. illucens*: P = 0.051; *A. domesticus* P = 1.000; *R. differens*: P = 0.148; *S. littoralis* P = 1.000). There was a significant reduction in CF upon oven drying in all the edible insects in the study, with the exception of *S. littoralis* whose CF reduction of 2.5% was insignificant (P = 1.000). Toasting had a higher CF loss ranging from 29.6% to 51.7% compared to boiling, which led to a CF loss ranging from 6.7% to 31.2%. The CA was a function of the interaction of insect species and processing method (F = 298.0; df = 19; $P < 0.001$), with significant differences in the main effects as well (processing method: F = 38.2; df = 4; $P < 0.001$; insect species: F = 562.7; df = 3; $P < 0.001$). Boiling decreased the CA significantly ($P < 0.001$), with *H. illucens* having the highest decrease at 24.3%. However, toasting significantly increased ($P < 0.001$) the CA in all the edible insects in this study with an increase range of 9.5% in *H. illucens* to 40.6% in *R. differens*. Both solar and oven drying had no significant effect on the CA of all the edible insects in the study.

Table 4.1: Proximate composition of the four raw and processed edible insect species

	Insect species				[SEM]
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	
			Moisture content (%)		
Raw	67.5^{Cb}	65.5^{Ca}	65.4^{Ca}	74.0^{Cc}	1.2
Boiled	74.2^{Db} (+9.9)	71.3^{Da} (+8.9)	76.5^{Dc}(+17.0)	87.2^{Dd}(+17.8)	1.8
Toasted	32.7^{Ba} (-52.5)	44.2^{Bb} (-32.5)	46.1^{Bb} (-39.5)	32.6^{Ba}(-55.9)	1.9
Solar-dried	10.1^{Ad} (-85.0)	9.9^{Ac} (-84.9)	9.8^{Ab} (-85.0)	9.1^{Aa}(-87.7)	0.2
Oven-dried	8.9^{Ac} (-86.8)	8.8^{Ac} (-86.6)	8.3^{Ab} (-87.3)	7.2^{Aa}(-90.3)	0.2
[SEM]	7.4	7.0	7.5	8.8	
			Crude protein (% dry matter)		
Raw	36.3^{Aa}	52.3^{Ad}	43.8^{Ac}	38.5^{Ab}	1.9
Boiled	39.6^{Ca}(+9.1)	57.5^{Cd}(+9.9)	48.8^{Bc}(+11.4)	41.9^{Db}(+8.9)	2.1
Toasted	41.3^{Da}(+13.7)	59.5^{Dd}(+13.7)	53.5^{Cc}(+22.1)	43.8^{Eb}(+13.7)	2.2
Solar-dried	37.0^{Ba}(+1.9)	53.1^{ABd}(+1.5)	42.3^{Ac}(+3.4)	39.7^{Bb}(+3.1)	1.8
Oven-dried	37.9^{Ca}(+4.4)	53.8^{Bd}(+2.9)	44.4^{Ac}(+1.4)	41.1^{Cb}(+7.1)	1.8
[SEM]	0.5	0.7	1.1	0.5	
			Crude fat (% dry matter)		
Raw	29.6^{Db}	18.3^{Da}	28.4^{Db}	17.4^{Ca}	1.7
Boiled	20.3^{Bb}(-31.4)	16.5^{Ba}(-9.8)	24.9^{Bc}(-12.3)	16.3^{Ba}(-6.3)	1.1
Toasted	14.3^{Ab}(-51.7)	10.5^{Aa}(-42.6)	20.1^{Ac}(-29.2)	9.7^{Aa}(-44.3)	2.5
Solar-dried	28.2^{Dc}(-4.7)	17.9^{CDa}(-2.2)	27.5^{CDb}(-3.3)	17.3^{Ca}(-0.8)	1.3
Oven-dried	26.9^{Cb}(-9.2)	17.1^{BCa}(-6.6)	26.7^{Cb}(-6.1)	16.9^{BCa}(-2.9)	1.5
[SEM]	1.5	0.8	0.8	0.8	
			Crude ash (% dry matter)		
Raw	3.9^{Bb}	3.6^{Bab}	3.1^{Ba}	7.6^{Bc}	0.5
Boiled	2.9^{Aa}(-25.6)	3.0^{Aa} (-16.7)	2.7^{Aa}(-12.9)	6.7^{Ab}(-11.8)	0.5
Toasted	4.3^{Ca}(+10.3)	4.2^{Ca} (+16.7)	4.3^{Ba}(+38.7)	9.5^{Cb}(+25.0)	0.7
Solar-dried	3.8^{Ba}(-2.5)	3.6^{Ba} (0)	3.8^{Ba}(+22.5)	7.6^{Bb}(0)	0.6
Oven-dried	3.9^{Ba}(0)	3.5^{Ba} (-2.8)	3.7^{Ba}(+19.3)	7.7^{Bb}(+1.3)	0.5
[SEM]	0.1	0.1	0.7	0.3	
			Crude fibre (% dry matter)		
Raw	8.6^{Bc}	8.1^{BCc}	4.2^{Ba}	6.4^{Bb}	0.5
Boiled	7.9^{Ac} (-8.1)	7.4^{Ac} (-8.6)	3.9^{Aa} (-7.1)	6.5^{BCb} (+1.6)	0.5
Toasted	10.6^{Cd} (+23.2)	8.2^{Cc} (+1.2)	4.5^{Ca} (+7.1)	6.2^{Ab} (-3.1)	0.7
Solar-dried	8.7^{Bc} (+1.2)	8.1^{BCc} (0.0)	4.2^{Ba} (0.0)	6.6^{Cb} (+3.1)	0.5
Oven-dried	8.5^{Bc} (-1.2)	8.0^{Bc} (-1.2)	4.1^{Ba} (-2.4)	6.6^{Cb} (+3.1)	0.5
[SEM]	0.3	0.1	0.1	0.1	
			Available carbohydrate (% dry matter)		
Raw	21.6^{Ab}	17.7^{Da}	20.4^{Cb}	30.1^{Cc}	1.4
Boiled	29.3^{Cc}(+35.6)	15.6^{Aa}(-11.9)	19.7^{Bb}(-3.4)	28.6^{Bc}(-4.9)	1.8
Toasted	29.5^{Cb}(+36.6)	17.6^{CDa}(-0.6)	17.7^{Aa}(-13.2)	30.7^{Cb}(+2.0)	1.9
Solar-dried	22.3^{ABb}(+3.2)	17.3^{Ba}(-2.3)	22.2^{Eb}(+8.8)	28.9^{Bc}(-4.0)	1.3
Oven-dried	22.8^{Bb}(+5.6)	17.5^{BCa}(-1.1)	21.2^{Db}(+3.9)	27.6^{Ac}(-8.3)	1.1
[SEM]	1.0	0.3	0.4	0.4	

For each parameter, means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw product. SEM is standard error of the means; DM is dry matter; BSF is black soldier fly; CLW is cotton leaf worm

The CFR of the processed edible insect varied with processing method (F = 5.6; df = 4; P < 0.001) and insect species (F = 289.1; df = 3; P < 0.001), with the interaction effect of processing method and insect species being significant as well (F = 168.2; df = 19; P < 0.001). With the exception of *S. littoralis*, boiling significantly decreased the CFR (7.1-8.6%) while toasting significantly increased the CFR by 1.2-23.2%. Unlike boiling and toasting, solar and oven drying did not affect the CFR. The AC was dependent on the interaction of processing method and insect species (F= 26.3; df = 19; P < 0.001), with significant differences in the main effects as well (insect species: F = 460; df = 3; P < 0.001; processing method: F= 7.9; df = 4; P < 0.001). There was however no clear trend in the increase or decrease in AC across all edible insects when similar processes were applied.

4.3 Effect of processing methods on the fatty acid profile and fatty acid groups of edible insects oil

The fatty acid profiles of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* are presented in Tables 4.2, 4.3, 4.4 and 4.5 respectively, while their fatty acid groups are shown in Figures 4.2, 4.3, and 4.4 respectively. The initial saturated fatty acids (SAFA) content for all the edible insects ranged from 32.36% to 62.84% and were statistically different (P < 0.001). Raw *H. illucens* and *S. littoralis* had lauric (15.23-30.22%) and palmitic acids (15.9-17.66%), while raw *A. domesticus* and *R. differens* had palmitic (22.05-31.51%) and stearic acids (4.75-7.28%) as their pre-dominant SAFA. Other SAFA that were present were capric (0.03-2.11%), myristic (0.04-5.59%) and arachidic acids (0.10-2.22%). Lauric acid was not detected in raw *A. domesticus* and *R. differens*, but was detected in low levels after processing the two insect species.

The initial unsaturated fatty acids (UFA) content that ranged from 37.16% to 67.64% were statistically different (P < 0.001) in all the edible insects in this study. All the raw insects had oleic acid (20.07-25.96%) and α-linolenic acid (7.84-37.17%) as their pre-dominant monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, with the

exception of *S. littoralis* which had α-linolenic acid (19.18%) as the pre-dominant PUFA. Other PUFA that were present in raw insect products were arachidonic (0.04-1.11), eicosapentaenoic (EPA) (0.03-0.96) and docosahexaenoic (DHA) (0.02-0.83). The initial PUFA/SAFA (P/S) ratio followed the order A. *domesticus* (1.28) > *R. differens* (0.94) > *S. littoralis* (0.89) > *H. illucens* (0.14), while the n-6/n-3 ratio followed the order A. *domesticus* (11.72) > *R. differens* (8.69) > *H. illucens* (0.99) > *S. littoralis* (0.80).

Table 4.2: Fatty acid profile (% of total fatty acids) of raw and processed *H. illucens* (black soldier fly)

Fatty acid	R	B	T	Solar-dried	Oven-dried	B & SD	T & SD	B & OD	T & OD	[SEM]
Capric	1.44^{a}	1.61^{d}	1.88^{g}	1.48^{b}	1.52^{c}	1.75^{e}	1.92^{h}	1.79^{f}	1.99^{i}	0.04
Lauric	30.22^{a}	31.34^{d}	32.67^{f}	30.71^{b}	31.12^{c}	32.34^{e}	32.79^{g}	32.53^{f}	33.37^{h}	0.20
Myristic	5.59^{a}	6.15^{d}	6.86^{f}	5.81^{b}	5.99^{c}	6.68^{e}	7.39^{g}	6.91^{f}	7.63^{h}	0.13
Palmitic	15.90^{a}	17.27^{d}	18.88^{g}	16.20^{b}	16.51^{c}	17.61^{e}	19.02^{h}	18.01^{f}	19.11^{h}	0.23
Stearic	9.53^{a}	11.02^{c}	13.06^{e}	9.70^{a}	10.06^{b}	11.92^{d}	13.87^{f}	12.05^{d}	14.14^{g}	0.32
Oleic	22.01^{e}	20.62^{d}	16.91^{b}	21.86^{e}	21.78^{e}	18.44^{c}	16.07^{a}	18.06^{c}	15.82^{a}	0.47
Linoleic	7.84^{h}	6.01^{f}	3.99^{c}	7.31^{g}	7.12^{g}	5.22^{e}	3.44^{b}	4.85^{d}	2.96^{a}	0.33
α-linolenic	1.01^{h}	0.57^{e}	0.41^{d}	0.87^{g}	0.76^{f}	0.26^{c}	0.18^{b}	0.22^{bc}	0.09^{a}	0.06
Arachidic	0.16^{a}	0.29^{c}	0.36^{d}	0.20^{b}	0.21^{b}	0.39^{de}	0.42^{ef}	0.44^{f}	0.58^{g}	0.03
Arachidonic	0.04^{c}	0.01^{b}	Nd^{a}	0.03^{c}	0.03^{c}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.01
EPA	0.03^{c}	Nd^{a}	Nd^{a}	0.01^{b}	0.01^{b}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.00
DHA	0.02^{c}	Nd^{a}	Nd^{a}	0.01^{b}	0.01^{b}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.00
P/S ratio	0.14	0.10	0.06	0.13	0.12	0.08	0.05	0.07	0.04	
Total n-6	1.05	0.58	0.41	0.90	0.79	0.26	0.18	0.22	0.09	
Total n-3	1.06	0.57	0.41	0.89	0.78	0.26	0.18	0.22	0.09	
n-6/n-3 ratio	0.99	1.01	1	1.01	1.01	1	1	1	1	

Means in the same row followed by the same letters are not significantly different ($P > 0.05$; n = 3). Nd is not detected; R is raw; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; SEM is standard error of mean; EPA is eicosapentaenoic acid; DHA is docosahexaenoic acid; P/S ratio is PUFA/SAFA ratio; n-6 is sum of linoleic and arachidonic acids; n-3 is sum of α-linolenic, EPA and DHA acids. Unidentified fatty acid methyl esters accounted for 4.31-6.21% of the total fatty acids across the treatments

Table 4.3: Fatty acid profile (% of total fatty acids) of raw and processed *A. domesticus* (cricket)

Fatty acid	R	B	T	Solar-dried	Oven-dried	B & SD	T & SD	B & OD	T & OD	[SEM]
Capric	0.03^{a}	0.10^{b}	0.22^{c}	0.05^{ab}	0.07^{ab}	0.16^{c}	0.78^{e}	0.35^{d}	0.8^{e}	0.06
Lauric	Nd^{a}	Nd^{a}	0.01^{b}	Nd^{a}	Nd^{a}	0.01^{b}	0.03^{c}	0.01^{b}	0.04^{d}	0.00
Myristic	0.77^{a}	1.05^{b}	2.14^{d}	0.89^{ab}	0.97^{b}	1.51^{c}	3.17^{e}	1.56^{c}	3.57^{f}	0.19
Palmitic	22.05^{a}	24.35^{c}	25.55^{d}	22.21^{a}	22.92^{b}	25.45^{d}	26.45^{e}	25.56^{d}	26.91^{f}	0.34
Stearic	7.28^{a}	8.30^{b}	14.51^{d}	7.37^{a}	7.56^{a}	8.48^{b}	15.41^{e}	9.36^{c}	15.46^{e}	0.67
Oleic	24.40^{f}	23.30^{d}	22.17^{b}	24.26^{f}	24.15^{e}	23.04^{c}	21.66^{a}	23.14^{c}	21.47^{a}	0.21
Linoleic	37.17^{e}	34.51^{d}	27.37^{b}	37.01^{e}	37.03^{e}	33.72^{d}	25.15^{a}	32.51^{c}	25.13^{a}	0.93
α-linolenic	1.47^{d}	1.04^{b}	1.02^{b}	1.42^{d}	1.34^{c}	1.01^{b}	1.01^{b}	0.98^{b}	0.18^{a}	0.07
Arachidic	2.22^{a}	3.52^{c}	4.63^{d}	2.21^{a}	2.46^{b}	3.62^{c}	4.91^{f}	4.64^{de}	4.86^{ef}	0.22
Arachidonic	1.11^{d}	1.06^{d}	0.74^{b}	1.06^{d}	0.94^{c}	1.02^{cd}	Nd^{a}	Nd^{a}	Nd^{a}	0.09
EPA	0.96^{f}	0.83^{e}	0.23^{b}	0.88^{e}	0.67^{d}	0.48^{c}	Nd^{a}	Nd^{a}	Nd^{a}	0.07
DHA	0.83^{e}	0.02^{b}	Nd^{a}	0.70^{d}	0.50^{c}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.06
P/S ratio	1.28	1.00	0.62	1.25	1.19	0.92	0.52	0.81	0.49	
Total n-6	38.28	35.57	28.11	38.07	37.97	34.74	25.14	32.51	25.13	
Total n-3	3.27	1.89	1.24	2.99	2.51	1.49	1.01	0.98	0.18	
n-6/n-3	11.72	18.83	22.51	12.71	15.13	23.32	24.81	33.17	139.61	

Means in the same row followed by the same letters are not significantly different ($P > 0.05$; n = 3). Nd is not detected; R is raw; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; SEM is standard error of mean; EPA is eicosapentaenoic acid; DHA is docosahexaenoic acid; P/S ratio is PUFA/SAFA ratio; n-6 is sum of linoleic and arachidonic acids; n-3 is sum of α-linolenic, EPA and DHA acids. Unidentified fatty acid methyl esters accounted for 1.40-1.94% of the total fatty acids across the treatments

Table 4.4: Fatty acid profile (% of total fatty acids) of raw and processed *R. differens* (grasshopper)

Fatty acid	R	B	T	Solar-dried	Oven-dried	B & SD	T & SD	B & OD	T & OD	[SEM]
Capric	0.06^{a}	0.07^{a}	0.85^{a}	0.06^{a}	0.06^{a}	0.09^{a}	0.16^{b}	0.09^{a}	0.59^{c}	0.03
Lauric	Nd^{a}	Nd^{a}	0.03^{b}	Nd^{a}	Nd^{a}	0.04^{b}	0.09^{c}	0.08^{c}	0.09^{c}	0.01
Myristic	0.61^{a}	0.81^{c}	1.02^{d}	0.66^{ab}	0.71^{bc}	1.62^{e}	1.91^{f}	1.69^{e}	2.04^{g}	0.11
Palmitic	31.51^{a}	33.53^{c}	34.40^{e}	31.67^{a}	32.19^{b}	34.07^{d}	34.77^{f}	34.49^{e}	35.29^{g}	0.26
Stearic	4.75^{a}	6.11^{bc}	12.59^{e}	4.95^{ab}	5.51^{b}	6.61^{cd}	13.05^{e}	6.97^{d}	15.17^{f}	0.74
Oleic	25.96^{g}	24.44^{e}	23.55^{c}	25.62^{f}	25.17^{f}	24.08^{d}	23.30^{b}	24.01^{d}	22.82^{a}	0.20
Linoleic	31.39^{e}	30.34^{d}	23.18^{b}	30.94^{de}	30.76^{de}	29.51^{c}	22.82^{b}	29.06^{c}	20.02^{a}	0.80
α-linolenic	2.38^{e}	1.09^{c}	1.02^{c}	2.27^{e}	1.91^{d}	0.81^{b}	0.72^{b}	0.68^{ab}	0.55^{a}	0.13
Arachidic	0.66^{a}	0.75^{b}	1.36^{d}	0.65^{a}	0.67^{a}	1.20^{c}	1.38^{de}	1.23^{c}	1.45^{e}	0.07
Arachidonic	0.29^{g}	0.19^{de}	0.17^{d}	0.23^{f}	0.20^{e}	0.13^{c}	0.09^{b}	Nd^{a}	Nd^{a}	0.02
EPA	0.91^{e}	0.63^{d}	0.59^{d}	0.87^{e}	0.85^{e}	0.39^{c}	0.03^{b}	Nd^{a}	Nd^{a}	0.07
DHA	0.35^{f}	0.21^{d}	0.19^{d}	0.31^{e}	0.30^{e}	0.16^{c}	0.02^{b}	Nd^{a}	Nd^{a}	0.02
P/S ratio	0.94	0.79	0.51	0.91	0.87	0.71	0.46	0.66	0.38	
Total n-6	31.68	30.53	23.35	31.17	30.96	29.64	22.91	29.06	20.02	
Total n-3	3.65	1.94	1.79	3.45	3.05	1.36	1.10	0.68	0.55	
n-6/n-3	8.69	15.76	12.98	9.03	10.14	21.85	20.76	42.74	36.4	

Means in the same row followed by the same letters are not significantly different ($P > 0.05$; n = 3). Nd is not detected; R is raw; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; SEM is standard error of mean; EPA is eicosapentaenoic acid; DHA is docosahexaenoic acid; P/S ratio is PUFA/SAFA ratio; n-6 is sum of linoleic and arachidonic acids; n-3 is sum of α-linolenic, EPA and DHA acids. Unidentified fatty acid methyl esters accounted for 1.12-1.98% of the total fatty acids across the treatments

Table 4.5: Fatty acid profile (% of total fatty acids) of raw and processed *S. littoralis* (cotton leaf worm)

Fatty acid	R	B	T	Solar-dried	Oven-dried	B & SD	T & SD	B & OD	T & OD	[SEM]
Capric	2.11^{a}	3.04^{c}	3.67^{d}	2.22^{ab}	2.29^{b}	3.81^{e}	3.91^{ef}	3.99^{f}	4.07^{g}	0.15
Lauric	15.23^{a}	18.05^{b}	21.02^{c}	16.01^{a}	16.04^{a}	18.51^{b}	21.25^{c}	18.22^{b}	21.91^{c}	0.91
Myristic	0.04^{a}	0.10^{c}	0.19^{f}	0.06^{ab}	0.07^{b}	0.13^{d}	0.22^{g}	0.16^{e}	0.28^{h}	0.02
Palmitic	17.66^{a}	21.64^{d}	22.28^{e}	18.47^{b}	18.93^{c}	22.28^{e}	22.83^{f}	22.76^{f}	23.04^{f}	0.39
Stearic	4.50^{a}	6.06^{c}	8.07^{e}	5.11^{b}	5.61^{c}	6.44^{c}	10.04^{f}	6.89^{d}	10.97^{g}	0.41
Oleic	20.07^{f}	17.13^{c}	16.72^{b}	19.17^{e}	18.86^{d}	16.92^{bc}	16.11^{a}	16.14^{a}	16.05^{a}	0.28
Linoleic	15.27^{g}	12.67^{d}	9.01^{b}	13.76^{f}	13.10^{e}	12.03^{c}	8.91^{b}	11.75^{c}	7.99^{a}	0.46
α-linolenic	19.18^{h}	17.70^{e}	15.00^{c}	18.59^{g}	18.07^{f}	16.59^{d}	12.81^{b}	16.24^{d}	12.04^{a}	0.48
Arachidic	0.10^{a}	0.23^{c}	0.33^{e}	0.13^{b}	0.14^{b}	0.25^{c}	0.41^{f}	0.28^{d}	0.49^{g}	0.02
Arachidonic	0.28^{e}	0.11^{c}	0.08^{b}	0.22^{d}	0.20^{d}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.02
EPA	0.23^{e}	0.08^{c}	0.01^{b}	0.15^{d}	0.16^{d}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.02
DHA	0.04^{d}	0.01^{b}	Nd^{a}	0.03^{c}	0.03^{c}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.01
P/S ratio	0.89	0.62	0.43	0.78	0.73	0.56	0.37	0.54	0.33	
Total n-6	15.55	12.78	9.08	13.98	13.30	12.03	8.91	11.75	7.99	
Total n-3	19.45	17.79	15.01	18.77	18.26	16.59	12.81	16.24	12.04	
n-6/n-3	0.80	0.72	0.60	0.74	0.73	0.73	0.69	0.72	0.66	

Means in the same row followed by the same letters are not significantly different ($P > 0.05$; n = 3). Nd is not detected; R is raw; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; SEM is standard error of mean; EPA is eicosapentaenoic acid; DHA is docosahexaenoic acid; P/S ratio is PUFA/SAFA ratio; n-6 is sum of linoleic and arachidonic acids; n-3 is sum of α-linolenic, EPA and DHA acids. Unidentified fatty acid methyl esters accounted for 3.04-6.10% of the total fatty acids across the treatments

Processing led to a concomitant increase in the SAFA content and a decrease in the UFA content of all the edible insects in this study (Table 4.3, 4.4, 4.5 and 4.6). Fatty acids content were dependent on the interaction of processing method and insect species (capric: F = 22029.93; df = 35; P < 0.001; lauric: F = 5.1*10^6; df = 35; P < 0.001; myristic: F = 1425.82; df = 35; P < 0.001; palmitic: F = 3768.38; df = 35; P < 0.001; stearic: F = 555.52; df = 35; P < 0.001; oleic: F = 1788.31; df = 35; P < 0.001; linoleic: F = 39547.97; df = 35; P < 0.001; α-linolenic: F = 25415.40; df = 35; P < 0.001; arachidic: F = 553.29; df = 35; P < 0.001; arachidonic: F = 1178.90; df = 35; P < 0.001; EPA: F = 353.82; df = 35; P < 0.001; DHA: F = 313.63; df = 35; P < 0.001). Therefore, the fatty acid groups were also a function of the interaction of processing method and insect species (SAFA: F = 3183.44; df = 35; P < 0.001; UFA: F = 13829.92; df = 35; P < 0.001; MUFA: F = 1788.31; df = 35; P < 0.001; PUFA: F = 23311.45; df = 35; P < 0.001). There were significant differences (P < 0.001) in the main effects for all the fatty acids and fatty acid groups as well (P < 0.001). *Hermetia illucens* had higher SAFA levels (Figure 4) compared to the other edible insect species in all the processing methods employed.

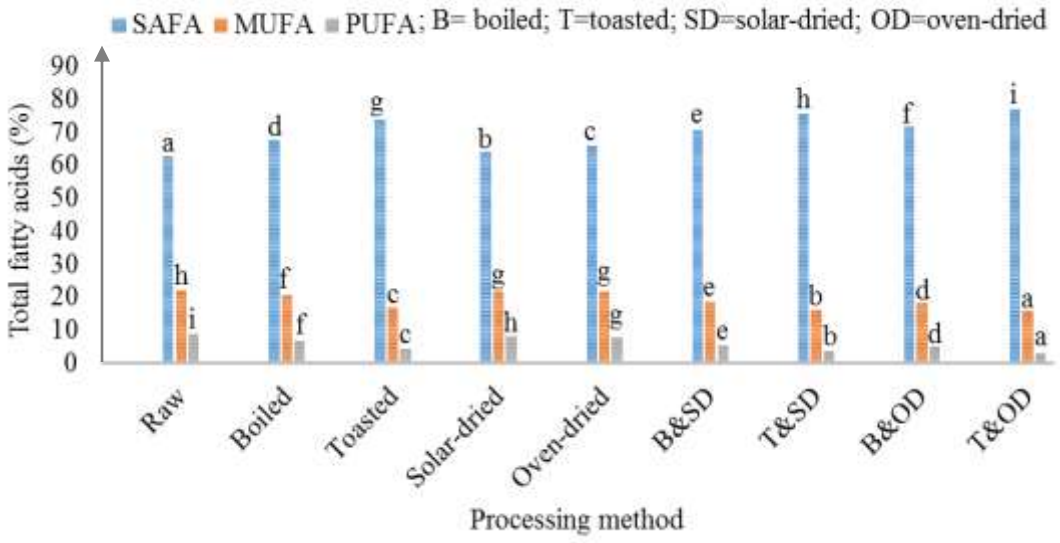

Figure 4.3: SAFA, MUFA and PUFA of raw and processed *H. illucens* (black soldier fly)

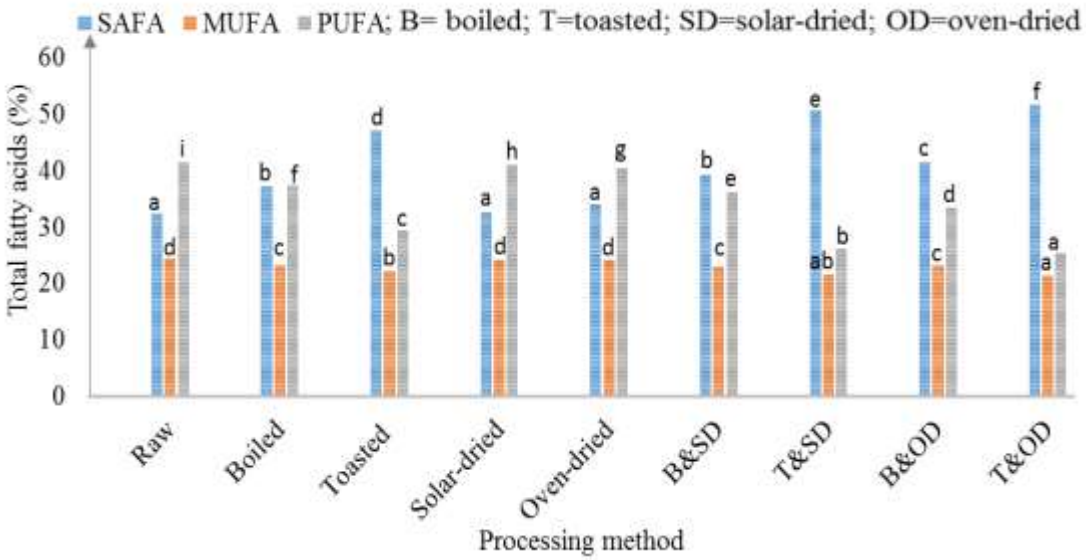

Figure 4.4: SAFA, MUFA and PUFA for raw and processed *A. domesticus* (cricket)

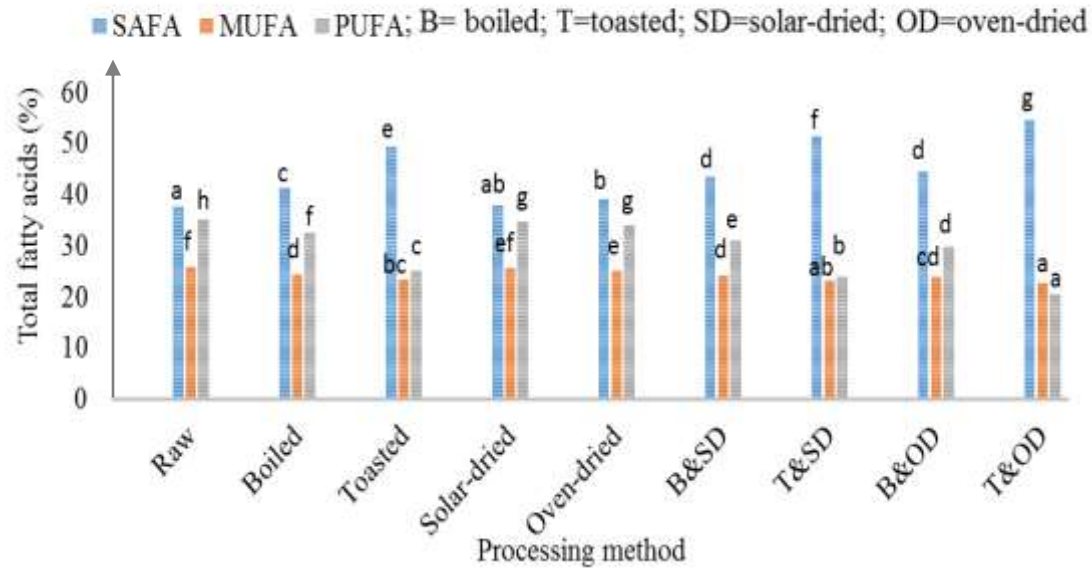

Figure 4.5: SAFA, MUFA and PUFA for raw and processed *R. differens* (grasshopper)

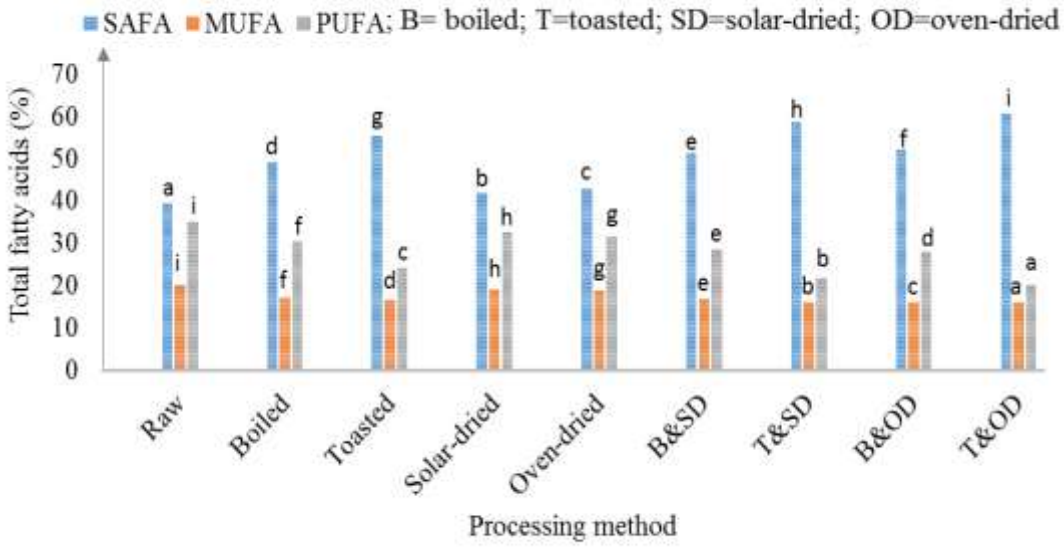

Figure 4.6: SAFA, MUFA and PUFA for raw and processed *S. littoralis* (cotton leaf worm)

Solar and oven drying had the least effect on the fatty acids (Table 4.3 -4.6) and fatty acid groups (Figure 4.3-4.7) in all the insect species. Toasting had a greater effect on the fatty acids and fatty acid groups compared to boiling in all the insect species with SAFA being more and UFA being the least in all the toasted edible insect samples than in their boiled counterparts. Stearic acid increased significantly ($P < 0.001$) during both boiling and toasting. There was however a sharp decrease in linoleic acid in all the toasted samples, bringing its levels to a range of between 3.9% in *H. illucens* and 27.4% in *A. domesticus*. DHA was not detected in all the toasted edible insect samples except in toasted *R. differens* (0.2%).

Drying post boiling and toasting had the greatest significant effect on the fatty acids ($P < 0.001$) and the fatty acid groups ($P < 0.001$), with the greatest effect found in toasted oven-dried edible insect samples. In the SAFAs category, stearic acids increased the most as compared to other SAFAs, with toasted oven-dried *A. domesticus* (15.5%) having the highest stearic acid. The pre-dominant MUFA, oleic acid, decreased

significantly in all the samples ($P < 0.001$), with toasted oven-dried *S. littoralis* (16.1%) having the lowest oleic acid. Linoleic acid, which was the pre-dominant PUFA in *H. illucens, A. domesticus* and *R. differens*, also decreased significantly with toasted oven-dried *H. illucens* (2.9%) having the least linoleic acid. On the other hand, α-linolenic acid, which was the pre-dominant PUFA in *S. littoralis*, was lowest in its toasted oven-dried product (12.0%). Arachidonic, EPA and DHA were not detected in all the samples, where oven drying post boiling or toasting was done.

Processing decreased the P/S ratio by 0.28-0.92, 0.38-1, 0.4-0.96 and 0.37-0.87 factors in *H. illucens, A. domesticus, R. differens* and *S. littoralis* respectively. Toasted and dried edible insect samples had the least P/S ratio ranging from 0.04 in *H. illucens* to 0.49 in *A. domesticus*. The n-6/n-3 ratio increased in processed *H. illucens, A. domesticus* and *R. differens* insect samples by 1, 1.08-11.91 and 1.04-4.92 factors respectively, while in processed *S. littoralis* samples, the ratio decreased by 0.75-0.93 factors.

4.4 Effect of processing techniques on the peroxide, iodine and saponification values of edible insects oil

The initial peroxide value (PV), iodine value (IV) and saponification value (SV) of *H. illucens, A. domesticus, R. differens* and *S. littoralis* ranged from 0.5-5.8 mEq O_2/Kg, 107.9-349.2 g I_2/100g and 226.7-251.5 mg KOH/g respectively (Tables 4.6, 4.7 and 4.9). Processing led to a decrease in IV but an increase in PV and SV in all the edible insect species in this study. All the chemical stability parameters were a function of the interaction of processing method and insect species (PV: $F = 43.74$; df = 35; $P < 0.001$; IV: $F = 3327.40$; df = 35; $P < 0.001$; SV: $F = 12568.80$; df = 35; $P < 0.001$), with significant differences in the main effects as well ($P < 0.001$).

Considering the processes, solar and oven drying had the least effect on PV and SV. The PV increase reported in solar-dried *H. illucens* ($P = 0.405$) and solar-dried *S. littoralis* ($P = 0.057$) was insignificant. In addition, the SV increase reported in all the edible insect

species was insignificant. Solar drying had a greater impact on IV reduction with the exception of *H. illucens*, whose solar-dried sample had a higher IV (100.3 g I_2/100g) compared to its oven-dried counterpart (97.7 g I_2/100g). When heat treatment was employed through boiling and toasting, there was an increase in PV of all the four insect species. Toasting had a greater effect on PV, IV and SV compared to boiling. Toasted insect samples had a PV increase of between 110.2-1320%, with *H. illucens* and *S. littoralis* having the lowest and highest PV of 7.1 mEq O_2/Kg and 13.0 mEq O_2/Kg respectively. Boiling increased the PV of the edible insects by 46.6-600%. Toasting reduced the IV of the insects by 17.3-58.2%, while the IV reduction upon boiling ranged from 12.4% to 45%. Boiling and toasting increased the SV of all the four edible insect species significantly, with the exception for boiled *H. illucens* and *R. differens*, whose 12.9% and 7.0% increase respectively were not significantly different from their raw counterparts.

Table 4.6: Peroxide values (mEq O_2/Kg) of oil from raw and processed edible insect species

Process	*H. illucens* (BSF)	*A. domesticus* (cricket)	*R. differens* (grasshopper)	*S. littoralis* (CLW)	[SEM]
Raw	0.5^{Aa}	4.9^{Ac}	1.9^{Ab}	5.8^{Ad}	0.7
Boiled	3.5^{Ca} (+600)	8.4^{Cc} (+71.4)	5.3^{Cb} (+178.9)	8.5^{Cc} (+46.6)	0.7
Toasted	7.1^{Da} (+1320)	10.3^{Db} (+110.2)	7.6^{Da} (+300)	13.0^{Db} (+124.1)	0.8
Solar-dried	1.0^{ABa} (+100)	6.3^{Bc} (+28.6)	3.9^{Bb} (+105.3)	6.5^{ABc} (+12.1)	0.7
Oven-dried	1.7^{Ba} (+240)	5.9^{Bc} (+20.4)	3.8^{Bb} (+100)	6.9^{Bd} (+19.0)	0.6
B&SD	8.4^{Ea} (+1580)	12.9^{Ec} (+163.3)	9.7^{Eb} (+410.5)	13.3^{DEc}(+129.3)	0.8
T&SD	10.5^{Fb} (+2000)	13.6^{Fc} (+177.6)	9.3^{Ea} (+389.5)	15.3^{Fd} (+163.8)	0.8
B&OD	8.2^{Ea} (+1540)	12.6^{Eb} (+157.1)	8.3^{Da} (+336.8)	13.9^{Ec} (+139.7)	0.9
T&OD	12.7^{Ga} (+2440)	16.4^{Gb} (+234.7)	12.7^{Fa}(+568.4)	18.7^{Gc} (+222.4)	0.8
[SEM]	0.8	0.8	0.7	0.8	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent increase relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

Table 4.7: Iodine value (g I2/100g) of oil from raw and processed edible insect species

Process	*H. illucens* (BSF)	*A. domesticus* (cricket)	*R. differens* (grasshopper)	*S. littoralis* (CLW)	[SEM]
Raw	107.9^{Ha}	349.2^{Gd}	114.4^{Fb}	295.9^{Fc}	32.4
Boiled	91.7^{Ea}(-15.0)	251.8^{Dd}(-27.9)	100.2^{Db}(-12.4)	162.7^{Dc}(-45.0)	19.3
Toasted	87.2^{Da}(-19.2)	229.9^{BCc}(-34.1)	94.6^{Ca}(-17.3)	123.7^{Cb}(-58.2)	17.3
Solar-dried	100.3^{Ga}(-7.0)	290.3^{Ed}(-16.8)	108.9^{Eb}(-4.8)	197.8^{Ec}(-33.2)	23.2
Oven-dried	97.7^{Fa}(-9.5)	299.5^{Fd}(-14.2)	109.7^{Eb}(-4.1)	200.5^{Ec}(-32.2)	24.5
B&SD	80.1^{Ca}(-25.8)	248.8^{Dd}(-28.8)	98.8^{Db}(-13.6)	113.1^{Cc}(-61.8)	20.1
T&SD	66.6^{Aa}(-38.3)	227.6^{ABc}(-34.8)	87.9^{Bb}(-23.2)	93.9^{Bb}(-68.3)	19.2
B&OD	71.5^{Ba}(-33.7)	236.3^{Cc}(-32.3)	90.4^{Bb}(-21.0)	95.3^{Bb}(-67.8)	19.8
T&OD	66.4^{Aa}(-38.5)	216.1^{Ac}(-38.1)	78.4^{Ab}(-31.5)	71.6^{Ab}(-75.8)	18.8
[SEM]	2.8	8.0	2.2	13.2	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

Combined processing methods (drying post boiling and toasting) had the greatest effect on the PV, IV and SV in all the four edible insect species in this study. Toasted + oven-dried (T&OD) samples had a significantly higher PV compared to their Toasted + solar-dried (T&SD) counterparts. Boiled + oven-dried (B&OD) had a lesser impact on the PV of edible insects compared to their boiled + solar-dried (B&SD) counterparts, except in the case of *R. differens*. The IV of T&SD *R. differens* ($P = 0.002$) and T&SD *S. littoralis* ($P < 0.001$) were significantly higher than those of their T&OD counterparts. B&OD had a greater impact on IV reduction rate of all the four insect species (20.9-67.8%) compared to their B&SD counterparts, whose IV reduction rate ranged between 13.6% in *A. domesticus* to 61.8% in *S. littoralis*. B&SD and T&SD had a lesser impact on SV of all the four insect species, when compared to their B&OD and T&OD counterparts. B&SD *A. domesticus* had the least SV increase (55.7%), while T&SD *R. differens* had the highest SV increase (151.6%) among all the other solar-dried insect samples. T&OD

had a greater influence on the SV of all the four insects compared to their B&OD counterparts. T&OD insect products had SV increase ranging from 157.8% in *A. domesticus* and 293.1% in *H. illucens*, while B&OD insect samples had SV increase ranging from 70.1% in *S. littoralis* and 148.2% in *R. differens*.

Table 4.8: Saponification values (mg KOH/g) of oil from raw and processed edible insect species

Process	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
Raw	251.5^{Ad}	231.8^{Ab}	239.0^{Ac}	226.7^{Aa}	2.9
Boiled	284.1^{ABb}(+12.9)	286.6^{Bb} (+23.6)	255.8^{Aa} (+7.0)	324.6^{Bc} (+43.2)	7.4
Toasted	316.2^{Ba} (+25.7)	306.1^{Ba} (+32.1)	448.5^{Bc} (+87.7)	371.2^{Cb} (+63.7)	17.1
Solar-dried	262.9^{Ad} (+4.5)	239.8^{Aa} (+3.5)	243.5^{Ab} (+1.9)	253.8^{Ac} (+12.0)	2.8
Oven-dried	265.4^{Ac} (+5.5)	244.5^{Aa} (+5.5)	253.8^{Ab} (+6.2)	252.6^{Ab} (+11.4)	2.4
B&SD	459.5^{Cb} (+82.7)	360.8^{Ca} (+55.7)	541.7^{Cc}(+126.7)	366.3^{Ca} (+61.6)	22.5
T&SD	590.8^{Dc}(+134.9)	510.8^{Eb}(+120.4)	601.5^{Dd}(+151.7)	453.2^{Da} (+99.9)	18.3
B&OD	503.4^{Cc}(+100.2)	499.1^{Db}(+115.4)	593.3^{Dd}(+148.2)	385.5^{Ca} (+70.0)	22.2
T&OD	988.6^{Ed}(+293.1)	596.4^{Fa}(+157.3)	693.7^{Ec}(+190.3)	662.0^{Eb}(+192.0)	45.3
[SEM]	44.6	25.4	34.0	24.7	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent increase relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.5 Effect of processing methods on the cholesterol level of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*

The cholesterol level of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.9. Raw insect samples had cholesterol levels ranging from 10.3 mg/100g in *S. littoralis* to 33.4 mg/100g in *H. illucens*. Cholesterol level was dependent on the interaction of processing method and insect species (F = 2534.28; df = 35; $P < 0.001$), with significant differences in the main effects ($P < 0.001$).

Table 4.9: Cholesterol content (mg/100g) (wet basis) of raw and processed edible insect species

Process	*H. illucens* (BSF)	*A. domesticus* (cricket)	*R. differens* (grasshopper)	*S. littoralis* (CLW)	[SEM]
Raw	33.4^{Gd}	21.4^{Fb}	26.3^{Ec}	10.3^{Ea}	2.5
Boiled	30.6^{Dd} (-8.3)	17.9^{Db} (-16.4)	19.6^{Cc} (-25.5)	9.7^{Ca} (-5.6)	2.2
Toasted	27.6^{Ad} (-17.4)	10.4^{Ab} (-51.4)	12.2^{Ac} (-53.6)	8.6^{Aa} (-16.8)	2.3
Solar-dried	35.4^{Hd} (+6.0)	24.8^{Hb} (+15.9)	29.4^{Fc} (+11.8)	10.6^{Fa} (+2.3)	2.8
Oven-dried	35.9^{Hd}(+7.5)	22.8^{Gb} (+6.5)	28.4^{Fc} (+8.0)	11.0^{Ga} (+6.7)	2.8
B&SD	31.1^{Ef} (-6.9)	20.8^{EFb} (-2.8)	23.8^{Dc} (-9.5)	10.0^{Da} (-2.9)	2.3
T&SD	28.1^{Bd} (-15.9)	15.8^{Cb} (-26.2)	17.3^{Bc} (-34.2)	8.9^{Ba} (-12.9)	2.1
B&OD	31.6^{Fd} (-5.4)	20.1^{Eb} (-6.1)	23.0^{Dc} (-12.5)	10.2^{Ea} (-0.8)	2.3
T&OD	29.0^{Cd} (-13.2)	13.3^{Bb} (-37.9)	16.4^{Bc} (-37.6)	9.0^{Ba} (-12.7)	2.2
[SEM]	0.6	0.9	1.1	0.2	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent increase relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

There was a significant increase ($P < 0.001$) in the cholesterol level in all edible insect species when the raw insects were dried either in an oven or in a solar dryer. This increase ranged from 2.3% in solar-dried *S. littoralis* to 15.9% in *A. domesticus*. On the other hand, there was a significant decrease ($P < 0.001$) in cholesterol level in all the insect species when the raw samples were either boiled or toasted. While toasted *H. illucens* had the highest cholesterol level (27.6 mg/100g), toasted *R. differens* had the highest cholesterol reduction level (53.6%). When the edible insects samples in this study were subjected to combined processes (drying post boiling and toasting), a significant increase in their cholesterol level was realised compared to their boiled and toasted counterparts. In relation to the raw insects, B&OD *S. littoralis* had the lowest decrease in cholesterol level (0.8%), while the highest decrease in cholesterol level was realised by both T&OD *A. domesticus* and T&OD *R. differens*.

4.6 Effect of processing methods on the mineral content of *H. illucens, A. domesticus, R. differens* and *S. littoralis*

The mineral composition of raw and processed *H. illucens, A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.10, 4.11, 4.12 and 4.13 respectively. All the four raw edible insect species samples had potassium (K) as their most abundant mineral with a range of 335-542.4 mg/100g. This was followed by sodium (Na) (86.5 mg/100g) and magnesium (Mg) (37.5 mg/100g) in *H. illucens*, phosphorus (P) (299.9 mg/100g) and Na (127.3 mg/100g) in *A. domesticus*, Na (300.9 mg/100g) and P (141.8 mg/100g) in *R. differens* and Mg (187.7 mg/100g) and Na (47.7 mg/100g) in *S. littoralis*. Micro-minerals such as iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) ranged from 0.4-8.6 mg/100g in *H. illucens*, 0.2-7.4 mg/100g in *A. domesticus*, 0.4-21 mg/100g in *R. differens* and 0.9-1.4 mg/100g in *S. littoralis*. The varied mineral content in all the insect species studied were significantly different ($P < 0.001$), with *S. littoralis* having the highest amount of K (542.4 mg/100g), Mg (187.7 mg/100g), Mn (3.6 mg/100g) and Cu (0.9 mg/100g), *R. differens* having the highest content of Na (300.9 mg/100g), Fe (21.0 mg/100g) and Zn (16.8 mg/100g) and *A. domesticus* having the highest amount of Ca

(38.4 mg/100g) and P (299.9 mg/100g). The total mineral concentration in the raw insect samples followed the order; *R. differens* > *S. littoralis* > *A. domesticus* > *H. illucens*.

Table 4.10: Mineral composition (mg/100g) (wet basis) of raw and processed *H. illucens* (black soldier fly)

Mineral	Raw	Boiled	Toasted	Solar-dried	Oven-dried	B&SD	T&SD	B&OD	T&OD	[SEM]
Sodium	86.5^{c}	80.9^{a}(-6.5)	89.6^{d} (+3.5)	87.2^{c} (+0.8)	86.9^{c} (+0.4)	81.6^{ab} (-5.7)	90.8^{e} (+4.9)	81.7^{b} (-5.5)	91.7^{f} (+6)	0.7
Potassium	468.0^{b}	451.7^{a} (-3.5)	476.4^{c} (+1.8)	469.3^{b} (+0.3)	469.7^{b} (+0.4)	452.4^{a} (-3.3)	477.2^{c} (+2)	453.0^{a} (-3.2)	477.8^{c} (+2.1)	2.0
Calcium	15.5^{c}	12.4^{a}(-20.4)	18.9^{e} (+21.6)	16.1^{d} (+3.5)	16.2^{d} (+4.5)	13.7^{b} (-11.9)	19.3^{ef} (+24.5)	13.9^{b} (-9.9)	19.7^{f} (+27)	0.4
Magnesium	37.5^{b}	32.8^{a}(-12.5)	41.7^{d} (+11.4)	37.4^{bc} (-0.2)	38.4^{c} (+2.6)	33.3^{a} (-11.1)	42.8^{e} (+14.3)	33.5^{a} (-10.5)	42.6^{e} (+13.7)	0.7
Phosphorus	10.9^{b}	9.9^{a} (-9.5)	11.8^{c} (+8.1)	11.1^{b} (+0.9)	11.0^{b} (+0.5)	10.1^{a} (-8.3)	11.9^{c} (+9)	10.1^{a} (-8.1)	11.9^{c} (+9)	0.2
Iron	6.7^{c}	4.2^{a} (-38.5)	8.7^{e} (+29.3)	7.4^{d}(+10.4)	7.5^{d}(+11.2)	4.9^{b} (-27.9)	9.4^{f} (+39)	5.2^{b} (-23.7)	9.7^{f} (+43)	0.3
Zinc	8.6^{c}	5.5^{a} (-35.7)	11.3^{e} (+31.4)	9.0^{cd} (+4.8)	9.2^{d} (+6.3)	6.2^{b} (-27.5)	11.6^{e} (+34.5)	5.9^{ab} (-30.9)	12.4^{f} (+43.7)	0.4
Manganese	2.6^{c}	1.0^{a} (-59.8)	3.8^{e} (+49.5)	2.7^{cd} (+5)	2.9^{d}(+12.5)	1.5^{b} (-41)	4.2^{f} (+64.2)	1.7^{b}(-34.2)	4.5^{g} (+77.4)	0.2
Copper	0.4^{c}	0.1^{a} (-67.6)	0.9^{e} (+155.6)	0.4^{c}(+18.5)	0.6^{d}(+52.8)	0.3^{b} (-8.3)	1.1^{g} (+194.4)	0.3^{b}(-25)	1.0^{f}(+175.9)	0.06

Means in the same row with the same superscript letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw insect product. SEM is the standard error of mean; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried.

Table 4.11: Mineral composition (mg/100g) (wet basis) of raw and processed *A. domesticus* (cricket)

Mineral	Raw	Boiled	Toasted	Solar-dried	Oven-dried	B&SD	T&SD	B&OD	T&OD	[SEM]
Sodium	127.3^{c}	120.9^{a} (-5)	135.8^{d} (+6.6)	127.8^{c} (+0.4)	128.2^{c} (+0.7)	122.1^{ab} (-4.1)	136.3^{de} (+7)	122.5^{b} (-3.8)	137.5^{e} (+8)	1.2
Potassium	335.0^{b}	320.9^{a} (-4.2)	340.4^{c} (+1.6)	335.5^{b} (+0.2)	335.4^{b} (+0.1)	321.5^{a} (-4)	340.8^{c} (+1.7)	321.2^{a} (-4.1)	341.2^{c} (+1.9)	1.6
Calcium	38.4^{c}	30.6^{a}(-20.4)	42.5^{e} (+10.7)	39.3^{cd} (+2.2)	39.8^{d} (+3.6)	31.7^{b} (-17.5)	43.5^{f} (+13.2)	32.0^{b} (-16.7)	43.9^{f} (+14.3)	0.9
Magnesium	22.4^{c}	16.6^{a}(-26.1)	29.2^{e} (+30.3)	23.5^{d} (+4.7)	23.2^{cd} (+3.6)	18.2^{b} (-19)	31.1^{f} (+38.6)	17.9^{b} (-20.4)	31.5^{f} (+40.5)	1.0
Phosphorus	299.9^{b}	276.2^{a} (-7.9)	313.6^{c} (+4.5)	300.6^{b} (+0.2)	301.0^{b} (+0.3)	277.2^{a} (-7.6)	315.2^{c} (+5.1)	277.6^{a} (-7.4)	315.7^{c} (+5.2)	3.0
Iron	1.5^{b}	0.9^{a} (-41.1)	3.0^{d} (+96.8)	1.9^{c}(+23.4)	1.6^{b} (+5.6)	1.0^{a} (-35.5)	4.1^{e} (+168.2)	1.1^{a} (-28.4)	4.5^{f}(+193.9)	0.2
Zinc	7.4^{c}	5.1^{a} (-30.4)	13.1^{e} (+77.9)	8.1^{cd} (+9.5)	8.3^{d}(+13.2)	5.9^{b} (-18.7)	14.0^{f} (+90.7)	5.8^{b} (-21.1)	14.4^{f} (+96.3)	0.6
Manganese	1.0^{b}	0.6^{a} (-38)	1.8^{d} (+80)	1.6^{c}(+55.1)	1.5^{c}(+43.6)	0.9^{b} (-8.2)	2.2^{f} (+118)	0.9^{b} (-14.1)	2.0^{e} (+94.1)	0.1
Copper	0.2^{b}	0.1^{a} (-58.5)	0.7^{d} (+230.8)	0.3^{c}(+58.5)	0.3^{c}(+58.5)	0.1^{a} (-58.5)	0.9^{e} (+326.2)	0.2^{b} (0)	0.9^{e}(+326.2)	0.06

Means in the same row with the same superscript letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw insect product. SEM is the standard error of mean; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried.

Table 4.12: Mineral composition (mg/100g) (wet basis) of raw and processed *R. differens* (grasshopper)

Mineral	Raw	Boiled	Toasted	Solar-dried	Oven-dried	B&SD	T&SD	B&OD	T&OD	[SEM]
Sodium	300.9^{b}	287.7^{a} (-4.4)	311.2^{c} (+3.4)	302.6^{b} (+0.6)	301.5^{b} (+0.2)	288.2^{a} (-4.2)	313.6^{d} (+4.2)	288.5^{a} (-4.1)	313.9^{d} (+4.3)	1.9
Potassium	341.3^{b}	332.5^{a} (-2.6)	348.6^{c} (+2.1)	341.9^{b} (+0.2)	342.3^{b} (+0.3)	332.9^{a} (-2.4)	349.8^{c} (+2.5)	333.1^{a} (-2.4)	349.4^{c} (2.4)	1.3
Calcium	29.5^{c}	22.4^{a}(-23.8)	33.4^{e} (+13.1)	30.6^{d} (+3.6)	30.9^{d} (+4.6)	23.5^{b} (-20.2)	33.9^{ef}(+14.7)	23.2^{ab} (-21.2)	34.3^{f} (+16)	0.8
Magnesium	30.9^{c}	22.9^{a}(-25.9)	34.0^{d} (+9.8)	31.5^{c} (+1.9)	31.8^{c} (+2.8)	23.6^{ab}(-23.7)	34.4^{d}(+11.1)	23.9^{b} (-22.6)	34.6^{d} (+11.9)	0.9
Phosphorus	141.8^{b}	134.9^{a} (-4.9)	152.6^{c} (+7.6)	142.5^{b} (+0.5)	143.0^{b} (+0.8)	135.4^{a} (-4.6)	152.9^{c} (+7.8)	135.7^{a} (-4.3)	153.2^{c} (+8)	1.4
Iron	21.0^{b}	16.6^{a} (-21)	24.3^{d} (+15.3)	22.1^{c} (+5.1)	21.8^{c} (+3.7)	16.8^{a} (-0.1)	25.3^{e}(+20.2)	17.1^{a} (-18.9)	24.8^{de} (+17.7)	0.6
Zinc	16.8^{b}	12.4^{a}(-26.4)	20.2^{d} (+20.4)	17.1^{bc} (+1.8)	17.5^{c} (+4.3)	12.9^{a} (-2.8)	20.6^{d} (+23)	12.5^{a} (-25.3)	21.3^{e} (+27.2)	0.6
Manganese	2.7^{c}	0.8^{a} (-69.5)	4.0^{e} (+45.2)	3.2^{d} (+19.2)	3.4^{d}(+25.7)	1.3^{b} (-53.5)	3.9^{e}(+46.5)	1.4^{b} (-49.9)	4.0^{e} (+48)	0.2
Copper	0.4^{c}	0.1^{a} (-80.2)	0.6^{d} (+55.2)	0.4^{c} (0)	0.4^{c} (0)	0.1^{a} (-80.2)	0.7^{e}(+85.3)	0.2^{b} (-44.8)	0.9^{f}(+122.4)	0.04

Means in the same row with the same superscript letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw insect product. SEM is the standard error of mean; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried.

Table 4.13: Mineral composition (mg/100g) (wet basis) of raw and processed *S. littoralis* (cotton leaf worm)

Mineral	Raw	Boiled	Toasted	Solar-dried	Oven-dried	B&SD	T&SD	B&OD	T&OD	SEM
Sodium	47.7^{c}	36.1^{a} (-24.2)	51.2^{d} (+7.4)	48.6^{c} (+1.9)	48.0^{c} (+0.7)	37.6^{b} (-21.2)	51.8^{ef} (+8.8)	37.7^{b} (-20.9)	52.6^{f}(+10.3)	1.2
Potassium	542.4^{b}	498.4^{a} (-8.1)	567.4^{d} (+4.6)	546.2^{bc} (+0.7)	549.6^{c} (+1.3)	500.5^{a} (-7.7)	568.4^{d} (+4.8)	499.9^{a} (-7.8)	568.7^{d} (+4.8)	5.6
Calcium	22.4^{c}	17.4^{a} (-22.5)	26.7^{e} (+19)	22.6^{cd} (+0.5)	23.2^{d} (+3.3)	18.0^{ab} (-19.8)	28.1^{f} (+25)	18.4^{b} (-18.1)	28.5^{f}(+27.1)	0.7
Magnesium	187.7^{b}	133.1^{a} (-29.1)	198.2^{c} (+5.6)	188.5^{b} (+0.4)	187.1^{b} (-0.3)	134.5^{a} (-28.3)	198.6^{c} (+5.8)	134.8^{a} (-28.2)	198.6^{c} (+5.8)	5.5
Phosphorus	16.6^{b}	12.5^{a} (-25.2)	17.8^{c} (+7)	16.7^{b} (+0.3)	16.8^{b} (+0.9)	12.6^{a} (-24.3)	17.8^{c} (+7.2)	12.7^{a} (-23.6)	17.9^{c} (+7.7)	0.4
Iron	1.4^{b}	0.9^{a} (-38.4)	1.9^{d} (+29.3)	1.6^{c} (+11.4)	1.5^{bc} (+6.3)	0.8^{a} (-40.9)	2.3^{e} (+60.2)	0.8^{a} (-40.2)	2.3^{e}(+59.1)	0.1
Zinc	12.8^{b}	8.6^{a}(-32.8)	15.6^{c} (+21.8)	13.3^{b} (+3.5)	13.3^{b} (+3.5)	8.9^{a} (-30.9)	15.9^{cd} (+23.9)	9.0^{a} (-29.5)	16.3^{d}(+27.2)	0.5
Manganese	3.6^{c}	2.8^{a} (-22.5)	5.0^{e} (+38.5)	3.7^{cd} (+2.2)	3.8^{d} (+3.7)	3.3^{b} (-10.4)	5.4^{f} (+48.9)	3.4^{b} (-7.2)	5.7^{g}(+56.4)	0.1
Copper	0.9^{c}	0.7^{a} (-29)	1.6^{e} (+75.7)	0.9^{c} (0)	1.0^{d} (+12.7)	0.8^{b} (-10.5)	1.9^{f}(+106.2)	0.9^{c}(-5.4)	2.1^{g}(+130.8)	0.09

Means in the same row followed by the same superscript are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw insect product. SEM is the standard error of mean; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried.

Processing influenced the mineral content in all the edible insect species studied. Mineral composition was a function of the interaction of insect species and processing method (Na: $F = 54973.43$; df = 35; $P < 0.001$, K: $F = 9179.55$; df = 35; $P < 0.001$, Ca: $F = 1302.34$; df = 35; $P < 0.001$, Mg: $F = 21897.02$; df = 35; $P < 0.001$, P: $F = 52492.06$; df = 35; $P < 0.001$, Fe: $F = 4207.24$; df = 35; $P < 0.001$, Zn: $F = 1185.34$; df = 35; $P < 0.001$, Mn: $F = 136.41$; df = 35; $P < 0.001$, Cu: $F = 154.36$; df = 35; $P < 0.001$). Significant differences ($P < 0.001$) in the processing method as a main effect were observed in all the minerals analysed. Solar and oven drying had the least positive impact on the minerals analysed with a change range of 0-58.5%. Toasting led to a 1.6-230.8% increase in the mineral content, with toasted + dried insect samples having the highest positive mineral content change of between 1.7% and 362.2% among the four edible insect types. On the other hand, boiling led to a 3.5-80.2% decrease in the insects' mineral content, with boiled + dried insect products having the least negative impact on the edible insects' mineral content (0-80.2%).

4.7 Effect of processing methods on the vitamins of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*

4.7.1 *Influence of processing methods on fat soluble vitamins of H. illucens, A. domesticus, R. differens and S. littoralis*

The retinol content of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* is shown in tables 4.14. Raw insect products had retinol content following the order *H. illucens* (0.3 μg/g) < *S. littoralis* (0.9 μg/g) < *A. domesticus* (1.8 μg/g) < *R. differens* (2.5 μg/g). The retinol content of the processed edible insect samples varied with processing method ($F = 22.20$; df = 8; $P < 0.001$) and insect species ($F = 20.01$; df = 3; $P < 0.001$), and the interaction of processing method and insect species was significant ($F = 107.04$; df = 35; $P < 0.001$). Toasting had a more profound effect on the retinol content of all the edible insect types in this study compared to boiling. Of the two drying techniques, solar drying reduced the retinol content more, with a range of 91.6-100%, compared to oven drying, whose reduction rate ranged from 66.1-94.7%. There

was no detection of retinol when samples of all four insect types were subjected to combined processes (drying post boiling or toasting).

Table 4.14: Retinol content (µg/g) of raw and processed edible insects

Process	*H. illucens* (BSF)	*A. domesticus* (cricket)	*R. differens* (grasshopper)	*S. littoralis* (CLW)	[SEM]
Raw	0.3^{Ca}	1.8^{Ec}	2.5^{Dd}	0.9^{Cb}	0.3
Boiled	0.02^{Ba} (-93.1)	1.4^{Db} (-24.6)	1.8^{Cc} (-28.3)	0.1^{Ba} (-84.9)	0.2
Toasted	Nd^{Aa} (-100)	0.7^{Cb} (-58.2)	1.0^{Bc} (-61.7)	Nd^{Aa} (-100)	0.1
Solar-dried	Nd^{Aa} (-100)	0.08^{Ab} (-95.6)	0.2^{Ac} (-91.6)	Nd^{Aa} (-100)	0.0
Oven-dried	0.02^{Ba} (-93.1)	0.3^{Bb} (-83.9)	0.8^{Bc} (-66.1)	0.1^{Ba} (-94.7)	0.1
B&SD	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	0.0
T&SD	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	0.0
B&OD	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	0.0
T&OD	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	0.0
[SEM]	0.0	0.1	0.2	0.1	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

The α-tocopherol content of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.15.). Raw insect samples had α-tocopherol content ranging from 148.8 µg/g in *R. differens* to 624.5 µg/g in *A. domesticus* The α-tocopherol content was dependent on the interaction of processing method and insect species ($F = 676.87$; df = 35; $P < 0.001$), with significant differences in the main effects (Processing method: $F = 35.14$; df = 8; $P < 0.001$; insect species: $F = 31.18$; df = 3; $P < 0.001$). With the exception of *R. differens*, toasting had a more profound effect on the α-tocopherol content in the four edible insect types in this study compared to boiling. Of the two drying techniques, solar drying reduced the α-tocopherol content more, with a

range of 60.9-81.8% compared to oven drying whose α-tocopherol reduction rate ranged from 46.9-69.2% across the four edible insect types. Combined processing (drying post boiling or toasting) had the most reduction rate in all the four edible insects studied, with a range of 74.4-100%, with the most effect being reported in solar-dried post boiled /toasted insect products.

Table 4.15: α-tocopherol content (µg/g) of raw and processed edible insects

Process	*H. illucens* (BSF)	*A. domesticus* (cricket)	*R. differens* (grasshopper)	*S. littoralis* (CLW)	[SEM]
Raw	412.1^{Gb}	624.5^{Fc}	148.8^{Fa}	174.4^{Ha}	58.6
Boiled	319.7^{Fc} (-22.4)	311.5^{Ec} (-50.1)	87.1^{Da} (-41.5)	126.5^{Gb}(-27.4)	31.8
Toasted	254.4^{Eb} (-38.3)	269.6^{Db} (-56.8)	106.8^{Ea}(-28.2)	113.8^{Fa} (-34.8)	23.0
Solar-dried	88.7^{Cc} (-78.5)	174.4^{Cd} (-72.1)	27.1^{Ba} (-81.8)	68.2^{Db} (-60.9)	16.4
Oven-dried	148.4^{Dc} (-63.9)	267.5^{Dd} (-57.2)	45.8^{Ca} (-69.2)	92.5^{Eb} (-46.9)	25.2
B&SD	53.6^{ABb}(-86.9)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	7.0
T&SD	48.9^{Ab} (-88.1)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	6.4
B&OD	76.5^{BCc}(-81.4)	69.7^{Bc} (-88.8)	Nd^{Aa} (-100)	44.6^{Cb} (-74.4)	9.0
T&OD	66.7^{ABCc} (83.8)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	23.6^{Bb} (-86.5)	8.2
[SEM]	24.7	38.1	10.3	11.2	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.7.2 Influence of processing methods on water soluble vitamins of H. illucens, A. domesticus, R. differens and S. littoralis

The thiamine and riboflavin contents of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.16. Raw insect samples had thiamine content ranging from 0.9 mg/100g in *H. illucens* to 1.7 mg/100g in *A. domesticus*, while their riboflavin content ranged from 1.0 mg/100g in *H. illucens* to 2.7 mg/100g in *R. differens*. Both vitamins were a function of the interaction of processing method and insect species (thiamine: $F = 551.72$; df = 35; $P < 0.001$; riboflavin: $F = 611.99$; df = 35; $P < 0.001$). There were significant differences in the main effects for thiamine

(processing method: $F = 47.24$; df = 8; $P < 0.001$; insect species: $F = 256.13$; df = 3; $P < 0.001$) and riboflavin as well (processing method: $F = 32.17$; df = 8; $P < 0.001$; insect species: $F = 122.81$; df = 3; $P < 0.001$).

Table 4.16: Thiamine and riboflavin content (mg/100g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Thiamine		
Raw	0.9^{Fa}	1.7^{Dc}	1.2^{Eb}	1.1^{Fb}	0.1
Boiled	0.4^{Aa} (-55.6)	0.9^{Ac} (-47.1)	0.7^{Ab} (-41.7)	0.5^{Aa} (-54.5)	0.1
Toasted	0.8^{Ea} (-11.1)	1.5^{Cc} (-11.8)	1.0^{Db} (-16.7)	0.9^{Dab}(-18.2)	0.1
Solar-dried	0.7^{Da} (-22.2)	1.6^{CDb} (-5.9)	0.7^{Aa} (-41.7)	0.8^{Ca} (-27.3)	0.1
Oven-dried	0.8^{Ea} (-11.1)	1.9^{Ec} (+11.8)	1.2^{Eb} (0)	1.0^{Eb} (-9.1)	0.1
B&SD	0.5^{Ba} (-44.4)	1.0^{Ad} (-41.2)	0.7^{Ac} (-41.7)	0.6^{Bb} (-45.5)	0.05
T&SD	0.7^{Da} (-22.2)	1.7^{Dd} (0)	0.9^{Cc} (-25)	0.8^{Cbc}(-27.3)	0.1
B&OD	0.6^{Ca} (-33.3)	1.3^{Bc} (-23.5)	0.8^{Bb} (-33.3)	0.6^{Ba} (-45.5)	0.1
T&OD	1.1^{Gb} (+22.2)	1.9^{Ec} (+11.8)	0.9^{Ca} (-25)	1.0^{Eab} (-9.1)	0.1
[SEM]	0.04	0.1	0.03	0.04	
			Riboflavin		
Raw	1.0^{Ga}	2.5^{Fc}	2.7^{Fd}	1.3^{Fb}	0.2
Boiled	0.8^{Ea} (-20)	2.1^{Dc} (-16)	1.9^{Dc} (-29.6)	1.1^{Eb} (-15.4)	0.2
Toasted	0.7^{Da} (-30)	1.8^{Cc} (-28)	1.7^{Cc} (-37)	0.9^{Cb} (-30.8)	0.1
Solar-dried	0.5^{Ba} (-50)	1.7^{Cc} (-32)	0.7^{Ab} (-74.1)	0.7^{Ab} (-46.2)	0.1
Oven-dried	1.0^{Ga} (0)	2.3^{Ec} (-8)	2.4^{Ec} (-11.1)	1.3^{Fb} (0)	0.2
B&SD	0.4^{Aa} (-60)	1.5^{Bc} (-40)	0.6^{Ab} (-77.8)	0.7^{Ab} (-46.2)	0.1
T&SD	0.6^{Ca} (-40)	1.2^{Ac} (-52)	0.6^{Aa} (-77.8)	0.8^{Bb} (-38.5)	0.1
B&OD	0.9^{Fa} (-10)	2.0^{Db} (-20)	2.0^{Db} (-25.9)	1.0^{Da} (-23.1)	0.2
T&OD	0.5^{Ba} (-50)	1.7^{Cd} (32)	1.1^{Bc} (-59.3)	0.9^{Cb} (-30.8)	0.1
[SEM]	0.04	0.1	0.1	0.04	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

Boiling had a profound reduction rate on the thiamine content (41.7–55.6%) compared to toasting, whose reduction rate was 11.1% to 18.2%. On the other hand, riboflavin was found to be lesser in all the toasted samples (0.7-1.8 mg/100g) compared to their boiled counterparts (0.8-2.1 mg/100g). Solar drying reduced both the thiamine and riboflavin contents profoundly compared to oven drying, with solar-dried *R. differens* having the highest reduction rate for both vitamins. Of all the combined processes that the insects in this study were subjected to, B&SD had the greatest reduction effect on thiamine content, with *S. littoralis* having the highest reduction rate at 45.5%. Solar drying post boiling of both *H. illucens* and *S. littoralis* had the same effect on riboflavin as in thiamine. However, for both *A. domesticus* and *R. differens*, T&SD had a more reduction rate on riboflavin content. Generally, B&SD and T&SD had more reduction rates on both vitamins as compared to B&OD and T&OD.

The niacin and pyridoxine contents of raw and processed *H. illucens, A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.17. Raw edible insect samples had niacin content ranging from 2.4 mg/100g to 5.9 mg/100g, and pyridoxine content ranging from 0.7 mg/100g to 1.3 mg/100g. The interaction effect of processing method and insect species was significant in both niacin (F = 3634.00; df = 35; $P < 0.001$) and pyridoxine (F = 718.22; df = 35; $P < 0.001$). There were significant differences in the main effects for niacin (processing method: F = 58.82; df = 8; $P < 0.001$; insect species: F = 17.10; df = 3; $P < 0.001$) and pyridoxine (processing method: F = 58.07; df = 8; $P < 0.001$; insect species: F = 155.99; df = 3; $P < 0.001$).

Boiling had a greater effect on the reduction of both niacin and pyridoxine in all edible insect types in this study compared to toasting, with boiled *H. illucens* and *S. littoralis* having 100% niacin reduction rates and boiled *S. littoralis* having the highest pyridoxine reduction rate (100%). When the raw edible insect samples were dried, the solar-dried samples had lesser niacin and pyridoxine content compared to their oven-dried counterparts. Solar drying post boiling had the highest niacin reduction rate of all the combined processes, leading to non-detectable levels in all species samples. This was closely followed by B&OD, which led to niacin reduction rates ranging from 96.9-

100%. Pyridoxine was not detected in *S. littoralis* after applying all the combined processes except in T&OD where 0.1 mg/100g was observed. Both B&SD and T&SD had a greater effect on pyridoxine reduction in the other edible insect species compared to their B&OD and T&OD counterparts.

Table 4.17: Niacin and pyridoxine content (mg/100g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Niacin		
Raw	5.9^{Fc}	3.2^{Eb}	3.2^{Fb}	2.4^{Ea}	0.4
Boiled	Nd^{Aa} (-100)	0.2^{Ab} (-93.8)	0.2^{Ab}(-93.8)	Nd^{Aa} (-100)	0.03
Toasted	2.9^{Ed} (-50.8)	2.8^{Dcd}(-12.5)	2.6^{Eb} (-18.8)	1.0^{Da}(-58.3)	0.2
Solar-dried	0.9^{BCb}(-84.7)	2.0^{Cc} (-37.5)	1.8^{Cc} (-43.8)	0.5^{Ba}(-79.2)	0.2
Oven-dried	1.1^{Cb}(-81.4)	2.6^{Dd} (-18.8)	1.9^{CDc}(-40.6)	0.8^{Ca}(-66.7)	0.2
B&SD	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	Nd^{Aa} (-100)	0.0
T&SD	0.7^{Bb}(-88.1)	1.6^{Bd} (-50)	0.9^{Bc} (-71.9)	0.4^{Ba} (-83.3)	0.1
B&OD	Nd^{Aa} (-100)	0.1^{Ab} (-96.9)	0.1^{Ab}(-96.9)	Nd^{Aa} (-100)	0.02
T&OD	2.5^{Dc}(-57.6)	2.1^{Cb} (-34.4)	2.1^{Db}(-34.4)	0.9^{CDa}(-62.5)	0.2
[SEM]	0.3	0.2	0.2	0.1	
			Pyridoxine		
Raw	1.3^{Eb}	0.9^{Fb}	0.7^{Ga}	0.7^{Da}	0.1
Boiled	0.5^{ABd}(-61.5)	0.2^{BCb}(-77.8)	0.3^{Cc} (-57.1)	Nd^{Aa} (-100)	0.05
Toasted	1.2^{DEc}(-7.7)	0.8^{EFb}(-11.1)	0.6^{Fa} (-14.3)	0.5^{Ca}(-28.6)	0.1
Solar-dried	0.9^{Cc} (-30.8)	0.5^{Db} (-44.4)	0.5^{Eb} (-28.6)	0.1^{Ba}(-85.7)	0.1
Oven-dried	1.1^{Dd}(-15.4)	0.7^{Ec} (-22.2)	0.7^{Gb} (0)	0.5^{Ca}(-28.6)	0.1
B&SD	0.4^{Ac}(-69.2)	Nd^{Aa} (-100)	0.1^{Ab}(-85.7)	Nd^{Aa} (-100)	0.04
T&SD	0.8^{Cc} (-38.5)	0.1^{ABab}(-88.9)	0.2^{Bb} (-71.4)	Nd^{Aa} (-100)	0.1
B&OD	0.6^{Bc} (-53.8)	0.3^{Cb} (-66.7)	0.4^{Db} (-42.9)	Nd^{Aa} (-100)	0.1
T&OD	1.6^{Fd} (-23.1)	0.8^{EFc} (-11.1)	0.6^{Fb} (-14.3)	0.1^{Ba}(-85.7)	0.2
[SEM]	0.1	0.1	0.04	0.05	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

The folic and ascorbic acid contents of raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* are shown in Tables 4.18. Raw insects had folic acid content ranging from 0.5 mg/100g in *S. littoralis* to 1.6 mg/100g in *R. differens*. They also had ascorbic acid content ranging from 0.6 mg/100g in *S. littoralis* to 9.6 mg/100g in *A. domesticus*. The interaction effect of processing method and insect species was significant in both folic acid (F = 256.34; df = 35; $P < 0.001$) and ascorbic acid (F = 4842.43; df = 35; $P < 0.001$). There were significant differences in the main effects as well for folic acid (processing method: F = 52.77; df = 8; $P < 0.001$; insect species: F = 174.88; df = 3; $P < 0.001$) and ascorbic acid (processing method: F = 10.50; df = 8; $P < 0.001$; insect species: F = 195.45; df = 3; $P < 0.001$).

Boiling of the edible insects led to a more reduction level for both folic (45.5-100%) and ascorbic acids (41.7-60.9%) compared to toasting, which had reduction level of 0-40% and 3.2-16.6% respectively. Solar drying of the raw samples led to a more profound reduction rate for both folic (36.4-60%) and ascorbic acids (32.3-65.2%) compared to oven drying. In the combined processes, B&SD and B&OD had higher reduction rates of folic acid in all the four insect types compared to both T&SD and T&OD. For ascorbic acid, B&SD had the highest reduction rate in all the insect species, except for *A. domesticus*, whose highest reduction rate was in T&SD.

Table 4.18: Folic acid and ascorbic acid content (mg/100g) of raw and processed edible insects

	Insect species				
	H. illucens **(BSF)**	***A. domesticus*** **(cricket)**	***R. differens*** **(grasshopper)**	***S. littoralis*** **(CLW)**	**[SEM]**
			Folic acid		
Raw	1.2^{Eb}	1.1^{Fb}	1.6^{Fc}	0.5^{Fa}	0.1
Boiled	0.5^{Bb}(-58.3)	0.6^{Bb} (-45.5)	0.8^{BCc}(-50)	Nd^{Aa} (-100)	0.1
Toasted	1.2^{Ec} (0)	1.0^{Eb} (-9.1)	1.1^{Dbc}(-31.3)	0.3^{Da} (-40)	0.1
Solar-dried	0.7^{Cb}(-41.7)	0.7^{Cb} (-36.4)	0.8^{BCc}(-50)	0.2^{Ca} (-60)	0.1
Oven-dried	1.1^{Eb} (-8.3)	1.1^{Fb} (0)	1.4^{Ec} (-12.5)	0.4^{Ea} (-20)	0.1
B&SD	0.3^{Ac} (-75)	0.2^{Ab} (-81.8)	0.5^{Ad} (-68.8)	Nd^{Aa} (-100)	0.05
T&SD	0.9^{Dc} (-25)	0.8^{Db} (-27.3)	0.9^{Cc} (-43.8)	0.1^{Ba} (-80)	0.1
B&OD	0.8^{CDc}(-33.3)	0.8^{Dc} (-27.3)	0.7^{Bb} (-56.3)	Nd^{Aa} (-100)	0.1
T&OD	1.4^{Fc}(-16.7)	1.0^{Eb} (-9.1)	0.9^{Cb} (-43.8)	0.5^{Fa} (0)	0.1
[SEM]	0.1	0.04	0.1	0.03	
			Ascorbic acid		
Raw	2.3^{Fb}	9.6^{Gc}	3.1^{Eb}	0.6^{Ea}	1.0
Boiled	0.9^{Bab}(-60.9)	5.6^{Cc} (-41.7)	1.4^{Ab} (-54.8)	0.3^{Ba} (-50)	0.6
Toasted	2.1^{Eb} (-8.7)	8.4^{Fc} (-12.5)	3.0^{Eb} (-3.2)	0.5^{Da} (-16.6)	0.9
Solar-dried	0.8^{Aba}(-65.2)	5.6^{Cc} (-41.7)	2.1^{Cb} (-32.3)	0.3^{Ba} (-50)	0.6
Oven-dried	1.5^{Db}(-34.8)	6.5^{Dd} (-32.3)	2.8^{Dc} (-9.7)	0.3^{Ba} (-50)	0.7
B&SD	0.7^{Ab} (-69.6)	4.8^{Bd} (-50)	1.3^{Ac} (-58.1)	0.1^{Aa} (-83.3)	0.5
T&SD	1.1^{Cb} (-52.2)	2.7^{Ad} (-71.9)	1.6^{Bc} (-48.4)	0.5^{Da} (-16.7)	0.2
B&OD	1.6^{Dc} (-30.4)	2.9^{Ad} (-69.8)	1.3^{Ab} (-58.1)	0.4^{Ca} (-33.3)	0.3
T&OD	1.2^{Cb} (-47.8)	7.3^{Ed} (-23.9)	1.6^{Bc} (-48.4)	0.1^{Aa} (-83.3)	0.8
[SEM]	0.1	0.4	0.1	0.03	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.8 Effect of processing techniques on the amino acid composition of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*

The amino acid profiles for raw 4.19 and 4.20 Essential amino acids like valine (Val), methionine (Met), leucine (Leu), isoleucine (Ile) and phenylalanine (Phe) were initially detected in significantly ($P < 0.01$) varied proportions in all the four insect species. For instance, *A. domesticus* had the highest amount of all the essential amino acids (17.4-51.5 mg/g) except for Phe, where it had the least amount (15.4 mg/g). On the other hand, *S. littoralis* had the least amounts of all the essential amino acids, with non-detectable levels of Ile. However, it had the highest amount of Phe (43.3 mg/g). Other essential amino acids like lysine (Lys) and histidine (His) were not detected in all the edible insect samples. Processing altered the composition of the essential amino acids in all samples (Tables 22). Essential amino acid content was a function of the interaction of insect species and processing method (Val: $F = 2329.43$; df = 11; $P < 0.001$; Met: $F = 220.38$; df = 11; $P < 0.001$; Ile: $F = 4394.29$; df = 11; $P < 0.001$; Leu: $F = 14781.49$; df = 11; $P < 0.001$; Phe: $F = 4708.19$; df = 11; $P < 0.001$). There were significant differences in processing method as a main effect in all the essential amino acids ($P < 0.001$). Significant differences in insect type as a main effect were recorded in Val ($P = 0.022$), Met ($P < 0.001$), Ile ($P = 0.004$) and Phe ($P = 0.007$). However, Leu ($P = 0.125$) had no significant differences in insect type as a main effect. When comparing the processes to the raw insect samples, B&SD insect samples had a -73.4% to 2.2% change in Val and reductions in Met (14.2-54.1%), Leu (65.9-89.2%), Ile (71.9-100%) and Phe (43.2-86.3%), with (60.8-72.8%) reduction in total essential amino acids. Similarly, B&OD edible insect samples had a -87.7% to 4.7% change in Leu and reductions in Val (20.7-83.3%), Met (19.5-100%), Ile (42.3-100%) and Phe (55-73.7%), with (43.3-79.2%) reduction in total essential amino acids.

Table 4.19: Essential amino acids content (mg/g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Valine		
Raw	28.8 ^{Bb}	51.5 ^{Cd}	42.9 ^{Bc}	14.4 ^{Ca}	5.3
B&SD	29.5 ^{Bc} (+2.2)	13.7 ^{Ab} (-73.4)	12.5 ^{Ab} (-70.9)	6.7 ^{Aa} (-53.8)	3.2
B&OD	18.4 ^{Ad} (-36)	14.4 ^{Bc} (-72)	7.2 ^{Aa} (-83.3)	11.4 ^{Bb} (-20.7)	1.6
[SEM]	2.3	7.9	7.0	1.4	
			Methionine		
Raw	14.4 ^{Ca}	17.4 ^{Cb}	14.2 ^{Ca}	13.6 ^{Ca}	0.6
B&SD	6.6 ^{Aa} (-54.1)	14.9 ^{Bc} (-14.2)	11.3 ^{Bb} (-20.3)	6.4 ^{Ba} (-53.3)	1.4
B&OD	11.6 ^{Bc} (-19.5)	13.6 ^{Ac} (-21.6)	5.7 ^{Ab} (-60)	Nd ^{Aa} (-100)	2.0
[SEM]	1.4	0.7	1.6	2.4	
			Leucine		
Raw	30.8 ^{Cb}	51.4 ^{Cc}	50.7 ^{Cc}	19.8 ^{Ba}	5.1
B&SD	5.8 ^{Aa} (-81.1)	5.6 ^{Aa} (-89.2)	17.3 ^{Bb} (-65.9)	6.0 ^{Aa} (-69.7)	1.9
B&OD	12.7 ^{Bb} (-58.5)	19.8 ^{Bc} (-61.5)	6.3 ^{Aa} (-87.7)	20.7 ^{Bc} (+4.7)	2.2
[SEM]	4.7	8.6	8.5	3.0	
			Isoleucine		
Raw	22.7 ^{Cc}	33.6 ^{Cd}	17.0 ^{Cb}	Nd ^{a}	4.6
B&SD	6.4 ^{Ab} (-71.9)	5.7 ^{Bb} (-83)	Nd ^{Aa} (-100)	Nd ^{a}	1.1
B&OD	13.1 ^{Bc} (-42.3)	Nd ^{Aa} (-100)	5.4 ^{Bb} (-67.9)	Nd ^{a}	2.1
[SEM]	3.0	6.6	3.2	0	
			Phenylalanine		
Raw	41.4 ^{Bc}	15.4 ^{Ba}	24.1 ^{Cb}	43.3 ^{Cc}	4.4
B&SD	5.8 ^{Aa} (-85.9)	6.1 ^{Aa} (-60.1)	13.7 ^{Bb} (-43.2)	5.9 ^{Aa} (-86.3)	1.3
B&OD	11.7 ^{Ab} (-71.7)	4.3 ^{Aa} (-72.1)	6.3 ^{Aa} (-73.7)	19.5 ^{Bc} (-55)	2.2
[SEM]	7.0	2.1	3.3	6.9	
			Total essential amino acids		
Raw	138.1	169.3	148.9	91.1	
B&SD	54.1 (-60.8)	46.0 (-72.8)	54.8 (-63.2)	25.0 (-72.5)	
B&OD	67.5 (-51.1)	52.1 (-69.2)	30.9 (-79.2)	51.6 (-43.3)	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw product. SEM is standard error of the means; Nd is not detected; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

Non-essential amino acids like arginine (Arg), proline (Pro), Glutamic acid (Glu), tyrosine (Tyr) and hydroxyproline (Hxp) were initially detected in significantly varied proportions in all the four edible insect species studied ($P < 0.001$). For example, *R. differens* had the highest amount of Arg (268.6 mg/g), while *H. illucens* had the least amount (11.1 mg/g). Both *H. illucens* and *A. domesticus* had Glu in varied amounts (67.5 mg/g) and (18.7 mg/g) respectively, while both *R. differens* and *S. littoralis* had non-detectable levels. Pro, Tyr and Hxp acids ranged from 12.3-74.7 mg/g in all the raw edible insect samples. Other non-essential amino acids like glutamine (Gln) and serine (Ser) were not detected in all samples. Processing altered the composition of the non-essential amino acids in all the four edible insect species (Table 4.20). Non-essential amino acids were a function of the interaction of insect species and processing method (Arg: $F = 52205.42$; df = 11; $P < 0.001$; Glu: $F = 29751.42$; df = 11; $P < 0.001$; Pro: $F = 20334.26$; df = 11; $P < 0.001$; Tyr: $F = 877.78$; df = 11; $P < 0.001$; Hxp: $F = 355.73$; df = 11; $P < 0.001$). Differences due to processing method were significant in Glu ($P = 0.042$), Pro ($P < 0.001$), Tyr ($P = 0.001$) and Hxp ($P = 0.010$) but not significant in Arg ($P = 0.092$). On the other hand, differences due to insect species were significant in Arg ($P = 0.049$), Pro ($P = 0.008$) and Tyr ($P < 0.001$) but not significant in Glu ($P = 0.067$) and Hxo ($P = 0.954$). Boiling + drying led to an increase in Arg content in *H. illucens*, but a decrease was observed in the other edible insect types by 22.4% to 82.4%. There was a 100% reduction in Glu content in boiled + dried *H. illucens* and *A. domesticus*, although a 25.8% increase was observed in B&SD *A. domesticus*. Both processes reduced Pro, Tyr and Hxp contents by 0.9-100% in all the edible insect species samples except for B&SD *R. differens* and B&OD *A. domesticus*, where Hxp increased by 1.3% and 8.9% respectively.

Table 4.20: Non-essential amino acids content (mg/g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Arginine		
Raw	11.1 ^{Aa}	133.9 ^{Cc}	268.6 ^{Cd}	67.2 ^{Cb}	36.3
B&SD	48.7 ^{Ba}(+340.7)	51.7 ^{Aa}(-61.4)	101.6 ^{Bb}(-62.2)	52.2 ^{Ba}(-22.4)	8.3
B&OD	111.5 ^{Cc}(+908)	67.2 ^{Bc}(-49.8)	47.3 ^{Ab}(-82.4)	32.1 ^{Aa}(-52.2)	11.3
[SEM]	18.5	15.9	42.1	6.4	
			Glutamic acid		
Raw	67.5 ^{Bc}	18.7 ^{Bb}	Nd ^{a}	Nd ^{a}	10.4
B&SD	Nd ^{Aa}(-100)	23.6 ^{Cb}(+25.8)	Nd ^{a}	Nd ^{a}	3.8
B&OD	Nd^{a}(-100)	Nd^{a}(-100)	Nd	Nd	0
[SEM]	14.2	4.5	0	0	
			Proline		
Raw	38.6 ^{Ca}	74.7 ^{Cc}	64.7 ^{Bb}	68.4 ^{Bb}	5.2
B&SD	27.2^{b}(-29.3)	24.4 ^{Ac}(-67.3)	15.8 ^{Ab}(-75.5)	8.2 ^{Aa}(-88.1)	2.8
B&OD	23.6 ^{Ab}(-38.8)	68.4 ^{Bc}(-8.5)	7.9 ^{Aa}(-87.8)	13.2 ^{Aa}(-80.7)	9.0
[SEM]	2.9	10.0	11.2	12.2	
			Tyrosine		
Raw	22.5 ^{Bd}	14.7 ^{Cb}	18.4 ^{Cc}	13.3 ^{Ca}	1.3
B&SD	11.2 ^{Ac}(-50.2)	8.5 ^{Ab}(-42.1)	11.3 ^{Bc}(-38.6)	6.1^{Ba}(-54.1)	0.8
B&OD	22.9 ^{Bd}(-19.5)	13.3 ^{Bc}(-9.1)	6.9 ^{Ab}(-62.6)	Nd ^{Aa}(-100)	3.1
[SEM]	2.4	1.2	2.1	2.4	
			Hydroxyproline		
Raw	13.5 ^{Bc}	11.4 ^{Ba}	12.3 ^{Bb}	12.4 ^{Cb}	0.3
B&SD	6.0 ^{Aa}(-55.5)	6.6 ^{Aa}(-42.3)	12.5 ^{Bb}(+1.3)	5.9 ^{Aa}(-52.1)	1.0
B&OD	13.1 ^{Bb}(-2.9)	12.4 ^{Bb}(+8.9)	5.6 ^{Aa}(-54.3)	12.3 ^{Bb}(-0.9)	1.1
[SEM]	1.5	1.1	1.4	6.9	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw product. SEM is standard error of the means; Nd is not detected; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.9 Microbiological quality of raw and processed *H. illucens, A. domesticus, R. differens* and *S. littoralis*

4.9.1 Effect of processing techniques on the total viable count (TVC) and total yeast and mould count (YMC) of H. illucens, A. domesticus, R. differens and S. littoralis

Table 4.21 shows the total viable count (TVC) and the total yeast and mould count (YMC) for raw and processed *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*. Raw edible insect samples had TVC ranging from 7.0 Log cfu/g to 9.1 Log cfu/g, and YMC ranging from 6.4 Log cfu/g to 8.2 Log cfu/g. Processing influenced the TVC and YMC of all edible insect species in this study. The interaction effect of processing method and insect species was significant in both TVC (F = 1207.6; df = 35; $P < 0.001$) and YMC (F = 11739.6; df = 35; $P < 0.001$). Further, the main effects were significant for both TVC (processing method: F = 505.5; df = 8; $P < 0.001$; insect species: F = 10.26; df = 3; $P < 0.001$) and YMC (processing method: F = 526.1; df = 8; $P < 0.001$; insect species: F = 13.1; df = 3; $P < 0.001$).

Solar drying increased the TVC of all the four edible insect species in the study but these increments were not statistically significant ($P > 0.05$). On the other hand, oven drying significantly decreased the TVC of all the four insect types ($P < 0.001$) with *R. differens* having the highest TVC reduction rate at 25.3%. The YMC reduced upon solar drying, although the reductions in *R. differens* (2.4%) and *S. littoralis* (1.5%) were not significantly low (*R. differens*: P = 1.000; *S. littoralis*: P = 1.000). Oven drying significantly reduced ($P < 0.001$) the YMC in all the insect species, with the reduction ranging from 32.9% in *R. differens* to 59% in *A. domesticus*. Boiling and toasting reduced the TVC of all the insect species by 4-6 log cycles and completely eliminated yeast and moulds. Boiling was more effective at lowering the TVC (66.2-72.3%) compared to toasting (57.1-68.1%), with boiled *A. domesticus* having the lowest TVC of 2.3 Log cfu/g and the highest TVC reduction rate of 72.3%. Generally, solar drying post boiling/ toasting increased, while oven drying post boiling/ toasting lowered the TVC compared to their boiled and toasted counterparts. Toasted + oven-dried and boiled +

oven-dried samples had a TVC reduction rate of between 70-84% and 76-87% respectively. There was however no detection of YMC when all the combined processes were employed.

Table 4.21: Total viable counts and yeast and mould counts (Log cfu/g) on raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Total viable count		
Raw	$7.7^{E\,b}$	$8.3^{F\,c}$	$9.1^{E\,d}$	$7.0^{E\,a}$	0.2
Boiled	$2.6^{B\,b}$ (-66.2)	$2.3^{B\,a}$ (-72.3)	$2.8^{B\,c}$ (-69.2)	$2.6^{B\,b}$ (-62.9)	0.1
Toasted	2.8^{BCa}(-63.6)	$3.1^{C\,d}$ (-62.7)	$2.9^{B\,b}$ (-68.1)	3.0^{BCc}(-57.1)	0.1
Solar-dried	$7.8^{E\,b}$ (+1.3)	$8.8^{F\,c}$ (+6.0)	$9.2^{E\,d}$ (+1.1)	$7.4^{E\,a}$ (+5.7)	0.2
Oven-dried	$6.2^{D\,a}$ (-19.4)	$6.8^{E\,b}$ (-18.1)	$6.8^{D\,b}$ (-25.3)	$6.4^{D\,c}$ (-8.6)	0.1
B&SD	$3.1^{C\,a}$ (-59.7)	$3.9^{D\,b}$ (-53.0)	$3.2^{B\,a}$ (-64.8)	$3.1^{C\,a}$ (-55.7)	0.1
T&SD	3.0^{BCab}(-61.0)	$3.1^{C\,b}$ (-62.6)	$3.9^{C\,c}$ (-57.1)	2.9^{BCa}(-58.6)	0.1
B&OD	$1.6^{A\,b}$ (-79.2)	$1.0^{A\,a}$ (-87.9)	$1.8^{A\,c}$ (-80.2)	1.7^{Abc}(-75.7)	0.1
T&OD	$1.8^{A\,b}$ (-76.6)	$1.3^{A\,a}$ (-78.3)	$2.0^{A\,c}$ (-78.0)	2.1^{Ac} (-70.0)	0.1
[SEM]	0.5	0.5	0.5	0.5	
			Yeasts and moulds		
Raw	$7.6^{D\,b}$	$7.8^{D\,b}$	$8.2^{C\,c}$	$6.4^{C\,a}$	0.3
Boiled	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
Toasted	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
Solar-dried	$6.5^{C\,a}$(-14.5)	$7.0^{C\,b}$ (-10.2)	$8.0^{C\,c}$ (-2.4)	$6.3^{C\,a}$ (-1.5)	0.3
Oven-dried	$4.2^{B\,b}$(-44.7)	$3.2^{B\,a}$ (-59.0)	$5.5^{B\,c}$ (-32.9)	$3.0^{B\,a}$ (-53.1)	0.3
B&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
T&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
B&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
T&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	-
[SEM]	0.6	0.6	0.7	0.5	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent change relative to the raw product. SEM is standard error of the means; Nd is not detected; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.9.2 Effect of processing techniques on Enterobacteriaceae count of H. illucens, A. domesticus, R. differens and S. littoralis

The *Enterobacteriaceae* count of raw and processed *H. illucens, A. domesticus, R. differens* and *S. littoralis* is presented in Table 4.22. Raw insects had an *Enterobacteriaceae* count ranging from 5.3-9.6 Log cfu/g. *Enterobacteriaceae* count reduced with processing and was a function of the interaction of processing method and insect species (F = 1377.8; df = 35; $P < 0.001$), with significant differences being reported in the main effects as well (processing method: F = 223.2; df = 8; $P < 0.001$; insect species: F = 12.9; df = 3; $P < 0.001$). Oven drying reduced the *Enterobacteriaceae* count better than solar drying, as it led to a 24.7-52.8% reduction as opposed to 17-34.2% reduction achieved in solar-dried samples. However, boiling, toasting and drying post boiling/toasting were the most effective in *Enterobacteriaceae* reduction, as they achieved 100% elimination in all samples.

Table 4.22: *Enterobacteriaceae* count (Log cfu/g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
Raw	$7.6^{D\,d}$	$7.9^{D\,b}$	$8.9^{C\,c}$	$5.3^{D\,a}$	0.4
Boiled	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Toasted	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Solar-dried	$5.5^{C\,b}$ (-27.6)	$5.2^{C\,b}$ (-34.2)	$7.1^{B\,c}$ (-20.2)	$4.4^{C\,a}$ (-17)	0.3
Oven-dried	$3.2^{B\,b}$ (-58)	$4.3^{B\,c}$ (-45.6)	$6.7^{B\,d}$ (-24.7)	$2.5^{B\,a}$ (-52.8)	0.5
B&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
B&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
[SEM]	0.5	0.6	0.7	0.4	

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; Nd is not detected; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.9.3 Effect of processing techniques on indicator micro-organisms (faecal coliforms and Lac+ bacteria) of H. illucens, A. domesticus, R. differens and S. littoralis

Table 4.23 presents the faecal coliforms and Lac+ bacterial counts of raw and processed edible insect samples. Raw edible insect samples had faecal coliforms ranging from 3.4 Log cfu/g in *S. littoralis* to 8.4 Log cfu/g in *R. differens*, while their Lac+ bacterial count ranged from non-detectable levels in *S. littoralis* to 1.5 Log cfu/g in *A. domesticus*. Processing decreased the faecal coliforms and Lac+ bacteria in all samples and were a function of the interaction of insect species and processing method (faecal coliforms: $F = 483.4$; df = 35; $P < 0.001$; Lac+ bacteria: $F = 5138.1$; df = 35; $P < 0.001$). Further, significant differences in the main effects were reported as well for both faecal coliforms (processing method: $F = 101.5$; df = 8; $P < 0.001$; insect species: $F = 13.2$; df = 3; $P < 0.001$) and Lac+ bacteria (processing method: $F = 35.4$; df = 8; $P < 0.001$; insect species: $F = 9.8$; df = 3; $P < 0.001$).

Solar drying had the least effect on faecal coliform and Lac+ bacterial reduction of all the processes employed. Solar-dried *H. illucens* had the least faecal coliform reduction at 28.1%, while solar-dried *A. domesticus* had the least Lac+ bacterial reduction at 54.1%. Oven drying was more effective in the reduction of these indicator micro-organisms, as it achieved 72.6-100% reduction levels in all samples, as opposed to solar drying. The difference between the two drying processes in eliminating indicator micro-organisms was significant ($P < 0.05$). Boiled, toasted and dried post boiled/toasted samples had non-detectable levels of the indicator micro-organisms, hence being the most effective methods in their elimination.

Table 4.23: Faecal coliforms and Lac+ bacteria counts (Log cfu/g) of raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Faecal coliforms		
Raw	$6.4^{D\,c}$	$6.2^{D\,b}$	$8.4^{D\,d}$	$3.4^{C\,a}$	0.6
Boiled	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Toasted	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Solar-dried	$4.6^{C\,c}$ (-28.1)	$3.7^{C\,b}$ (-40.3)	$5.6^{C\,d}$ (-33.3)	$1.4^{B\,a}$ (-58.8)	0.5
Oven-dried	$1.1^{B\,b}$ (-82.8)	$1.6^{B\,c}$ (-74.2)	$2.3^{B\,d}$ (-72.6)	$Nd^{A\,a}$ (-100)	0.3
B&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
B&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
[SEM]	0.5	0.4	0.6	0.2	
			Lac+ bacteria		
Raw	$6.4^{D\,b}$	$6.1^{D\,b}$	$5.9^{D\,b}$	$Nd^{A\,a}$	0.8
Boiled	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Toasted	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
Solar-dried	$2.1^{C\,b}$ (-67.2)	$2.8^{C\,c}$ (-54.1)	$2.3^{C\,b}$ (-60.1)	$Nd^{A\,a}$	0.3
Oven-dried	$1.1^{B\,b}$ (-82.8)	$1.5^{B\,c}$ (-75.4)	$1.0^{B\,b}$ (-83.1)	$Nd^{A\,a}$	0.2
B&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&SD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
B&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
T&OD	Nd^{A}	Nd^{A}	Nd^{A}	Nd^{A}	
[SEM]	0.4	0.4	0.4		

Means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; Nd is not detected; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

4.9.4 Effect of processing techniques on pathogenic micro-organisms (Staphylococcus aureus and Salmonella spp*) of H. illucens, A. domesticus, R. differens and S. littoralis*

Table 4.24 shows the *St. aureus* counts and presence (+) or absence (-) of *Salmonella* spp on raw and processed edible insect samples. Raw edible insect samples had *St. aureus* count ranging from 7.7 Log cfu/g in *S. littoralis* to 9.8 Log cfu/g in *R. differens* and also tested positive for *Salmonella* spp. Processing lowered *St. aureus* count by 0.9-4.5 log cycles and was dependent on the interaction of processing method and insect species (F = 3110.7; df = 35; $P < 0.001$). Further, both main effects were significant in the reduction of *St. aureus* (processing method: F = 517.9; df = 8; $P < 0.001$; insect species: F = 100.6; df = 3; $P < 0.001$).

Solar drying had the least effect on *St. aureus* count, with solar-dried *R. differens* having the lowest reduction of 50.5%. Moreover, both solar and oven drying did not eliminate *Salmonella* spp, hence having the least effect on the bacteria. Boiled and toasted samples had *St. aureus* reduction levels of 62.3-74.7% and 68.1-80.1% respectively. Solar-dried post boiled/toasted samples had higher *St. aureus* count compared to their boiled and toasted counterparts. However, the *St. aureus* count increase in B&SD *H. illucens* (P = 1.000), B&SD *S. littoralis* (P = 1.000) and T&SD *R. differens* (P = 0.136) were not significant compared to their boiled and toasted counterparts. On the other hand, oven drying post boiling/toasting further reduced *St. aureus* in all the samples compared to their boiled and toasted counterparts, except for T&OD *S. littoralis*, whose 0.2 Log cfu/g reduction from its toasted product was not significant (P = 0.350). *Salmonella* spp was completely eliminated in all the boiled, toasted and dried post boiled/toasted insect products.

Table 4.24: Counts of *Staphylococcus aureus* (Log cfu/g) and presence (+) or absence (-) of *Salmonella* spp on raw and processed edible insects

	Insect species				
	***H. illucens* (BSF)**	***A. domesticus* (cricket)**	***R. differens* (grasshopper)**	***S. littoralis* (CLW)**	**[SEM]**
			Staphylococcus aureus		
Raw	$7.9^{F\,a}$	$8.3^{F\,b}$	$9.1^{E\,c}$	$7.7^{E\,a}$	0.3
Boiled	$2.5^{D\,b}$ (-68.4)	$2.1^{CD\,a}$ (-74.7)	$3.3^{B\,d}$ (-63.7)	$2.9^{C\,c}$ (-62.3)	0.2
Toasted	$1.5^{B\,a}$ (-80.1)	$1.9^{C\,b}$ (-77.1)	$2.9^{B\,c}$ (-68.1)	$1.8^{A\,b}$ (-76.6)	0.2
Solar-dried	$3.0^{E\,a}$ (-62.0)	$2.9^{E\,a}$ (-65.1)	$4.5^{D\,c}$ (-50.5)	$3.8^{D\,b}$ (-50.6)	0.2
Oven-dried	$1.7^{BC\,a}$(-78.5)	$1.6^{BC\,a}$ (-80.7)	$3.9^{C\,c}$ (-57.1)	$3.2^{C\,b}$ (-58.4)	0.3
B&SD	$2.6^{D\,a}$ (-67.1)	$3.0^{E\,b}$ (-63.8)	$4.0^{C\,c}$ (-56.0)	$3.1^{C\,b}$ (-59.7)	0.2
T&SD	$2.0^{C\,a}$ (-74.9)	$2.4^{D\,b}$ (-71.1)	$3.1^{B\,c}$ (-65.9)	$2.1^{B\,a}$ (-72.7)	0.1
B&OD	$1.2^{AB\,a}$(-84.8)	$1.3^{AB\,a}$(-84.3)	$2.1^{A\,c}$ (-76.9)	$1.9^{AB\,b}$ (-75.3)	0.1
T&OD	$0.9^{A\,a}$ (-88.6)	$1.0^{A\,a}$ (-87.9)	$2.0^{A\,c}$ (-78.0)	$1.6^{A\,b}$ (-79.2)	0.1
[SEM]	0.4	0.4	0.4	0.4	
			Salmonella spp		
Raw	+	+	+	+	
Boiled	-	-	-	-	
Toasted	-	-	-	-	
Solar-dried	+	+	+	+	
Oven-dried	+	+	+	+	
B&SD	-	-	-	-	
T&SD	-	-	-	-	
B&OD	-	-	-	-	
T&OD	-	-	-	-	

For *St. aureus*, means in the same column followed by the same capital letters, and means in the same row followed by the same small letters are not significantly different ($P > 0.05$; n = 3). Values in parentheses are the percent decrease relative to the raw product. SEM is standard error of the means; B is boiled; T is toasted; SD is solar-dried; OD is oven-dried; BSF is black soldier fly; CLW is cotton leaf worm

CHAPTER FIVE

DISCUSSION

5.1 Consumption and post-harvest handling practices of insects in western Kenya

Edible insects are traditional foods in many cultures and they often play a role in human nutrition, as they have much nutrients to offer (Banjo, Lawal, & Songonuga, 2006). Edible insects are acquired through wild collection using strategies such as hand picking, net and light trapping, semi-domestication in appropriate substrates or through buying them in open markets (Meutchieye, Tsafo, & Niassy, 2016; Mmari et al., 2017; Mutungi et al., 2019; van Huis et al., 2013). This was the case in western Kenya, where wild collection and together with buying were reported as the main methods of acquiring edible insects. Rearing of edible insects is not being practiced in the region probably because of lack of interest, as they can obtain the edible insects from the wild with little effort or probably due to lack of awareness and expertise. However, through institutions of higher learning and research centres such Jaramogi Oginga Odinga University of Science and Technology (JOOUST), Jomo Kenyatta University of Agriculture and Technology (JKUAT) and International Centre for Insect Physiology and Ecology (*icipe*), who offer capacity building services like training and research, the culture of insect rearing for food and feed is being reported to have emerged and is being embraced as a viable source of food security and livelihood within the western region (Homa-bay, Siaya and Kisumu counties) and throughout the country (Halloran et al., 2018; Kamau et al., 2021; Kelemu et al., 2015; Nischalke et al., 2020). The main insects consumed within the region were among the over 470 and 1800 edible insects reported to be consumed in Africa and globally respectively (Jongema, 2017; Kelemu et al., 2015). The decline reported in the number of some edible insects like locusts (*Locusta migratoria*) could be attributed to climatic changes and urbanization, which have been shown to negatively influence some insects (Ayieko, 2014; Bale et al., 2002; Ramos-Elorduy, 2006).

Women and children have also been reported to be the most involved in the collection, post-harvest handling and the sale of edible insects by other authors like Mmari et al. (2017) and Mutungi et al. (2019). Elsewhere, Pambo, Okello, Mbeche, & Kinyuru (2017), revealed that the responsibility of food purchases and preparation would often solely lie on women, hence their higher involvement in edible insect handling. Further, the involvement of women in edible insect trade has been reported by Kozanayi and Frost (2002) and such endeavors have been shown to be of importance in the families' livelihood (Mmari et al., 2017). All the post-harvest handling techniques used by the residents in Western Kenya were among the processing techniques reported elsewhere (Kelemu et al., 2015; Kinyuru et al., 2018; Mutungi et al., 2019). Sun drying has also been reported to be the most widely used method of preservation of edible insects among rural areas (Kinyuru, Kenji, Njoroge, & Ayieko, 2009) probably due to its simplicity. Nonetheless, the shelf life of sun-dried insect products is short (7-14 days) as reported by the respondents probably due to microbial spoilage brought about by contamination and/or recontamination from environmental factors during unhygienic open sun drying (Braide et al., 2011). Lack of electricity at household level in such rural areas could be the main reason why refrigeration was not common among the respondents (Ayieko et al., 2010). The formation of objectionable secondary and tertiary lipid oxidation products like aldehydes, ketones and alkenals (Guillén & Cabo, 2002) could be the reason why the respondents could identify spoiled edible insects via smelling.

5.2 Effect of processing methods on the proximate composition of *H. illucens, A. domesticus, R. differens* and *S. littoralis*

Edible insects have been reported to be highly nutritious with their profiles being largely dependent on the edible insect species (Rumpold & Schlüter, 2013), age (Kipkoech et al., 2017), habitat, collection site and swarming seasons (Ssepuuya, Smets, Nakimbugwe, Van Der Borght, & Claes, 2019), sex (Kulma et al., 2019), diet (Chia et al., 2020; Ewald et al., 2020) and post-harvest handling techniques (Dobermann et al., 2019; Escamilla-Rosales et al., 2019). All these factors could probably be the reason

why all the raw and processed edible insects in this study had varied nutritional composition. The moisture content (MC) of raw edible insects in this study was comparable to 61.2%, 60.2% and 74.8% MC of black soldier fly larvae (*Hermetia illucens*), tebo worm larvae (*Chilecomadia moorei*) and an adult housefly (*Musca domestica*) respectively as reported by Finke, (2012). The MC of raw *A. domesticus* was lower than that of the same species reported elsewhere (Barker, Fitzpatrick, & Dierenfeld, 1998; Pennino, Dierenfeld, & Behler, 1991). Raw *R. differens* had MC comparable to 66-72% reported by Kinyuru, Kenji, Muhoho, & Ayieko, (2010) for the same species. The MC of raw *S. littoralis* was the highest among the insects in the study, which could be attributed to the oozing off of its gut content when dead, therefore more moisture loss through evaporation. The higher MC reported in boiled insect products could have been due to hydration and entrapment of water by hydrophilic tissue components, while the lower MC reported in toasted insect products could have been due to evaporative loss. A moisture gain was also reported in boiled crickets (*Gryllodes sigillatus*) by Vandeweyer et al. (2018). Kamau et al. (2018b) reported dissimilarities in hydration properties of *A. domesticus* and *H. illucens* powders and attributed those differences to insect's nutrient composition, which influences the number and strength of water binding sites. This could be a possible explanation as to why *S. littoralis* had the most deviation in hydration properties compared to all the other edible insect species.

Oven-dried insect products had lower MC possibly due to the higher oven temperature as opposed to the temperature in the solar dryer. The least MC and reduction rates reported in both *A. domesticus* and *R. differens* as opposed to *H. illucens* and *S. littoralis* could be attribute to the presence of a developed wax covered exoskeleton that protected *A. domesticus* and *R. differens* from moisture loss to the environment (Nelson, Tissot, Nelson, Fatland, & Gordon, 2001; Patel, Nelson, & Gibbs, 2001) compared to the undeveloped exoskeleton of *H. illucens* and *S. littoralis* as they are in their larval stages (Opara, Sanyigha, & Okoli, 2012). In rural set ups, drying is used to increase the shelf life of edible insects by reducing their water activity (Kinyuru et al., 2018), with a

maximum of 10% MC being reported to be sufficient in achieving prolonged keeping quality in foods. In fact, Kamau et al. (2018b) concluded that a shelf life of up to 7 months was achievable when insect products are dried to < 5% MC.

The reported crude protein (CP) of raw *H. illucens, A. domesticus* and *R. differens* was within ranges reported elsewhere by Makkar et al., (2014) (38-48%), Rumpold and Schlüter (2013) (55-70%) and Ssepuuya et al. (2019) (34.2-45.8%) for similar edible insects respectively. However, raw *S. littoralis* had 0.75 times lower CP compared to the value reported by Sayed et al., (2019) for the same species. This difference could be attributed to variations in diet as the caterpillars reported by Sayed et al. (2019) were reared on castor bean (*Ricinus communis*) leaves, while black nightshade leaves (*Solanum nigrum*) were used in rearing the *S. littoralis* in this study. The CP of all the raw insects in this study were however within the range of 15-60% reported for seventy eight edible insect species in Mexico by Ramos-Elorduy (1997). The insects also had higher CP compared to values reported for beef (23.2%), veal (24.8%) and mutton (21.5%) (Williams, 2007). Therefore, with these edible insects' reported CP (36-52g/100g), they can be good protein substitutes in human diet, capable of satisfying the recommended daily allowance of 0.8 g per kg body weight (Food and Nutrition Board, Institute of Medicine, 2002) and thus could help curb protein malnutrition especially in under developed countries (Whyte & Kariuki, 1991).

Processes such as boiling and toasting could lead to a significant loss in other proximate components, such as fat, hence concentrating other proximate nutrients (Bai et al., 2017). This could be a possible explanation for the CP increment realised when all edible insect samples in this study were boiled, toasted or dried. Similar findings were reported by Megido et al. (2018), who also reported an elevated CP in mealworms (*T. molitor*) after boiling them for 1 min. On the contrary, authors like Manditsera et al. (2019) reported a decrease in CP when crickets (*Henicus whellani*) and beetles (*Eulepida Mashona*) were boiled for 30-60 minutes, but observed no significant change when they were toasted. Similarly, Egan et al. (2014) reported a decrease in CP in traditionally processed (sun-dried post boiling in salty water) edible caterpillar

(*Hemijana variegata*) as opposed to oven drying only. Lautenschläger, Neinhuis, Kikongo, Henle, & Förster (2017) however reported no significant difference in CP after processing (boiled and boiled + sun-dried) edible caterpillar (*Imbrasia epimethea*). A decrease in CP could occur possibly due to dissolution of protein or disintegration and loss of tissue as colloidal constituents in the boiling water. Thermal processes have also been shown to lead to loss of amines and amides through the formation of complexes with nutrients or lipid oxidation products, which ultimately reduce the nitrogen content of a product (Bai et al., 2017; Lira, Barros Silva, Figueirêdo, & Bragagnolo, 2014). In this study however, such effects could have been overshadowed by other factors like the loss of dry matter components, particularly fat, which in fact had a strong correlation with CP increase. This was in line with findings of Womeni et al. (2012), who reported a concomitant increase in CP in defatted palm weevils (*R. phoenicis*).

Fat is essential in the human diet as it offers more energy in the form of calories than both carbohydrates and proteins. It also makes many foods palatable and retains fat soluble nutrients and flavours, as well as forms part of the structural component of cells and helps in their biological functions (Ekpo & Onigbinde, 2007). The crude fat (CF) of raw *H. illucens* and *A. domesticus* was within the ranges 15-35% and 9.8-22.8% reported by authors like Makkar et al. (2014) and Rumpold and Schlüter (2013) respectively. Raw *R. differens* and *S. littoralis* had CF values comparable to 31.4% CF reported for termites (*Macrotermis bellicosus*) (Sallau, Mada, & Biola, 2012) and 17.7% CF reported for mealworms (*T. molitor*) (Oonincx & Dierenfeld, 2011) respectively. However, their CF was about half the levels reported elsewhere for *R. differens* (42.2-54.3%) (Ssepuuya et al., 2019) and *S. littoralis* (33%) (Sayed et al., 2019). These differences could be attributed to variations in feed substrate. Furthermore, Ssepuuya et al. (2019) reported that for wildly harvested insects, geographical area and swarming season could influence the type and abundance of different vegetation, which could potentially be a source of nutritional variations even in similar insect species.

The fat loss realised in boiled insect products in this study could be attributed to melting of fat globules into the boiling water, while the fat loss reported in toasted insect products could also be attributed to melting of fat globules which were exuded due to tissue contraction with some fat probably being lost through thermal decomposition (Knothe & Dunn, 2009). The lower CF in oven and solar-dried insect products may suggest that some of the fat may have transuded together with water vapor or oxidized into other compounds (Akonor, Ofori, Dziedzoave, & Kortei, 2016; Giami, Adindu, Akusu, & Emelike, 2000). Edible insects have different fatty acid profiles with varied physico-chemical properties (Guil-Guerrero et al., 2018), hence a possible explanation as to why the edible insects in this study had variable fat loss magnitudes during processing.

Crude ash (CA) is an estimated measure of inorganic matter, hence giving an estimate of how much minerals is contained in food. All the edible insects in this study had CA within the range (3.6-9.1%) reported elsewhere (Rumpold & Schlüter, 2013). Raw *S. littoralis* had the highest CA among all the edible insect species, but was comparable to that of dried pallid emperor moth larvae (*Cirina forda*) (7.1%) as reported by Akinnawo and Ketiku (2000). The CA values reported for raw *A. domesticus* and *H. illucens* were lower by about 0.7 and 0.3-0.6 factors compared to findings reported by Barker et al. (1998) and Chia et al. (2020) for the same insect species respectively. However, the CA of raw *R. differens* was comparable to values reported elsewhere (Ssepuuya et al., 2019). Variations in ash content among edible insects has been shown to be strongly influenced by the feed substrate edible insects are fed on (Chia et al., 2020; Ssepuuya et al., 2019). The lower CA reported in all the boiled insect products could be attributed to leaching (Manditsera et al., 2019). The increase reported in toasted insect products could be attributed to mineral concentration due to fat loss (Bai et al., 2017), which was contrary to findings of Madibela, Seitiso, Thema, & Letso (2007), who attributed the CA increase in their study to contamination from ash during hot-ash roasting of Mophane worm (*I. belina*).

Crude fibre (CFR) measures the amount of chitin in insects. The CFR of all the raw edible insects in this study were higher than values reported for fourteen insect species by Banjo et al. (2006). The CFR of raw *H. illucens, R. differens* and *S. littoralis* compared well with values reported elsewhere for similar insect species (Kinyuru et al., 2010; Makkar et al., 2014; Sayed et al., 2019; Ssepuuya et al., 2019). However, raw *A. domesticus* had about half the CFR values reported elsewhere by Rumpold and Schlüter (2013). Part of the fibre fraction may be a representation of the insects' gut content as their diet is rich in complex carbohydrates, such as cellulose and lignin (Madibela et al., 2007) hence, variability in CFR among edible insects may arise depending on the preparation processes such as starving the insects prior to harvest, degutting and removal of insect parts like wings and appendages (Wynants et al., 2018). The decrease in CFR reported for boiled *H. illucens, A. domesticus* and *R. differens* was in agreement with findings of Madibela et al. (2007) for cooked and roasted Mophane worm (*I. belina*). This CFR decrease during boiling could probably be due to dissolution of complex carbohydrates (Kutoš, Golob, Kač, & Plestenjak, 2003) and their washing off from insects' tissue into the boiling water. The increase in CFR that was reported in toasted insect products could have been due to the concomitant loss of fat (Bai et al., 2017) as well as a shift in the soluble to insoluble fibre ratio during thermal processing, which is brought about by the formation of protein-fibre complexes (Dhingra, Michael, Rajput, & Patil, 2012). The CFR drop in toasted *S. littoralis* could be attributed to its oozing off of the gut content and its ease of sticking to the toasting pan, hence loss of complex carbohydrates. There was no clear trend in the available carbohydrate (AC) content in all the insect species in this study, probably because AC determination was dependent on the increase or decrease in the other proximate components such as fat, which responded differently to the different processing methods used.

5.3 Effect of processing methods on the fatty acid profile and fatty acid groups of edible insects oil

All the edible insect species studied had different fatty acid proportions, potentially due to confounding factors such as insect species, their developmental stage, diet, physiology and their ability to synthesize some fatty acids (Ewald et al., 2020; Lehtovaara et al., 2017; Mariod, Abdel-Wahab, & Ain, 2011; Starčević, Gavrilović, Gottstein, & Mašek, 2017). Fatty acids are divided into groups based on their chain length or their unsaturation. Based on unsaturation, fatty acids are divided into saturated fatty acids (SAFA) and unsaturated fatty acids (UFA), which are further divided to mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). Oil from raw *H. illucens* had a lower lauric acid amount compared to lauric acid amount of the same species (35.6-47.7%) reported by both Li et al. (2011) and Matthäus, Piofczyk, Katz, & Pudel (2019), which could be attributed to diet differences (Ewald et al., 2020). However, both raw *H. illucens* and *S. littoralis* oils in this study had more capric and lauric acids compared to both *A. domesticus* and *R. differens*. This could probably be because they were in their developmental stage and organisms have been shown to use SAFAs and MUFAs as energy sources during their growth and development (Tocher, 2003). This could also be the reason why raw *H. illucens* oil had more SAFAs and a low PUFA to SAFA (P/S) ratio. Raw *S. littoralis* oil had a high P/S ratio, which could be a contribution from its high α-linolenic acid content, which is common to some insect species belonging to the Lepidoptera family, as reported also by Akinnawo and Ketiku (2000) for Westwood (*Cirina forda*) (45.3%) and by Yapo, Amara, & Tuo (2017) for shea caterpillar (*Cirina butyspermii*) (23.9%).

Edible insects are rich in long chain fatty acids. All the raw edible insect oils in this study had palmitic acid as their predominant SAFA, with raw *A. domesticus* having a similar palmitic acid amount to that of a Jamaican field cricket (*Gryllus assimilis*) (Starčević et al., 2017) and raw *R. differens* having a similar palmitic acid amount to that of the same species as reported by Kinyuru et al. (2009). Raw *H. illucens* had a higher palmitic acid content compared to 14.8% palmitic acid of the same species reported by

Li et al. (2011). All the edible insects in this study, with the exception of *H. illucens*, had lower SAFAs compared to conventional meats such as pork (44.1%) and beef (52.0%) (DeFoliart, 1991). Oleic acid was the most predominant MUFA in all the insect species in this study, which is in line with findings of Kinyuru et al. (2009) for grasshoppers (*R. differens*), Assielou, Due, Koffi, & Kouame (2015) for a rhinoceros beetle (*Oryctes owariensis*) and those of Yang, Siriamornpun, & Li (2006) for edible insects in Thailand.

Linoleic acid was the most predominant PUFA in all the edible insect species in this study, with the exception of *S. littoralis*, whose predominant PUFA was α-linolenic acid. Blackford, Clarke, & Dinan (1997) also reported α-linolenic acid as the most predominant PUFA in *S. littoralis* on two different diets. Raw *A. domesticus* had a similar amount of linoleic acid as Jamaican field crickets (*G. assimilis*) (37.3-37.7%) that were reared on feed containing either sunflower or pumpkin seeds oil (Starčević et al., 2017). Raw *R. differens* had a similar amount of linoleic acid to that of the same species (29.5-31.2%) reported by Kinyuru et al. (2010). The presence of both oleic and linoleic acids (essential acids) and a low SAFA percentage of raw *A. domesticus*, *R. differens* and *S. littoralis* gives these insects a high nutritional value in relation to atheriosclerosis problems (Mann, 1993). EPA and DHA, which are also essential fatty acids, are synthesized to some extent by human bodies from their precursors (linoleic and linolenic acids) (Glick & Fischer, 2013), but they can be supplemented in the diet, as they play a key role in children's brain growth and development (Wainwright, 1992). All the insects in this study were found to have low levels (<1%) of both EPA and DHA, which is line with a study done by Starčević et al. (2017), who found little amount of both EPA (0-1.16%) and DHA (0-0.2%) in Jamaican field crickets (*G. assimilis*) reared on different feed blends. These low levels could be due to the fact that these insects are terrestrial organisms as EPA and DHA have been shown to be present in abundance in aquatic organisms (Fontaneto et al., 2011; Hixson, Sharma, Kainz, Wacker, & Arts, 2015).

Processes such as boiling and toasting facilitate lipid oxidation reactions especially in long chain unsaturated fatty acids, which lead to the concentration of short chain fatty acids (Choe & Min, 2006; Dobermann et al., 2019). This could be the reason why there were significant increases in capric, myristic, palmitic, stearic and arachidic acids in all edible insects in this study. Lauric acid increased significantly in all the insect species samples after toasting, which could be due to the higher temperature that was employed during toasting (150°C) compared to temperature during boiling (96°C). Oxidation of long chain fatty acids due to the presence of their unstable double bond (Choe & Min, 2006) could be the reason there was a significant decrease in MUFAs and PUFAs in all the edible insect species samples in this study. Toasting had the most pronounced UFA reduction effect, with linoleic acid having a sharp decrease in all the insect species in this study which was in line with findings of Dobermann et al. (2019) for black crickets (*Gryllus bimaculatus*), where they found a significant drop in linoleic acid compared to other UFA, when a temperature of 120°C was used in processing. There could have been presence of conjugated linoleic acid, which has been shown to oxidise at a considerably faster rate compared to other PUFAs such linolenic acid, arachidonic acid (Zhang & Chen, 1997), hence a possible explanation for the significant drop in linoleic acid when toasting was done.

Drying (solar and oven) had the least effect on the fatty acid profile of all the insect species in this study which may be attributable to the lower drying temperature, whose effect on lipid oxidation was not as pronounced, since lipid oxidation is temperature dependent (Choe & Min, 2006). Of the two drying processes, oven drying had the most effect on the fatty acid profile in all the insects in this study possibly due to the higher oven temperature compared to that of the solar dryer. Exposure of the boiled or toasted insect to more heat and time during drying had the greatest effect on the fatty acids, as there was an increase in all the SAFAs, with stearic acid having the highest increase in all the edible insect samples. This could be due to saturation of the 18 carbon chained UFAs (Choe & Min, 2006; Dobermann et al., 2019). Arachidonic, EPA and DHA acids were not detected in all the boiled + oven-dreid (B&OD) and toasted + oven-dried

(T&OD) edible insect samples in this study, a result in agreement with a study carried out by Fombong, Van Der Borght, & Broeck (2017).The authors reported <1% levels of both arachidonic and EPA for oven-dried post blanched grasshopper (*R. differens*).

Health index factors associated with fatty acids are the PUFA/SAFA (P/S) ratio and the omega-6/omega-3 (n-6/n-3) fatty acid ratio. The P/S ratio is widely used to indicate the cholesterol lowering potential of foods (Mann, 1993). With the exception of *H. illucens*, all the raw edible insect samples in this study had a P/S ratio greater than 0.8, hence they could be associated with low levels of cholesterol and a lower risk of coronary heart diseases upon prolonged consumption (Mann, 1993). Processing decreased the P/S ratio in all the insect species samples in this study due to the significant increases in their SAFA content. Even after processing, the P/S ratio of *A. domesticus*, *R. differens* and *S. littoralis* was higher than 0.3, which is desirable as a P/S of less than 0.3 has been shown to be associated with atherogenesis (Mann, 1993). Thus, their consumption could be associated with desirable health effects and could be used in the dietetic management of certain coronary heart conditions (Womeni et al., 2009).

Prolonged consumption of foods with high amounts of n-6 fatty acids and low amounts of n-3 fatty acids has been associated with higher risks of cardiovascular diseases, inflammatory and other immunological disorders (Russo, 2009; Simopoulos, 2008). With the exception of *H. illucens* and *S. littoralis* whose initial n-6/n-3 ratios were about 1:1, the initial n-6/n-3 ratio for *A. domesticus* and *R. differens* were within the range 4:1 and 20:1 reported elsewhere for similar insect species (Fombong et al., 2017; Ssepuuya et al., 2019; Starčević et al., 2017; Yang et al., 2006) and values reported for pork (10:1-12:1) (Cai et al., 2010). The ratios reported were however lower and higher than the n-6/n-3 values reported for chicken (15:1) (Žlender, Holcman, Stibilj, & Polak, 2000) and beef (2:1) (Muchenje et al., 2009) respectively. The increase in n-6/n-3 ratio of processed *A. domesticus* and *R. differens* could be due to the significant decrease in linoleic acid during processing, while the decrease in n-6/n-3 ratio of processed *S. littoralis* could be due to the lesser effect of processing on α-linolenic acid. There are varied recommendations for the appropriate n-6/n-3 ratio for in the diet. For instance,

FAO/WHO (1994) recommended a ratio of <5:1, while countries like Britain and Sweden recommend a ratio of 7:1 and 5:1 respectively. Canada on the other hand gives a range (4:1-10:1) as their recommendation for a balanced n-6/n-3 ratio in diets (Shils & Shike, 2006). Such variations in recommendations could possibly indicate some uncertainty in the appropriate n-6/n-3 ratio that could have the most positive impact on human health. Nonetheless, a low n-6/n-3 ratio has been associated with positive health outcomes (Simopoulos, 2008) and could be achieved in edible insects through mass rearing and diet manipulations as reported by (Starčević et al., 2017).

5.4 Effect of processing on the peroxide, iodine and saponification values of edible insects oil

Foods with high fat contents such as the insects in this study are susceptible to lipid oxidation and rancidification (Choe & Min, 2006). Insect fat from all the four insect species in this study were found to have different iodine values (IV), peroxide values (PV) and saponification values (SV). This could probably be because of differences in their fatty acids composition which are dependent on insect species, age and feed substrate (Kinyuru et al., 2013; Kipkoech et al., 2017; Lehtovaara et al., 2017). While SV measures the inverse mean molecular weight of fatty acids present in lipids, IV is used to reflect the quantity of double bonds that are present in lipids and their susceptibility to oxidation, and PV expresses the amount of peroxides present in lipids that results from lipid oxidation and could be an indicator of spoilage due to rancidification (Assielou et al., 2015; Atinafu & Bedemo, 2011).

Raw *A. domesticus* oil was found to have the highest IV among all the insect species in this study, which therefore implies that raw *A. domesticus* contains more double bonds in its fatty acids compared to the other insect species in this study (Assielou et al., 2015). All the raw edible insect sample oils in this study had higher IV than that of a rhinoceros beetle (*O. owariensis*) (105.3 g I_2/100g) as reported by Assielou et al. (2015). The IV of both raw *A. domesticus* and *S. littoralis* oils were higher than the IV of a palm weevil (*R. phoenicis*) (192.25 g I_2/100g) as reported by Elemo et al. (2011). Raw *H. illucens* oil had

a higher IV compared to the IV of the same species (96.0 g I_2/100g) as reported by Li et al. (2011). These differences may be attributable to variations in their diets (Ewald et al., 2020). The high IV of these insects in this study makes them more desirable for consumption as high fat unsaturation levels have been closely associated with less risk for certain coronary heart diseases (Mann, 1993).

A high level of unsaturation gives oil a high oxidative power (Atinafu & Bedemo, 2011; Choe & Min, 2006), which ultimately leads to a high PV and a poor resistance to oxidation during storage (Assielou et al., 2015). All the raw insect oils in this study had higher PVs than that of *H. illucens* (0.04 mEq O_2/Kg) reared on restaurant waste Zheng, Li, Zhang, & Yu (2012). However, their PV was lower compared to 7.59 mEq O_2/Kg and 17.59 mEq O_2/Kg of termites (*Macrotermis bellicosus* and *Coptotermes gestroi*) reported by Sallau et al. (2012) and Mathew et al. (2013) respectively. The PV of raw *A. domesticus* and *S. littoralis* oils were higher than the PV of olive oil (1.7 mEq O_2/Kg), palm oil (2.2 mEq O_2/Kg) and sunflower oil (2.07 mEq O_2/Kg) reported by Atinafu and Bedemo (2011).The PV of all the raw edible insect oils in this study was nevertheless in line with that of the Codex general standards for fats and oils (10 mEq O_2/Kg) implying that these edible insects have a high oxidative stability, a resistance to oxidation during processing and storage (Guillén & Cabo, 2002). The SV of all the edible insects in this study was higher compared to the SV of rhinoceros beetles (*O. owariensis*) (120.1 mg KOH/g) and termites (*M. bellicosus*) (122.3 mg KOH/g) reported by Assielou et al. (2015) and Sallau et al. (2012) respectively. Similarly, Ekpo, Onigbinde, & Asia (2009) reported lower SV (187.17-198.9 mg KOH/g) for four edible insect species consumed in Nigeria.

During boiling and toasting, the presence of polyunsaturated fatty acids like linoleic, α-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids gives the edible insects oil a high oxidative power (Guillén & Cabo, 2002). The increase in SV that was realised in all the edible insect samples in this study could probably be due to the presence of less unsaturated bonds and formation of short chain fatty acids due to oxidation. The increase in PV that was also observed in all edible insect species in this

study could be an indication of formation of peroxides due to the oxidation process. The decrease in IV that was observed in all the edible insects in this study may suggest the possibility of the breakdown of double bonds to single bonds during the oxidation process (Kamau et al., 2018a). Generally, boiling had a lesser impact on the IV, PV and SV in all the species studied compared to toasting. This could be attributable to factors like higher heat and free atmospheric oxygen during toasting, which have been shown to have a high impact on lipid oxidation (Choe & Min, 2006).

The duration with which the samples were dried coupled with the drying temperatures and presence of oxygen could have initiated the induction stage of oxidation (Choe & Min, 2006), as there was an observed elevation in PV and a decrease in IV for all the solar and oven-dried edible insect samples. There could have been formation of free radicals during boiling or toasting of the raw edible insect species, which could have further catalyzed lipid oxidation when the boiled/toasted insect samples were further exposed to more heat, oxygen and time during drying (Choe & Min, 2006; Velasco, Andersen, & Skibsted, 2004) This could be a possible explanation for the significant changes in PV, IV and SV in all the dried post boiled/toasted edible insect samples. The significant increase in SV that was observed in all the dried post boiled/toasted edible insect samples could have been due to formation of short chain fatty acids, as well as formation of other oxidation products such as aldehydes and ketones (Kamau et al., 2018a). Nonetheless, even after processing, all the edible insects samples were safe for consumption because their PVs were less than 20 mEq O_2/Kg. Oils with a PV of between 20 and 40mEq/Kg are considered rancid as there could be a presence of secondary and tertiary products such as aldehydes, ketones and alkenals that are formed from hydro-peroxides (Choe & Min, 2006; Guillén & Cabo, 2002).

5.5 Effect of processing on the cholesterol level of *H. illucens, A. domesticus, R. differens* and *S. littoralis*

One of the main dietary aspects considered in relation to the risk of cardiovascular diseases is the composition of lipid fraction of insects, in which cholesterol falls (Lee, Lim, Seol, Erwanto, & Lee, 2006). All the raw edible insects in this study had lower cholesterol values compared to the cholesterol value range of 36 to 66mg/100g reported for different breeds of beef, lamb, mutton and turkey (Lee et al., 2006; Muchenje et al., 2009; Williams, 2007). These insects can therefore be incorporated in diets, as a 100g serving could contain less cholesterol compared to the recommended maximum cholesterol intake of 300mg per day by the American heart association (Lichtenstein et al., 2006).

Many chemical reactions take place when foods containing high levels of lipids and proteins, including these edible insects, are processed or stored. These reactions can lead to the formation of cholesterol oxidation products which are formed by mechanisms similar to those of lipid oxidation (Lee et al., 2006). The reduced cholesterol level that was obtained in boiled and toasted insect samples could have been due to significant losses of fat globules through melting during boiling and through both melting and volatilization during toasting (Knothe & Dunn, 2009; Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997). The increase in cholesterol level that was realised in solar and oven-dried edible insect samples could possibly be due to moisture loss by evaporation, which led to the concentration of other nutritive components including fat (Lee et al., 2006). Oven drying had a profound reduction in the cholesterol level compared to solar drying, probably due to the higher oven temperatures which could have led to a significant fat loss due to melting, therefore lowering the cholesterol levels.

Drying (solar and oven) after boiling or toasting led to an increased cholesterol level in all the insect species studied compared to their boiled or toasted counterparts. This can be attributed to concentration of fat due to moisture loss during the drying processes (Lee et al., 2006). Toasted and dried edible insect samples had consistently lower

cholesterol level compared to their boiled and dried counterparts, which could be attributed to the initial fat loss due to melting and volatilization during toasting. The higher oven temperature could have also led to more fat loss due to melting, hence lowering the cholesterol level (Rodriguez-Estrada et al., 1997). Due to oxidative processes during insect processing, which is aggravated by the presence of heat and oxygen, there could have been non-enzymatic formation of cholesterol oxidation products in the processed edible insect samples. Such oxides like oxysterols have been shown to alter metabolic pathways, modify proteins and increase intercellular oxidative stress within the body (Kulig, Cwiklik, Jurkiewicz, Rog, & Vattulainen, 2016).

5.6 Effect of processing methods on the mineral content of *H. illucens, A. domesticus, R. differens* and *S. littoralis*

Minerals are essential within the human diet as they help in the development of the skeletal tissue, act as pre-cursors for different metabolic reactions and help in osmoregulation (Lall & Prasad, 1989). The mineral concentration of edible insects has been shown to be largely dependent on the insect species, age and feed substrate they are fed on (Chia et al., 2020; Kipkoech et al., 2017; Sprangers et al., 2017). These factors could be the reason why all the raw edible insect species in this study had varied mineral contents. *Hermetia illucens* had potassium (K) as the most abundant mineral, which was in agreement with findings of (Sprangers et al., 2017) for the same insect species bred on restaurant waste substrate. This was however contrary to findings of other researchers, who reported calcium (Ca) as the abundant mineral for *H. illucens* bred on different substrates such as chicken feed, vegetable waste and brewers wastes (Chia et al., 2020; Sprangers et al., 2017). Sodium (Na) and magnesium (Mg) were the second and third most abundant mineral in *H. illucens* in this study, which was contrary to findings of Chia et al. (2020), Sprangers et al. (2017) and Finke (2012) who reported the two minerals to be third, fourth or fifth in the mineral profile of *H. illucens*. Raw *A. domesticus* had K, phosphorus (P) and Na as the abundant minerals, which corroborates with findings of Finke (2002, 2015a) for *A. domesticus* adults and nymphs. The Ca content for raw *A. domesticus* was within the range of 36.6-40.7 mg/100g reported by

Finke, (2002, 2015a), but lower and higher than the value reported by Christensen et al. (2006) and Kipkoech et al. (2017) respectively.

Raw *R. differens* had K, Na and P as the most abundant minerals, which was in agreement with findings of Kinyuru et al. (2010) for the same insect species. The initial K and Na contents for *R. differens* were within the range reported for 25 edible grasshopper species by Ramos-Elorduy, Moreno, & Camacho (2012), while Ca and Mg values were lower by 0.2-0.6 factors and 0.03-0.08 factors respectively compared to values reported by the same authors probably due to differences in their wild vegetation diet (Ssepuuya et al., 2019). All the mineral values reported for raw *R. differens* were however higher than values reported for two grasshopper species (*Spathosternum prasiniferum* and *Chrotogonus trachypterus*) by Das and Mandal (2013). Phosphorus (P) and K values were also higher than values reported for grasshopper (*Ruspolia nitidula*) by Ssepuuya, Mukisa, & Nakimbugwe (2017). Sodium was lower, while Mg, K and Ca were higher compared to values reported for grasshopper (*Sphenarium purpurascens*) collected from alfalfa (*Medicago sativa*) and maize (*Zea mays*) fields (Ibarra-herrera et al., 2020). Values reported for raw *S. littoralis* for all the minerals were within ranges reported by Bukkens (1997) for a variety of other Lepidopterans. All the differences noted could possibly be attributed to differences in the insects' feed substrates (Chia et al., 2020; Sprangers et al., 2017). All the insects had considerable amounts of micro-nutrients like iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu), which was in agreement with findings reported elsewhere for similar insect species (Bukkens, 1997; Chia et al., 2020; Sprangers et al., 2017). Both *R. differens* and *S. littoralis* had higher mineral values probably because of the vast vegetation that the *R. differens* fed on in the wild (Ssepuuya et al., 2019) and the nightshade leaves that the *S. littoralis* were fed on, which have been reported to contain more mineral compared to kales that both *H. illucens* and *A. domesticus* were fed (Akubugwo, Obasi, & Ginika, 2007; Thavarajah et al., 2016; Waterland, Moon, Tou, Pena-yewtukhiw, & Park, 2017).

Processing affected the mineral composition differently in all the edible insect species in this study which could be related to the nutritional matrix of the individual insect species (Manditsera et al., 2019). For instance, minerals have been shown to interact with proteins, carbohydrates and anti-nutrients during processing, which decreases their quantity and bioavailabity (El Hassan, Hamed, Hassan, Eltayeb, & Babiker, 2008; Gharibzahedi & Jafari, 2017). Moreover, mineral loss or gain during processing has been reported to be dependent on the initial mineral quantity, the chemical form of the mineral and the mineral's distribution within the insect (Manditsera et al., 2019; da Silva et al., 2017). These factors might explain the variability in the increase or decrease in mineral content in all the processed edible insect samples.

The decrease realised in the mineral content in all the boiled and dried post boiled edible insect samples in this study was in agreement with findings of Manditsera et al. (2019). In fact, Manditsera et al. (2019) reported a 0.03-12.1% loss in iron and zinc of boiled beetles (*Eulepida mashona*) and crickets (*Henicus whellani*). This decrease in mineral content after boiling could be attributed to leaching of minerals into the boiling water (da Silva et al., 2017). The increase that was realised in the mineral content in all the toasted and dried post toasted edible insect samples in this study similarly corroborate findings of Manditsera et al. (2019) for roasted beetles (*E. mashona*) and crickets (*H. whellani*). El Hassan et al. (2008) also reported higher mineral content in fried tree locusts (*Anacridium melanorhodon*) as opposed to boiled ones. This increase could be attributed to concentration of minerals, due to loss of macronutrients like fat, during toasting. This was contrary to findings of Madibela et al. (2007), who implicated contamination from ash as the main reason hot-ashed mophane worms (*I. belina*) had higher mineral content. Solar and oven drying did not have a significant impact on most of the minerals in all the dried edible insect samples in this study, which was in agreement with findings for oven-dried mealworms (*T. molitor*) by Megido et al. (2018).

5.7 Influence of processing methods on the vitamins of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*

The vitamin content of edible insects has been shown to be dependent on the insect species, their ecotype, age, dietary habits, swarming seasons and collection sites (Banjo et al., 2006; Kinyuru et al., 2009; Ssepuuya et al., 2019). In addition, most edible insects are subjected to various post-harvest handling techniques like removal of body parts and subjection to various processing/cooking methods (Egan et al., 2014; Kinyuru et al., 2009), hence a partial representation of the total vitamins in live whole insects. These factors could be a possible explanation as to why all the edible insect samples in this study had varied proportions of both fat and water soluble vitamins. The retinol content of the raw edible insects in this study, with the exception of raw *R. differens* was lower than that of termites (*Macrotermes subhylanus*) (2.24 μg/g) reported by Kinyuru et al. (2009). The retinol content of raw *R. differens* was within the range reported elsewhere for the same insect species (Kinyuru et al., 2009, 2010). During digestion, retinol is converted to preformed vitamin A, which when absorbed in the body, supports proper vision, immune and inflammatory systems as well as act as an antioxidant (Maqbool, Aslam, Akbar, & Iqbal, 2018). Using the conversion factor 0.3 μg retinol = 1 IU (Barker et al., 1998), a 100g raw edible insect portion of *H. illucens* (95.1 IU), *A. domesticus* (614.4 IU), *R. differens* (837.8 IU) and *S. littoralis* (318.9 IU) could contribute significantly to the daily vitamin A requirements for infants, adult female and lactating females (1249 IU, 2664 IU and 4329 IU respectively) (Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989). Therefore these insects, especially *A. domesticus* and *R. differens* can be good sources of vitamin A to supplement the widely consumed plant food sources.

All the processes reduced the retinol content in all edible insect samples studied. Toasted *R. differens* and *A. domesticus* had retinol contents comparable to values reported for toasted termites (*M. subhylanus*) (1.56 μg/g) and grasshoppers (*R. differens*) (0.82-1.82 μg/g) (Kinyuru et al., 2009). The higher retinol destruction level reported in toasted edible insect samples compared to boiled insect samples in this study could be attributed

to the higher toasting temperature, as vitamin destruction has been shown to accelerate with increase in temperature (Riaz, Asif, & Ali, 2009). Solar drying had a profound reduction on retinol content in all the solar-dried insect samples compared to oven-dried insect samples in this study probably because of the longer duration with which the insects were dried in the solar dryer as opposed to the oven dryer. There was no detection of retinol in all the dried post boiled/toasted edible insect samples probably because of the prolonged time-temperature combination during the processes. In general, *R. differens* had higher retinol content among all the insect species studied, which could be attributed to the fact that they were wild, hence could store more retinoids from their wide range of feed sources (Mlcek, Rop, Borkovcova, & Bednarova, 2014).

All the raw edible insects in this study had higher α-tocopherol content than values reported for termites (*M. subhylanus*) by Kinyuru et al. (2009). However, the α-tocopherol content of raw *R. differens* was lower compared to that of the same species reported by the same author probably due to geographical, swarming seasons and collection site differences (Ssepuuya et al., 2019). The raw edible insects in this study had comparable α-tocopherol content compared to beef, veal, lamb and mutton (Williams, 2007). With the conversion factor: 1mg α-tocopherol = 1.49 IU (Barker et al., 1998), a 100g raw edible insect portion of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis* will have vitamin E content of 61.4 IU, 93.1 IU, 22.2 IU and 26.0 IU respectively. This could significantly contribute to the recommended vitamin E daily requirement for humans, which ranges from 50 IU to 100 IU (Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989). Adequacy of vitamin E in the body has been shown to prevent cell oxidative stress and oxidation of cholesterol, which leads to atherosclerosis (Maqbool et al., 2018)

According to Charlton and Ewing (2007), vitamin E is stable to heat and moisture, but highly unstable to oxidation, which could be accelerated by heat, ultraviolet light and some oxidizing agents like metals. This could be a possible explanation for the higher α-tocopherol reduction rate when raw, boiled or toasted insect samples were solar-dried compared to their oven-dried counterparts. The general decline in the α-tocopherol

content in toasted edible insects compared to their boiled counterparts could be attributed to the higher toasting temperature, as high processing temperatures have been shown to increase vitamin E destruction through oxidation (Riaz et al., 2009). The decline in α-tocopherol content in all the processed edible insect samples could have also occurred probably due to their interaction with peroxides that may have been produced during lipid oxidation (Narciso-Gaytán et al., 2010).

All the raw edible insects in this study had substantial quantities of the B-vitamins, which were comparable to if not higher than those of other edible insects reported elsewhere. For example, the thiamine content of the raw insects in this study was higher than that reported for soldier flies (*H. illucens*) (Finke, 2012), pallid-winged grasshoppers (*T. pallidipennis*) (Finke, 2015b), crickets (*A. domesticus*) (Finke, 2015a) and beef (Egan et al., 2014). A serving of 100g of all the raw edible insects in this study capable of supplying the daily requirement for thiamine for both pregnant (1.4mg) and lactating women (1.5mg), hence reducing prevalence of thiamine deficiency, which mainly occurs in infants who are breastfed by mothers who are thiamine deficient (Joint FAO/WHO Expert Consultation, 2004). Thiamine is an integral nutrient in the kreb cycle and the electron transport chain, hence its deficiency severely impairs energy metabolism within the body (Maqbool et al., 2018). The riboflavin content of the raw insects in this study was within the range reported for 14 edible insects by (Banjo, Lawal, & Songonuga, 2006). They however had higher riboflavin than that reported for soldier flies (*H. illucens*) (Finke, 2012), pallid-winged grasshoppers (*T. pallidipennis*) (Finke, 2015b), crickets (*A. domesticus*) (Finke, 2015a) and beef (Egan et al., 2014). Raw *R. differens* in this study had a higher riboflavin content than that reported by Kinyuru et al. (2009) for the same species, attributable to the possible differences in wild vegetation in their different geographical habitat, swarming season and collection site (Ssepuuya et al., 2019). Raw *H. illucens* and *S. littoralis* had comparable riboflavin content to that reported for superworms (*Zophobas mori*) by (Finke, 2015a). Riboflavin promotes iron metabolism within the body and its deficiency could lead to increased risk of anaemia (Maqbool et al., 2018). In addition, inadequate intake of riboflavin could

lead to hyporiboflavinosis which is mostly aggravated by poor food processing and storage. In developing countries, children commonly present clinical symptoms of riboflavin deficiency when gastrointestinal infections are most prevalent (Joint FAO/WHO Expert Consultation, 2004). A serving of 100g of all the raw edible insect species in this study can therefore supply in excess of the recommended daily intake (RDI) of riboflavin for children (0.5-0.9mg) without problems of toxicity as its intestinal absorption is limited (Joint FAO/WHO Expert Consultation, 2004).

The niacin content of the raw *R. differens* in this study was comparable to that reported for the same species by Kinyuru et al. (2009), but lower than that of pallid-winged grasshoppers (*T. pallidipennis*) reported by Finke (2015b). Raw *H. illucens* and *A. domesticus* in this study had lower and higher niacin compared to the values reported for similar insect species by Finke (2012) and (Finke, 2015a) respectively. The raw edible insects in this study could offer a portion of the RDI of niacin for adult females (14mg) and males (16mg) as reported by the Joint FAO/WHO Expert Consultation (2004), hence reducing the prevalence of diseases like pellagra. In addition, niacin containing enzymes act as anti-oxidants within the body hence preventing tissue oxidative stress (Maqbool et al., 2018). The pyridoxine content of all the raw edible insects in this study was higher than values reported of other edible insects elsewhere (Finke, 2012, 2015a, 2015b). However, raw *R. differens* and *S. littoralis* had comparable pyridoxine content to that of mealworms (*T. molitor*) (Finke, 2015a). Within the human body, pyridoxine is important in the production of red blood cells, metabolism of carbohydrates and nervous system health (Maqbool et al., 2018). Pyridoxine deficiency is not common, as it is usually associated with the deficiency in other B-complex vitamins. All the raw edible insects in this study are capable of contributing a portion of the pyridoxine RDI of adults (1.3-1.7mg) as reported by the Joint FAO/WHO Expert Consultation (2004). Raw *H. illucens* and *R. differens* had higher folic acid content compared to values reported of same insect species by Finke (2012) and Kinyuru et al. (2009) respectively. Raw *A. domesticus* and *S. littoralis* had a folic acid value comparable to that of crickets (*A. domesticus*) and superworms (*Zophobas mori*) respectively as reported by (Finke,

2015a). Inadequate intake of folic acid results in folate-deficiency anemia, while a deficiency in pregnant women can cause improper growth of the fetus's spinal cord and brain (neural tube defect). With a daily requirement of 0.52-0.6mg for pregnant women (Food and Nutrition Board, Institute of Medicine, 2002), a 100g serving of the insects in this study are capable of satisfying this RDI. The oozing off of the gut content exhibited in raw *S. littoralis* could have been the reason why this edible insect had the least amount of most of the B-vitamins compared to the other edible insects in this study.

Some B-vitamins are relatively unstable and are destroyed by heat, light or even oxygen (Charlton & Ewing, 2007; Riaz et al., 2009), therefore a possible explanation for the loss of the B-vitamins in this study upon processing. The loss reported for B-vitamins like thiamine, niacin, pyridoxine and folic acid in all the boiled edible insect samples compared to toasted edible insect samples could be attributed to the sensitivity nature of these vitamins to moist heat, together with leaching off of the vitamins to the boiling water (Alajaji & El-Adawy, 2006). In addition, the vitamin loss could have been brought about by enzymatic reactions or presence of trace metal ions which catalyze the vitamins' degradation process (Rojas & Gerschenson, 1997b, 1997a). Riboflavin, which is relatively heat stable, was reduced more during toasting compared to boiling in all the edible insect samples. This could probably be due to the higher toasting temperature which could have led to its degradation. When the raw edible insects in this study were dried in a solar dryer, there was a greater loss in all the B-vitamins compared to when subjected to oven drying. This greater loss could be due to the vitamins' sensitivity towards ultraviolet rays in addition to the temperature (Charlton & Ewing, 2007), that the insects were exposed to in a solar dryer. This could also be a possible explanation as to why the solar-dried post boiled/toasted edible insect samples had the least B-vitamins than their oven-dried post boiled/toasted counterparts. In addition, the increase in the time-temperature combination could have been the reason why dried post boiled/toasted edible insect samples had less B-vitamin compared to their raw dried counterparts. These findings corroborate Kinyuru et al. (2009) findings, where they reported more

vitamin loss in toasted solar-dried termites (*M. subhylanus*) and grasshoppers (*R. differens*) compared to their raw solar-dried counterparts.

There is limited information on the ascorbic acid (vitamin C) content of wild insects possibly because most insects are generally not an efficient source of vitamin C (Kouřimská & Adámková, 2016) compared to its major sources (fruits and vegetables). In addition, vitamin C is very labile and its loss in food is influenced by post-harvest techniques, duration of cooking, storage and the pH of the cooking water (Charlton & Ewing, 2007). Inadequate intake of vitamin C can lead to a disease called scurvy, which can be reversed by its adequate intake (Joint FAO/WHO Expert Consultation, 2004). The vitamin C content in all the raw edible insects in this study was within the range reported elsewhere for 14 edible insect species by Banjo et al. (2006). These values could have been boosted by vitamin C content of the edible insects' gut contents (Egan et al., 2014). Raw *R. differens* had a higher vitamin C content than that reported of the same species by Kinyuru et al. (2009) probably because wild insects have been found to contain varied quantities of nutrients, depending on the season they were harvested (Kouřimská & Adámková, 2016; Ssepuuya et al., 2019). The higher reduction of vitamin C during boiling of the edible insects in this study, compared to toasting, could have probably been due to leaching of the vitamin into the boiling water combined with its chemical destruction, as it is heat sensitive (Alajaji & El-Adawy, 2006). Ascorbic acid is highly susceptible to light (especially ultraviolet) (Charlton & Ewing, 2007) and this could be a possible explanation as to why solar-dried edible insect samples had less vitamin C compared to oven-dried edible insect samples. The increase in the time-temperature combination during combined processing of the edible insects in this study could have been the reason dried post boiled/toasted edible insect samples had the least amount of vitamin C compared to their boiled/toasted counterparts. Losses in vitamin C in other foods due to drying have also been reported by Mziray, Imungi, & Jaruri (2000) and by Negi and Roy (2001).

5.8 Effect of processing techniques on the amino acid composition of *H. illucens*, *A. domesticus*, *R. differens* and *S. littoralis*

Authors like Finke (2007) suggest that amino acids contribute the highest proportion of insect nitrogen. Therefore, specific amino acid quantities determine the protein quality of a food. Variations in the amino acid profile of edible insects has been shown to be dependent on insect species, diets and age (Bednářová, Borkovcová, & Komprda, 2013; Finke, 2002, 2007, 2015). This could be the reason the raw edible insects in this study had varied proportions of amino acids. In addition, Finke (2007) observed that proteins present in the exoskeleton of insects could elevate the amino acid content in adult insects compared to their larval stages. This could be a possible explanation as to why raw adult *A. domesticus* and *R. differens* had higher amino acid quantities in most of the amino acids quantified than in raw *H. illucens* pre-pupae and *S. littoralis* instar. All the raw edible insect samples had lysine (Lys) and histidine (His) as the limiting amino acids which was contrary to findings of other researchers, who reported methionine (Met) and cystein (Cys) to be the limiting factors in other edible insects including *A. domesticus* (Finke, 2007, 2015a), *R. differens* (Finke, 2015b; Siulapwa, Mwambungu, Lungu, & Sichilima, 2014), *H. illucens* (Finke, 2012) and different edible worms (Finke, 2015a). Raw *A. domesticus* had a similar amount of valine (Val) and isoleucine (Ile) as values reported for the same species by Yi et al. (2013). The value reported for glutamic acid (Glu) was similar to values reported for the same species by Finke (2007, 2015a) but lower by 0.16 factors than the value reported by Yi et al. (2013). It also had a similar amount of Met as a value reported by Zielińska, Baraniak, Karaś, Rybczyńska, & Jakubczyk (2015) for crickets (*G. sigillatus*) but higher than values reported by Finke (2007, 2015a). All the amino acids that were quantified in raw *R. differens* were higher compared to values reported for edible grasshoppers (*R. differens* and *T. pallidipennis*) by Siulapwa et al. (2014) and Finke (2015b). Considering the recommended amino acid requirement for human nutrition (Joint WHO/FAO/UNU Expert Consultation, 2007), raw *A. domesticus* and *R. differens* had 1.1-1.32 times higher Val, but had 0.85-0.87 times and 0.51-0.8 times lower Leu and Phe respectively. Raw *A. domesticus* had higher

(about 1 factor), while raw *R. differens* had lower (0.56-0.88 factors) amounts of Ile and Met compared to their recommended values reported by the Joint WHO/FAO/UNU Expert Consultation (2007).

Raw *S. littoralis* had similar values for amino acids like valine (Val), tyrosine (Tyr) and leucine (Leu) to those reported for white-lined Sphinx moths (*Hyles lineata*) by Finke (2015b). In addition, the sum of Phe and Tyr (56.6) was about half the value reported by Yi et al. (2013) for mealworms (*T. molitor*), but about three times higher than values reported for three edible worms (*T. molitor*, *Zophobas mori* and *Galleria mellonela*) by Finke (2015a). The arginine (Arg) value reported for raw *H. illucens* was similar to that of the same species reported by Finke (2012). However, all the other amino acids that were quantified for raw *H. illucens* were higher than values reported by the same author. Raw *S. littoralis* and *H. illucens* had lower Val (0.36-0.73 factors), Met (0.85-0.87 factors), Leu (0.33-0.52 factors) and Ile (0-0.75 factors) compared to values recommended by the Joint WHO/FAO/UNU Expert Consultation (2007) for human nutrition. These two edible insects however had 1.38-1.44 times higher Phe compared to the recommended value of 30mg/g (Joint WHO/FAO/UNU Expert Consultation, 2007). Thermal processes such as boiling have been shown to lead to disintegration of protein or loss of tissue into the boiling water. In addition, there could be formation of complexes between proteins and other nutritive or non-nutritive compounds like primary and secondary lipid oxidation products, hence leading to loss of amides and amines (Bai et al., 2017; Lira et al., 2014; Manditsera et al., 2019). These factors could possibly be the reason all the dried post boiled edible insect samples had lower protein quality in terms of amino acids content. All the dried post boiled edible insect samples also had low totals of essential amino acids (0.09-0.25 factors) compared to the recommended value of 263 mg/g reported by Joint WHO/FAO/UNU Expert Consultation (2007).

Most of the staple foods in the world are cereal based, which are mostly deficient in one or more essential amino acids. Some insect species contain these lacking amino acids in good quantities (Bukkens, 2005), therefore could complement the staple foods. However, such recommendations regarding edible insects would entirely depend on the

commonly available staple foods in a given region, the locally available edible insects and their traditional use in diets. For instance, people in the Democratic Republic of Congo (DRC) complement their lysine-poor staple food with lysine-rich insects like the palm weevil (*R. phoenicis*) and other aquatic insects. Similarly, in Papua New Guinea, the palm weevil (*R. phoenicis*) often complements the leucine-poor tubers that are often consumed in the region (Bukkens, 2005). In countries like Kenya, Angola, Zimbabwe and Nigeria, where maize is a staple food, there are cases of tryptophan and lysine deficiencies especially in children. Edible insects like termites (*M. bellicosus*) can be incorporated in the diets in an attempt to alleviate such deficiencies, as they are readily acceptable in these communities (van Huis et al., 2013).

5.9 Microbiological quality of raw and processed *H. illucens, A. domesticus, R. differens* and *S. littoralis*

5.9.1 Microbiological quality of raw H. illucens, A. domesticus, R. differens and S. littoralis

The rich nutritional profile of edible insects provides a satisfactory environment for the survival and growth of many micro-organisms (Klunder et al., 2012). In addition to their nutrition, their habitats could also lead to their infection with micro-organisms (bacteria, yeasts and moulds) and could potentially cause foodborne diseases (Belluco et al., 2013; Imathiu, 2020). Contamination could also occur during post-harvest handling and processing (Braide et al., 2011; Mujuru, Kwiri, Nyambi, Winini, & Moyo, 2014). Although yeasts are not known to cause food poisoning, some mould strains are capable of producing mycotoxins, whose ingestion may be detrimental to human health (Mpuchane et al., 2000). All raw edible insect species in this study had total viable count (TVC) comparable to those reported elsewhere (Klunder et al., 2012; Vandeweyer et al., 2018; Wynants et al., 2018). Similarly, all the raw edible insect species had yeasts and moulds count (YMC) comparable to values reported by Vandeweyer et al. (2018) for industrially reared crickets (*G. sigillatus*). With the exception of raw *S. littoralis*, all the other raw edible insects in this study were not safe for consumption before processing,

since their TVC was higher than the maximum allowable TVC limit (7 Log cfu/g) of raw ready-to-eat foods (International Commission for Microbiological Specifications for Foods (ICMSF), 1998; Stannard, 1997). However, the YMC of raw *S. littoralis* exceeded the maximum recommended limit of 6 Log cfu/g for raw ready-to-eat foods (ICMSF, 1998; Stannard, 1997), hence the raw insect was not also fit for consumption.

Fresh food harvested from soil or having been in contact with soil material have been shown to have bacteria of the *Enterobacteriaceae* family (Klunder et al., 2012) and this was consistent with the results reported for all the raw edible insects in this study. Contamination with soil material could have possibly occurred through the insects feed (brewers waste or kitchen waste) and in the case of *R. differens*, its wild habitat. All the raw edible insects had higher *Enterobacteriaceae* counts than values reported for raw crickets (*A. domesticus*) *(*4.2-4.4 Log cfu/g) by (Klunder et al., 2012). Raw *S. littoralis* had lower *Enterobacteriaceae* count by about 1.5-2 log cycles than values reported for mealworm larvae (*T. moilitor*) by Klunder et al. (2012) and Vandeweyer et al. (2017), while raw *R. differens* had higher *Enterobacteriaceae* count by about 1.5 log cycles than values reported for mealworm larvae (*T. moilitor*) and crickets (*G. sigillatus*) (Vandeweyer et al., 2017, 2018). The *Enterobacteriaceae* counts reported for raw *H. illucens* and *A. domesticus* were however comparable to values reported elsewhere (Vandeweyer et al., 2017, 2018). Nonetheless, the allowable *Enterobacteriaceae* limit of 3 Log cfu/g (European Union, 2005) was exceeded by all the raw edible insect samples in this study, hence necessitating processing before their consumption.

Presence of indicator micro-organisms such as faecal coliforms and Lac+ bacteria in a food product is undesirable as it may indicate faecal contamination from soil, poor environmental hygiene and poor handling conditions (Belluco et al., 2013). Contamination from soil through the insects feed could possibly be the reason raw *H. illucens* and *A. domesticus* tested positive for these indicator micro-organisms, while in the case of raw *R. differens*, its habitat and handling conditions during harvesting could have been the source of contamination with faecal coliforms and Lac+ bacteria. The lowest count of indicator micro-organisms reported in raw *S. littoralis* in

this study could probably be attributable to the near absence of their gut matter, as they ooze it off when dead and hygienic conditions of rearing as there was limited or no contact with soil material from its feed (nightshade leaves).

All the raw edible insects in this study had higher *St. aureus* counts than the recommended limit of 4 Log cfu/g (Stannard, 1997). Such high counts could cause staphylococcal food poisoning (Hennekinne, De Buyser, & Dragacci, 2012). The natural habitat and the wild manual collection (Le Loir, Baron, & Gautier, 2003) involved in harvesting *R. differens* could have been the causes for their significantly higher *St. aureus* count compared to the other raw edible insect species in this study. The presence of pathogenic bacteria such as *Salmonella* spp in food is undesirable as it could cause illnesses such as salmonellosis and typhoid (D'Aoust & Maurer, 2007). All the raw insect samples tested positive for *Salmonella* spp, hence rendering all these raw edible insects unfit for consumption, as microbiological guidelines for ready-to-eat food require no detection of *Salmonella* in a 25g food sample (Kukier, Goldsztejn, Grenda, Kwiatek, & Bocian, 2013; Stannard, 1997). With the high microbial level in all the raw edible insects in this study, it should be noted that in general, insects themselves have been shown to naturally harbor dangerous pathogens intrinsically, which could be harmful for humans (Veldkamp et al., 2012).

5.9.2 Microbiological quality of processed H. illucens, A. domesticus, R. differens and S. littoralis

Insects cannot be fully excluded as carriers of pathogenic micro-organisms to humans and therefore Giaccone (2005) recommended cooking or pasteurization as risk minimizing steps. When all the edible insect types in this study were boiled and toasted at 96°C and 150°C respectively, most of the micro-organisms enumerated, including YMC, *Enterobacteriaceae*, faecal coliforms, Lac+ bacteria and *Salmonella* spp, were completely eliminated. This corroborates findings reported for boiled crickets (*G. sigillatus* and *A. domesticus*) (Klunder et al., 2012; Vandeweyer et al., 2018) boiled emperor moth (*I. belina*) (Gashe, Mpuchane, Siame, Allotey, & Teferra, 1997) and

toasted *R. differens* (Ng'ang'a et al., 2019). On the contrary, Klunder et al. (2012) noticed that there was no complete elimination of *Enterobacteriaceae* upon roasting of mealworm larvae (*T. molitor*) and crickets (*A. domesticus*). The TVC and *St. aureus* counts of all processed edible insects in this study were also reduced to safe ingestion levels, with regards to their respective recommended limits (ICMSF, 1996; Stannard, 1997). Klunder et al. (2012) and Ng'ang'a et al. (2019) also observed a TVC reduction in *A. domesticus* and *R. differens* after processing. The greater TVC reduction that was realised in boiled edible insect samples compared to toasted edible insect samples could have been due to better heat transfer through the insect tissues during boiling, compared to penetration of dry heat during toasting (Klunder et al., 2012). Similar to findings of this study, Mujuru et al. (2014) also observed partial elimination of *St. aureus* when mopani worms (*G. belina*) were drum roasted. Recontamination with *St. aureus* could have possibly occurred from the environment or contact during subsequent handling (Reij & Den Aantrekker, 2004).

Drying reduces the water activity necessary for microbial growth. Solar drying lowered the microbial counts in all the samples undergoing this treatment, although they did not meet their respective recommended limits (European Union, 2005; ICMSF, 1996; Stannard, 1997). However, the higher TVC levels that were reported in raw solar-dried insect products could suggest the presence of additional bacterial species, which could have been brought about by contamination from environmental factors and predators like ants. Conversely, oven drying led to attainment of allowable limits of most of the micro-organisms in all the samples undergoing this treatment probably due to the temperature difference in the two dryers. However, all the oven-dried edible insect samples were still unfit for consumption as the requirements for both *Enterobacteriaceae* and *Salmonella* spp were not met (European Union, 2005; Kukier et al., 2013). The effect of drying raw edible insects on their microbial quality has not been vastly documented in literature, however strains of moulds that could potentially produce mycotoxins were isolated in dried insect products by Mpuchane et al. (2000) and Braide et al. (2011). On the other hand, authors like Shigehisa, Nakagami, Taji, &

Sakaguchi (1985) reported that micro-organisms such as *E. coli*, *St. aureus* and *Salmonella* could be destroyed in beef chops when oven heated at a temperature close to 60°C. Therefore, the presence of *Salmonella* in all the oven-dried insect samples could probably indicate a rather resistant nature of the pathogen to dry heat (Shigehisa et al., 1985) or potential recontamination.

The TVC of all the solar-dried edible insect samples was higher than their respective initial counts. A similar trend was reported in the TVC and *St. aureus* counts on solar-dried post boiled/toasted edible insect samples compared to their boiled or toasted counterparts suggesting possible recontamination during solar drying. All these trends corroborate findings of Mujuru et al. (2014) who also reported a high TVC, and a partial elimination of *St. aureus*, when boiled mopani worms (*G. belina*) were dried in a solar dryer. The complete elimination of the other micro-organisms in dried post boiled/toasted edible insect samples are in line with findings of Gashe et al. (1997) who also reported absence of faecal coliforms, *E. coli* and *Salmonella* in boiled and dried emperor moth (*I. belina*). On the contrary, authors like Ali, Mohamadou, Saidou, Aoudou, & Tchiegang (2010) and Braide et al. (2011) reported presence of faecal coliforms, *E. coli*, *Salmonella* and fungi in thermally processed and sun-dried edible insects, a phenomena which they attributed to recontamination due to the unhygienic open sun drying processing and handling. Nonetheless, all the combined processes (solar and oven drying post boiling or toasting) maintained the microbiological quality of the boiled/toasted edible insect samples.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This research provides an overview of the commonly consumed insects and processing techniques used within the western region of Kenya. It also provides an overview of the nutritional and microbiological profile of raw and processed black soldier fly (*H. illucens*) pre-pupae, adult house cricket (*A. domesticus*), adult grasshopper (*R. differens*) and cotton leaf worm (*S. littoralis*) 5^{th} instar. Among the Luo and the Luhya communities, termites were considered a delicacy. The Luo people preferred consuming lakeflies to dung beetle larvae, while the Luhya people preferred the latter to the former. Most of the edible insects consumed were obtained from the wild through hand picking or use of traps. The majority of edible insect post-harvest handlers were found to be women, who used preparation methods such as toasting, boiling and frying to make delicacies. Sun drying was the common method for increasing edible insect shelf life, although spoilage rate was reported to be high (7-14 days). With the presence of initiatives spearheaded by higher learning institutions and research centres, local communities are able to access information through capacity building and training, regarding the use of edible insects in increasing their food security and economic wellbeing.

All the edible insect samples in this study were found to contain significant proportions of proteins, fat and other nutrients, which compared well with conventional animal protein. The protein quality of all the raw edible insects in this study was found to be high as they contained some essential amino acids, hence could complement the staple cereal-based foods that have limited essential amino acids. All the raw edible insects in this study had good quality fat in terms of fatty acids as they contained substantial proportions of desirable unsaturated fatty acids like omega 3 and omega 6 fatty acids. They also had lower cholesterol values compared to conventional animal protein. All the

raw edible insects in this study were generally found to be a valuable source of minerals with earlier reports suggesting that such minerals are highly bioavailable (Latunde-Dada, Yang, & Vera Aviles, 2016; Manditsera et al., 2019). This research also showed that the consumption of 100g of these edible insects could contribute significantly to the daily recommended intake requirement of nutrients such as proteins, minerals and vitamins.

Processing methods altered the nutritional composition of all the edible insects in this study. The nutritional composition of the final processed edible insects was found to be a function of the interaction of the processing method used and the insect species. In comparison to the raw edible insects, solar and oven drying outperformed the other processes, as they showed minimal rates of change in the nutrient composition of all the edible insects in this study, as well as the chemical stability parameters (peroxide, iodine and saponification values) determined. Either drying method could also be used to enhance edible insects' shelf life as they had the lowest peroxide values among all the other processes. Processes like boiling and toasting decreased the cholesterol level of the edible insects in this study but, the risk of ingesting cholesterol oxidation products, which can interfere with metabolic processes was also noted. Boiling was found to have a negative impact on minerals compared to toasting, while toasting was found to have a negative impact on the fatty acid profile of all the edible insects in this study compared to boiling. Combined processes (drying post boiling/toasting) were found to have the most negative impact on vitamins among all the other processes in this study.

All the raw edible insects in this study were found to have higher spoilage and pathogenic microbial loads compared to their respective ingestion limit requirements. Although drying is used to increase keeping quality, the microbial quality of the dried edible in this study was wanting, hence a conclusion that a heating process was necessary before ingestion. This research showed the importance of processing edible insects prior to consumption using actionable hazard control mechanisms like boiling and toasting. These two processes sufficiently reduced microbial load hence yielding safe insect products that met microbial ingestion limit standards. Combined processes

were found to be the most effective in increasing the microbial quality of all the edible insects in this study.

6.2 Recommendations

In light of the findings of this study, the following recommendations were made:

1. There is need for more initiatives geared towards empowerment and training for local communities so as to increase/encourage entomophagy, hence paving way for adoption of modern insect rearing methods.
2. Nutritional requirements of the final consumers need to be factored before recommending a processing method for edible insects. For instance, to boost the mineral intake through the consumption of the edible insects in this study, toasting would be the recommended option, as it increased mineral concentration. On the other hand, to increase the intake of healthy unsaturated fatty acids through consumption of the edible insects in this study, boiling would be the recommended option, as it preserved most of the essential fatty acids, particularly linoleic acid.
3. Combined processing (boiling/toasting + drying) could be recommended in improving the microbial quality of all the edible insects in this study while potentially lowering the edible insects' water activity.

Future research should consider:

1. Evaluating the post-harvest characteristics of other edible insects consumed globally together with assessment of modern processing methods like freeze drying, extrusion, microwaving and others as alternative ways of edible insect processing.
2. Evaluating the full potential in the development of insect-based nutraceutical foods, so as to take advantage of the edible insects' nutritional profiles in curbing malnutrition cases.

3. Assessing functional properties like antimicrobial and antioxidant capacity of edible insects. On the other hand, the anti-nutrient capacity should also be evaluated.
4. Evaluating microbial and chemical contaminants (spore forming bacteria, microbial spores, mycotoxins like aflatoxins and heavy metals) present in edible insects, along with how they are impacted by different post-harvest processing methods.
5. Assessing preservation techniques necessary for edible insects in terms of types of packages and preservation conditions, so as to increase the longevity of this otherwise seasonal food type.
6. Evaluating the substrate effects on nutrient level of edible insects.

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APPENDICES

Appendix I: questionnaire

The following questionnaire was used as a guide to determine the commonly consumed edible insects together with the post-harvest handling practices within the western region of Kenya.

Insects as food

1. Do you/your family consume edible insects?
2. Which are the commonly consumed edible insects in this region?
3. In which time of the year are the edible insects abundant?
4. How do you acquire the edible insects?
5. What are the common collection methods used to harvest the edible insects?
6. Who is most likely to handle the edible insects?
7. What are some of the post-harvest handling techniques used on edible insects prior to consumption?
8. What are the common methods of preservation used on the edible insects?
9. What is the average period for storing edible insects?
10. Are you able to tell when the edible insects have/are going bad?

Insects as feed

1. Do you have any domestic animals?
2. Do you use any of the edible insects as feed?
3. How are the edible insects for feed handled/used?
4. Do you buy any edible insects for feed purposes?