WILD HARVESTED EDIBLE INSECTS

Potential For Nutrition Security

Faith A Manditsera

Propositions

1. High nutritional content of edible insects is no guarantee for nutrition security.
   (this thesis)
2. Processing is a tool to modulate edible insects' nutritional quality.
   (this thesis)
3. Household power dynamics can be the cause of malnutrition.
4. Climate coping strategies by communities can improve household dietary diversity.
5. Publishing in a high impact journal is meaningless if the research outcomes are not accessible to the public.
6. Post-harvest management is key to biofortified crops in mitigating micronutrient deficiencies.
7. Starting a PhD is like embarking on a long international flight.

Propositions belonging to the thesis, entitled:

Wild harvested edible insects: potential for nutrition security

Faith Angeline Manditsera
Wageningen, 10 September 2019

Wild Harvested Edible Insects

Potential for Nutrition Security

Faith A. Manditsera

Thesis committee

Promotor
Prof. Dr V. Fogliano
Professor of Food Quality and Design
Wageningen University & Research

Co-promoters
Dr C.M.M. Lakemond
Assistant professor, Food Quality and Design
Wageningen University & Research

Dr P.A. Luning
Associate professor, Food Quality and Design
Wageningen University & Research

Other members
Prof. Dr J.J.A. van Loon, Wageningen University & Research
Dr A. Melse-Boonstra, Wageningen University & Research
Dr L. Gasco, University of Turin, Italy
Dr J.P. Schlebusch, Mars, Petcare, Verden, Germany

This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

Wild Harvested Edible Insects

Potential for nutrition security

Faith A. Manditsera

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 10 September 2019
at 1:30 p.m in the Aula.

Faith A. Manditsera
Wild harvested edible insects: potential for nutrition security, 129 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2019)
With references, with summary in English

ISBN: 978-94-6395-048-0
DOI: https://doi.org/10.18174/496746

TABLE OF CONTENTS

CHAPTER 1

General Introduction

1.1 Food and nutrition security and potential underutilised natural resources

The world's growing population necessitates the need for alternative food sources that are sustainable and able to meet the world's demands for food and nutrition security. In 2017, the United Nations expected the current world population of 7.6 billion to reach 8.6 billion in 2030 and 9.8 billion in 2050. In addition, food and nutrition insecurity problems remain a challenge especially in developing countries, despite these countries have vast of underutilised resources that can be used to alleviate the problem. An estimate of 821 million people are malnourished worldwide, with the majority in Africa, according to (FAO et al., 2018), with iron, zinc, and vitamin A deficiencies among the most common form of undernutrition. Food and nutrition security will thus be a great challenge.

FAO (2009) defined *food security* as 'when all people, at all times, have physical, social, and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life'. They defined *nutrition security* as the need to secure access to an appropriately nutritious diet, comprising all essential nutrients and water, coupled with a sanitary environment and adequate health services and care to ensure a healthy and active life for all household members (FAO 2012). Since food security cannot be achieved without nutrition security (Hwalla et al., 2016), it is of paramount importance to find solutions for both food and nutrition security.

Many proposed interventions towards improving food and nutrition security are mainly based on increased crop production and reduction of postharvest losses (Chegere, 2018; Kumar & Kalita, 2017; McNamara & Tata, 2015), whereas promoting use of edible non-wood forest products is also an option (Vinceti et al., 2013). FAO defined non-wood forest products (NWFPs) as "all goods of biological origin other than wood in all its forms, as well as services derived from forest or any land under similar use (FAO, 1995). Several studies have reported on NWFPs' direct and indirect contribution in improving food and nutrition security. For example, NWFPs can provide ready accessible, affordable, and, high nutritious foods (Vinceti et al., 2013), especially in lean seasons or times of low agricultural production. In addition, NWFPs can generate income to purchase other food items. Forest foods (NWFPs used as food) are of plant origin (e.g. fruits, seeds, nectars, tubers, and mushroom) or animal origin (e.g. bush meat, insects, and fish).

Edible insects are amongst the underutilised natural resources that can help in reducing problems of food security and malnutrition. Studies have reported that insects can contribute significantly to food security and livelihood in several transition countries, where they are consumed (Dube et al., 2013; Vantomme et al., 2012). FAO also reported that nutrients found in insects have a potential to reduce nutrient deficiencies in the population consuming them. In developing countries with food insecurity problems, edible insects can contribute to malnutrition reduction because of the high protein and micronutrient content, minimal ecological impact, availability, and above all the cultural

appropriateness for a large majority of the population (FAO 2012). As such, Kelemu et al. (2015) proposed a model (Figure 1.1) on the pathways to using edible insects to improve food security in Africa. The model emphasises on the multi-stakeholders (e.g. research institutions, private sector policy makers, etc.) approach to achieve the goal of attaining food security. They highlighted the importance of bottom up approach to improve food security in terms of quality and quantity of edible insects, through building up on existing knowledge to upscale mass rearing and processing of insects.

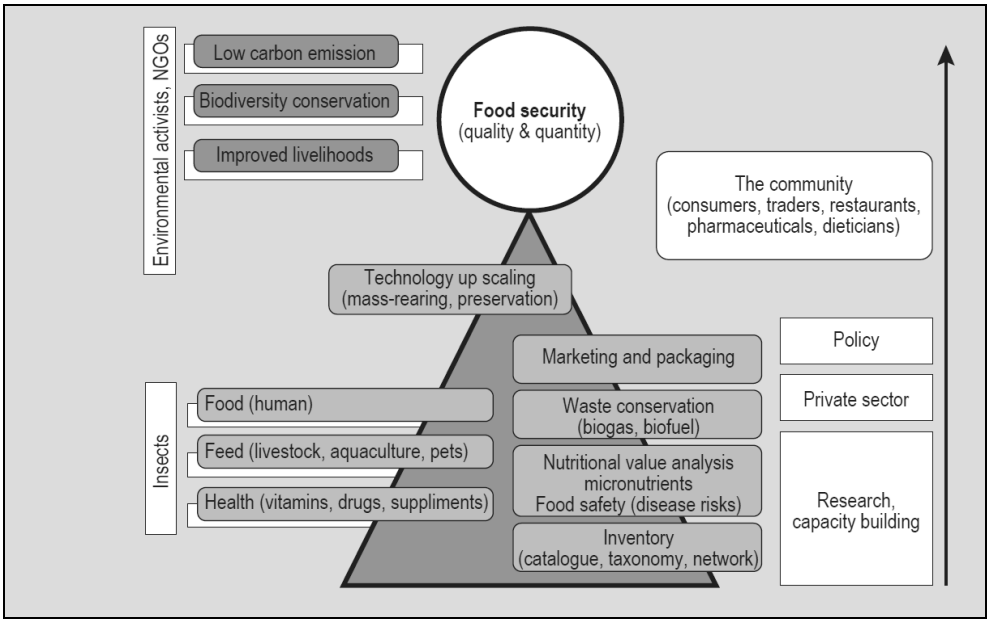

Figure 1.1: Pathways to using insects for food and feed to improve food security in Africa (Kelemu et al., 2015)

1.2 Promoting consumption of edible insects

There are limited studies on the current consumption practices of edible insects in relation to food and nutrition security. Some studies indicated a decline of the entomophagy in countries where eating insect is a traditional practice. The consumption of insects is ancient in human history and takes place mainly in Africa, Asia, and Latin America (van Huis, 2013), where inclusion of insects in human diets is common (Yen, 2009). Over 2000 species of insects have been documented as being consumed worldwide (Jongema, 2017) and in Africa alone, 470 species of edible insects are documented (Kelemu et al., 2015). Most of the insects consumed are seasonal and collected from the natural environments, also called wild harvested (Klunder et al., 2012; Mutungi et al., 2017). People can eat insects in different stages of development such as eggs, larvae, pupae, and adults (Chen et al., 2009; Mutungi et al., 2017; Julieta Ramos-Elorduy, 2002; Verkerk et al., 2007). The type of insect consumed

is dependent on the species and ethnic groups. Beetles, caterpillars, and ants are amongst the world's most frequently consumed species, followed by grasshoppers and locusts (Kouřimská & Adámková, 2016). Taste, nutritional value, and ethnic customs affect consumers' preferences for a certain insect species (Yen, 2009).

There are three reasons to promote entomophagy, i.e. health, environmental benefits, and livelihood improvement (social and economic factors) (van Huis et al., 2013a). Rearing insects is more environmentally friendly as it produces less greenhouse gases and requires less land as compared to livestock rearing (Oonincx et al., 2010). The growing population and need for alternative sustainable protein sources, is one of the major reasons for consumption of insects. As such, there has been an increase worldwide in the captive rearing of insects to meet the demand of emerging protein source. However, interventions to promote more or new consumption require an understanding of consumptions patterns of different communities and data on the nutritional composition of the insects.

1.3 Nutritional quality of edible insects

Understanding of the nutritional value of different edible insects is important for promoting consumption of insects as a nutrient source. Studies done across the world have proved that many edible insects are good sources of protein, fat, and micronutrients (Chen et al., 2009; DeFoliart, 1992; Obopile & Seeletso, 2013; Srivastava et al., 2009). However, there is high variability in the nutritional composition of insects. The nutritional value of insects depends on species, insect life stage, habitat, type of feed for the insects (van Huis et al., 2013a; Verkerk et al., 2007), origin (Rumpold & Schluter, 2013), and methods of preparation (Yhoung- Aree et al., 1997). Differences in nutritional composition exist among species (Bukkens, 1997; Kinyuru et al., 2013) and among species within same order (Bukkens, 1997; Rumpold & Schluter, 2013).

Proteins are the most important nutritional component of edible insects. The protein content of edible insects varies from species to species and can be up to 77% based on dry weight (Rumpold & Schluter, 2013). Insect protein contents are comparable to conventional protein sources such as beef and fish (Bukkens, 1997). Amino acid composition and protein digestibility determine protein quality of edible insects (Belluco et al., 2013; Boye et al., 2012; Sarwar Gilani et al., 2012). In general, edible insects are good sources of all essential amino acids, although different amino acid profiles exist for different species (Blásquez et al., 2012; Bukkens, 1997; Finke, 2007; Yi et al., 2013).

Lipids present the second largest nutritional component of insects. As such, edible insects can substantially contribute to the caloric value of meals. The crude fat content of edible insects varies between 10 and 70% (Finke and Oonincx, 2017). Moreover, fat content and fatty acid composition vary with species and are highly dependent on the diet and metamorphic stage of insects (Belluco et

al., 2013; Fontaneto et al., 2011; Komprda et al., 2013; St-Hilaire & Cranfill, 2007). Studies have reported that fatty acid profiles of edible insects are comparable to those of poultry, because of their unsaturated fatty acids. Additionally, differences exist in fat content and fatty acid composition between wild harvested insects and reared insects. Reared insects species have higher fat content and wild harvested insects have relatively higher amounts of linoleic acid and linoleic acid (Finke & Oonincx, 2017).

Further, edible insects are a good source of vitamins and minerals and their levels vary between species as well. The ash content of edible insects, which is an indication of the mineral content, varies between 1% and 25% (Rumpold & Schlüter, 2013). Several studies have shown edible insects as good sources of iron and zinc, though variation between species exists like for the macronutrients. Factors contributing to this variation between species are geographical place of harvest, seasonal and environmental factors, type of processing (Zhou & Han, 2006), and feed (Rumpold & Schlüter, 2013). There is limited data on the vitamin content of edible insects, though insects are generally low in retinol, but rich in riboflavin and pantothenic acid (van Huis, 2016). Variation also exists for chitin, which contributes significantly to the fibre content of insects. Finally, some bioactive components e.g. tannins and phenolic acids, are shown to be present in edible insects and can act as anti-nutritional factors. Despite several studies on nutritional value, the nutritional composition of many edible insect species consumed in the world is yet unknown (Nowak et al., 2016) as well as variation in composition of insects harvested from different regions. Furthermore, the nutritional quality of edible insects is beyond the nutrient content, as nutrient availability is also important, which can be influenced by processing.

1.4 Processing of edible insects and nutrient availability

Processing can influence the nutritional quality of insects and most edible insects require proper processing before their use or consumption. Edible insects, like any other foods are susceptible to microbial contamination (Klunder et al., 2012; Stoops et al., 2016). Therefore, processing is necessary to prevent spoilage and to ensure food safety (Rumpold & Schlüter, 2013). Moreover, processing improves the eating qualities of edible insects. Different processing pathways i.e. traditional and industrial processing both exist for edible insects (Figure 1.2). The elemental operations of traditional processing of insects involve cleaning followed by wet or dry heat treatments (Mutungi et al., 2017). The cleaning involves removing of extraneous matter, removing guts, wings, legs, and head depending on the species and washing with water. Boiling, steaming, frying, roasting, and drying (Kinyuru et al., 2010; Kinyuru et al., 2009; Obopile & Seeletso, 2013; Ramos- Elorduy, 1997) are amongst the commonly used traditional heating methods for processing insects. The techniques used vary depending on the insect species and geographical region.

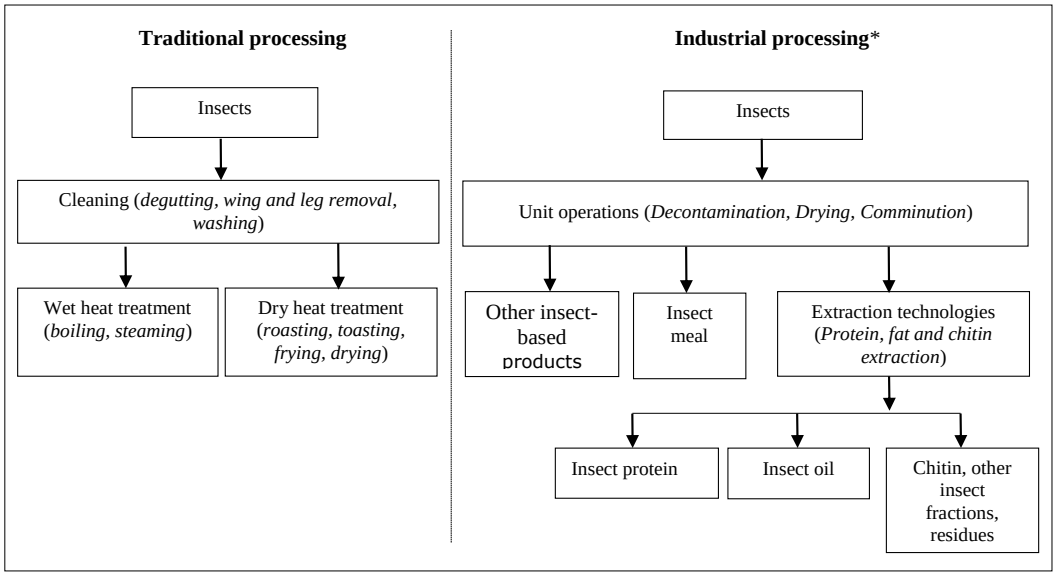

Figure 1.2: Traditional and industrial processing pathways for edible insects (*adapted from, Rumpold et al., 2017)

Over the years, there has been much interest in the captive rearing of insects and as a result insect processing industry advanced especially in the developed world (van Huis, 2016). Similar to traditional processes, industrial processes use wet and heat treatments to decontaminate insects. However, unlike in the traditional processing, where the processes are characterised by uncontrolled conditions, industrial processes are controlled, e.g. blanching, pasteurisation, sterilisation, and drying (Rumpold et al., 2017). In addition, decontaminated insects can undergo drying and grinding/milling to produce insect meal or insect-based products. Furthermore, some processors extract the major components of insects (protein, fat, and chitin) for use as ingredients in some food products.

Processing can also effect some changes to the properties of edible insects. For example, heat treatment can enhance or reduce protein digestibility and/or bioaccessibility, depending on the time-temperature combinations. Protein denaturation and reduction in the anti-nutritional factors enhance protein digestibility (Boye et al., 2012), as well as nutrient bioaccessibility although this is not well studied for insects so far. Furthermore, protein denaturation in general alters protein functionality with regard to solubility, gelling, emulsifying and foaming properties (Tiencheu et al., 2013). Traditional processing methods of insects are usually characterised by uncontrolled conditions. This may have detrimental effects on the nutritional quality of insects. For example, Kinyuru et al., (2009) reported a significant reduction in protein digestibility and decrease in vitamin for toasted and dried grasshoppers compared to fresh ones

According to Friedman (1996), a better understanding of the molecular changes that occur during processing is necessary to optimise the beneficial effects and minimise the formation of deleterious components. Different heat treatment techniques and processing parameters can have an effect on the final nutritional property of a product and/or bioaccessibility of nutrients. Several studies investigated the influence of traditional processing on bioaccessibility on other food products. However, there is limited information about the effects of traditional processing on insects' nutrients content and bioaccessibility.

Bioaccessibility of a particular nutrient gives a clear picture of nutritional quality (Fernandez-Garcia et al., 2009). Cardoso et al. (2015) defined bio-accessibility of a nutrient as the fraction that is soluble in the gastrointestinal environment and available for absorption. In addition to processing, bioaccessibility is influenced by the composition of the food matrix and interaction with other components (Fernandez-Garcia et al., 2009). For example, levels and/or type of fat and carbohydrates in a food can affect protein digestibility. Additionally, some anti-nutritional components either naturally present in food or formed during processing reduce protein digestibility (Boye et al., 2012) and mineral bioaccessibility. The presence of anti-nutritional components such as tannins and phenolic acids in some edible insects (Musundire et al., 2016) support the need for bioaccessibility studies.

1.5 Relevance of shelf life of edible insects to food and nutrition security

Harvested edible insects have a limited shelf life due to their biological nature. Since fresh wild harvested insects are seasonally available, deterioration in quality of harvested, processed and stored edible insects can limit their contribution to food and nutrition security. Thus, prolonging the shelf life of edible insects is an important aspect for improving their availability off-season and increasing nutrition security. Shelf life of food is the period of time where the product is safe to eat and has a quality that is acceptable to consumers (Fu & Labuza, 1997). Multiple chemical, physical, and biological processes can limit shelf life. Biological processes affect mainly food spoilage but the heat treatments applied to insects reduce the risk of biological spoilage. Drying is the traditional approach to extend shelf life of insects (Mutungi et al., 2017). Due to their low moisture content, dried insects can be susceptible to fat oxidation because of the high concentration of unsaturated fatty acids, thereby limiting the shelf life of stored dried insects. In addition, the presence of chitin can prevent fast evaporation of moisture from the insects (Chavunduka, 1975), thereby hampering the drying process. Limited information exists on shelf life of dried insects with few studies reporting on the stability of extracted insect oils. To extend the consumption of insects as a natural source for nutrition security all year round, there is a need to improve availability by extending the shelf life.

1.6 Problem statement

Zimbabwe, just like any other Sub Saharan country, is still facing food and nutrition insecurity. According to the World Food Program, more than 2.4 million people in Zimbabwe face food insecurity at the peak of the lean season of 2019. Micronutrient deficiencies, particular iron and zinc deficiency remain a public health concern amongst Zimbabweans (ZIMVAC, 2018). According to the ZIMVAC report 2018, meat and legumes, which are important protein sources, are the least consumed food groups by the rural and poor urban population. A major reason is that prices of these food items are too high for many people.

To improve nutrition security, edible insects could be used in fortified blended foods (FBFs), mainly because of the protein and mineral content. Van Huis et al. (2013) reported that in order to make recommendations regarding food enrichment, there is a need to explore traditional diets in their entirety and compare the diets' nutritional quality with that of local available insects. In Zimbabwe, there is high consumption of maize meal and green leafy vegetables and low rates of consumption of meat, milk, and eggs, especially in the rural areas. Maize meal is limiting in lysine and tryptophan (Friedman, 1996) and one needs to obtain these amino acids from other food sources. As such, wild harvested edible insects have the potential to alleviate malnutrition problem due to their high protein contents, which are comparable to that of other protein sources.

Edible insects are rich sources of iron and their inclusion in diets can improve iron status and prevent anaemia (van Huis, 2013). Consumption of edible insects could potentially reduce iron and zinc deficiency in developing countries (Mwangi et al., 2018; Rumpold & Schlüter, 2013b). However, not all insect species are able to meet all nutrient requirements. Furthermore, to reduce micronutrient deficiencies, the minerals in food should also be bioaccessible. Hence, there is a need to evaluate the nutritional quality and bioaccessibility of local available insect species.

Furthermore, the current traditional cooking methods of edible insects may result in the loss of nutritional quality. It is therefore important to apply optimal processes conditions (time, temperature, choice of processing method, and operations) to obtain high nutritional and sensorial quality, and safe insect food products. Wild harvested edible insects are seasonal, but for off-season consumption proper preservation and storage conditions need to be developed. Value addition to insects can create new marketing avenues in the cities and towns (Gahukar, 2011). Traditional knowledge of products and processing methods of insects can be used as a baseline for developing suitable and innovative processing technologies for improved nutritional value, acceptance, and enhanced economic value.

1.7 Case study species: *Eulepida mashona* and *Henicus whellani*

In Zimbabwe, more than 45 species of edible insects are consumed (DeFoliart, 1997; Kelemu et al., 2015), with Mopane worms and termites being the most common (Dube et al., 2013; Onigbinde & Adamolekun, 1998). To ascertain the suitability of wild harvested edible insects in alleviating malnutrition or complementing other foods in local diets, investigation of other less commonly consumed insects is also required. It is against this background that two different wild harvested species, *Eulepida mashona* and *Henicus whellani* were selected as case study in this thesis. Both insect species are consumed at adult stage (Figure 1.3 and 1.4) and belong to different orders. *Eulepida mashona*, also known as chaffer beetles (Chavunduka, 1975), belongs to the Coleoptera order of insects and is amongst the consumed edible insects in Zimbabwe (Chavunduka, 1975; DeFoliart, 2002; DeFoliart, 1997; Dube et al., 2013). The insects are prevalent in different agro-ecological regions of the country and are mainly associated with the *Brachytegia speciformis L*. and *Julbernadia globiflora L*. type of vegetation (Chavunduka, 1975). *Henicus whellani is* a ground edible cricket belonging to the Orthoptera order of insects, while chafer beetles are harvested from trees and bushes. *Henicus whellani* is harvested by digging them from the soil or picking from the ground when they emerge from their burrow soon after rains. Preparation steps of the insects include dewinging (*Eulepida mashona*), degutting (*Henicus whellani*), washing, boiling, followed by roasting.

Figure 1.3: *Eulepida mashona* a) in host tree b) freshly harvested c) prepared ready to eat

Figure 1.4: *Henicus whellani* a) freshly harvested b) degutted and c) prepared ready to eat

1.8 Thesis objectives and outline

Considering their diversity, wild harvested edible insects have a potential to contribute to food and nutrition security in developing countries. However, for most of these edible insects, the information on insects' suitability for use in improving nutrition of people who consume edible insects and consumption patterns is still lacking. The main objective of this thesis was therefore to investigate how wild harvested edible insects can contribute to nutrition security in developing countries, with Zimbabwe as a case study. The specific objectives were to:

a) Determine insect consumption patterns among rural and urban populations and identify factors that influence these patterns.
b) Investigate the variability in nutritional composition of *Eulepida mashona* and *Henicus whellani* amongst different districts of harvest.
c) Study the influence of domestic processing methods on protein and mineral retention, protein digestibility and mineral bioaccessibility of *Eulepida mashona* and *Henicus whellani*.
d) Examine effects of processing conditions (grinding, drying and storage temperature) on shelf stability of edible insects in terms of lipid oxidation.

This thesis consists of six chapters, four of which address the abovementioned specific objectives. **Chapter 1**, the general introduction, gives the background of the study, the need for research and outlines the objectives of this thesis. Most research on wild harvested edible insects focused on their nutritional value, ignoring the status quo consumption of edible insects. Thus, **Chapter 2** brings forward this evidence about current consumption patterns of edible insects in urban and rural areas of Zimbabwe and identified factors that influence the consumption of edible insects. However, promoting consumption of edible insects should be based on sound scientific knowledge on their nutritional value. Therefore, **Chapter 3** explores the nutritional potential of wild harvested *Eulepida mashona* and *Henicus whellani* and how their nutritional composition varies among different district of occurrence. To have a complete overview on the nutritional quality, not only the composition but also the bioaccessibility is an important parameter. Thus, **Chapter 4** explores how the common methods of processing insects (boiling and roasting) influence the nutrient content and bioaccessibility of the insects. In addition, seasonality of wild harvested insects can possibly limit potential contribution to food and nutrition security. This necessitates drying insects for longer storage. As such, **Chapter 5** investigated shelf stability of dried *Eulepida mashona* and *Henicus whellani* with respect to lipid oxidation. Finally, **Chapter 6** discussed the major findings and gives recommendation for further research. Figure 1.5 shows the overview of the four studies and how they connect to each other.

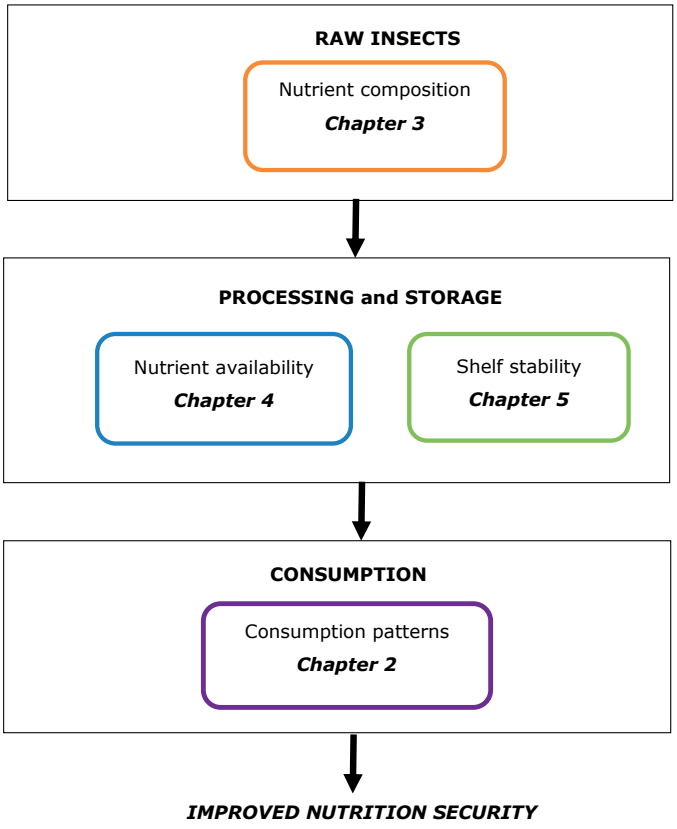

Figure 1.5: Schematic overview of the thesis

CHAPTER 2

Consumption Patterns of Edible Insects in Rural and Urban Areas of Zimbabwe: Taste, Nutritional Value, and Availability Are Key Elements For Keeping The Insect Eating Habit

This chapter has been published as: Manditsera, F. A., Lakemond, C. M. M., Fogliano, V., Zvidzai, C. J., & Luning, P. A. (2018). Consumption patterns of edible insects in rural and urban areas of Zimbabwe: taste, nutritional value and availability are key elements for keeping the insect eating habit. Food Security, 10(3), 561-570. doi: 10.1007/s12571-018-0801-8

Abstract

Edible insect consumption is a traditional practice in many countries and has the potential to contribute to food security. The aim of this study is to obtain insight into insect consumption patterns amongst rural and urban populations, and into factors that may influence these patterns. For this purpose, a case study was made in Zimbabwe. A literature-based conceptual model indicated that motives for consumption, individual characteristics, consumer environment, availability, food characteristics, and indigenous knowledge could affect edible insect consumption. A survey amongst 200 urban and 175 rural respondents showed that insect consumption was significantly higher in rural (89.7%) than in urban (80.0%) areas. Rural respondents (63.9%) consumed insects more than three times a week on average as compared to urban (14.5%) respondents. Quantities consumed as snacks are significantly different between urban and rural respondents. Taste was the main motive of respondents in both the rural (89.2%) and urban areas (74.4%). Respondents in urban areas more often reported nutritional value (74.4%) and medicinal properties (28.1%) as important motives for consumption compared to rural respondents (51.0% and 15.3%, respectively). For rural areas, socio-demographics did not relate to consumption of edible insects whereas in urban areas, insect consumption was negatively related to education, main livelihood source and monthly income. Availability of edible insects influences both urban (64.0%) and rural (83.0%) respondents' consumption of insects. The lower consumption of specific insect species in urban areas could hamper the potential contribution of insects to food security in these areas. Therefore, promotion of entomophagy by marketing and maintaining traditional knowledge on insect processing should target urban people through provision of tasty products, communicating nutritional value.

2.1. Introduction

Consumption of edible insects is a traditional practice in many African (van Huis, 2003), Asian (Yen, 2015) and Latin American (Costa-Neto, 2016) communities. More than 2000 species of insects are suitable for human consumption worldwide (Jongema, 2017). Edible insects have the potential to contribute to food security (Belluco et al., 2013; Ghaly, 2009). FAO is therefore promoting the consumption of insects from wild harvest or insect farming (Gahukar, 2011; Hanboonsong et al., 2013; van Huis et al., 2013b). The proportional contribution of edible insects to the diets of insect-eating populations ranges from minor to substantial and there can be variation in the contribution to different groups within communities (Raubenheimer & Rothman, 2013; van Huis et al., 2013b).

However, the potential contribution of edible insects to food security in continents such as Africa and Asia is under threat. A decrease in prevalence of traditional practices of entomophagy has been reported in communities in developing countries where insect consumption used to be common (Dube et al., 2013; Meyer-Rochow & Chakravorty, 2013; Obopile & Seeletso, 2013; Riggi et al., 2016; Yen, 2009). Reasons for this decrease include adoption of Western foods (Dube et al., 2013; Looy et al., 2013; Mlcek et al., 2014; Obopile & Seeletso, 2013; Yen, 2009) and decreased knowledge of preparation practices (Riggi et al., 2016). Other reported reasons include unavailability of the edible insects (Looy et al., 2013), uncontrolled harvesting (J. Ramos-Elorduy, 2006), and loss of habitats leading to extinction of some species (Dube et al., 2013; Meyer-Rochow & Chakravorty, 2013).

In developing countries, especially in urban areas and younger populations, there is a tendency to abandon the practice of entomophagy due to westernisation of traditional diets (Huis & Vantomme, 2014; Vantomme, 2015). In addition, a common belief is that traditional foods, like edible insects, are considered primitive and are not accepted by Western communities. This thinking leads to unwillingness of people to share experiences of these foods (Looy et al., 2013).
Multiple studies stress the importance of documenting traditional knowledge of edible insects to restore and promote entomophagy (Riggi et al., 2016; van Huis, 2015; Yen, 2009) and to disseminate information to new consumers, especially in urban areas (Gahukar, 2011). Traditional communities are well-enriched with local knowledge of the occurrence, methods of collection, processing and consumption (Riggi et al., 2016).

Many developing countries are still facing food insecurity despite an abundance of natural resources that can help to alleviate the problem. An estimated prevalence of 11.0% of the global population is undernourished and a higher 12.9 % is observed in developing countries (FAO, 2015). Food insecurity is not only limited to rural areas but also observed in urban areas (Frimpong, 2013; Tawodzera, 2011). Rural to urban migration is usually associated with changed consumptions habits (Frimpong, 2013; Puoane et al., 2006). The consumption of edible insects in many developing countries is documented

for specific insect species. However, to the best of our knowledge, it is not yet known how edible insect consumption differs between urban and rural areas.

The aim of the study was to obtain insight into the insect consumption patterns among rural and urban populations and to study the factors that influence these patterns. For this purpose, a case study was made in Zimbabwe. Although mopane worms and termites are the most popularly consumed edible insects in Zimbabwe (Gardiner & Gardiner, 2003; Onigbinde & Adamolekun, 1998), *Eulepida* species (Dube et al., 2013; Musundire et al., 2016; Onigbinde & Adamolekun, 1998) and *Henicus whellani* (Musundire et al., 2014a) are also commonly consumed in some regions of the country. The current study, next to overall insect consumption, focused on *Eulepida spp.* and *Henicus whellani*, because of their specific cultural value to local consumers and potential to contribute substantially to human nutrition.

2.2 Methodology

2.2.1 Study design

Data on consumption patterns and traditional processing of edible insects was collected through a survey. A questionnaire was administered in three urban towns and five rural districts of Zimbabwe through face-to-face interviews between July and October 2015. The questionnaire was based on a conceptual model that presents factors influencing consumption patterns of edible insects as described in the literature.

2.2.2 Questionnaire design

2.2.2.1 Conceptual model used to design the questionnaire

Figure 2.1 shows a conceptual model presenting possible factors influencing the consumption patterns of edible insects. The model is based on common food preference studies and literature on insect consumption. Food preferences play an important role in food consumption (Sijtsema et al., 2002) and can affect consumption patterns (Becker et al., 2000; Gerbens-Leenes & Nonhebel, 2005), which are defined as repeated arrangements in the food consumption of a population group (Gerbens-Leenes & Nonhebel, 2005). In the current study, consumption patterns refer to which insects are consumed, how much and when. Food preference can be affected by characteristics of the food itself (such as taste, flavour, appearance), consumer characteristics, such as gender, age and education (Han & Powell, 2013), and characteristics of the consumer's social environment such as individual upbringing and religion (Sijtsema et al., 2002). Particularly, religion can play an important role in the consumption of insects, and in some religious practices, entomophagy is strictly forbidden (Dube et al., 2013).

Important reasons for consuming insects are sensory/pleasure considerations and health (van Huis, 2013), nutritional value (Kinyuru et al., 2010a; Obopile & Seeletso, 2013) and medicinal properties

(Ayieko & Oriaro, 2008; Musundire et al., 2014b). Other studies indicate that insect availability influences preferences and consumption of edible insects (Chakravorty et al., 2013; Meyer-Rochow & Chakravorty, 2013; Obopile & Seeletso, 2013; Raubenheimer & Rothman, 2013). Also, indigenous knowledge on harvesting and processing have been mentioned as factors influencing the adoption and consumption of insect-based food (Gahukar, 2011; Kinyuru et al., 2010a; Obopile & Seeletso, 2013), and this factor is included in the model as well.

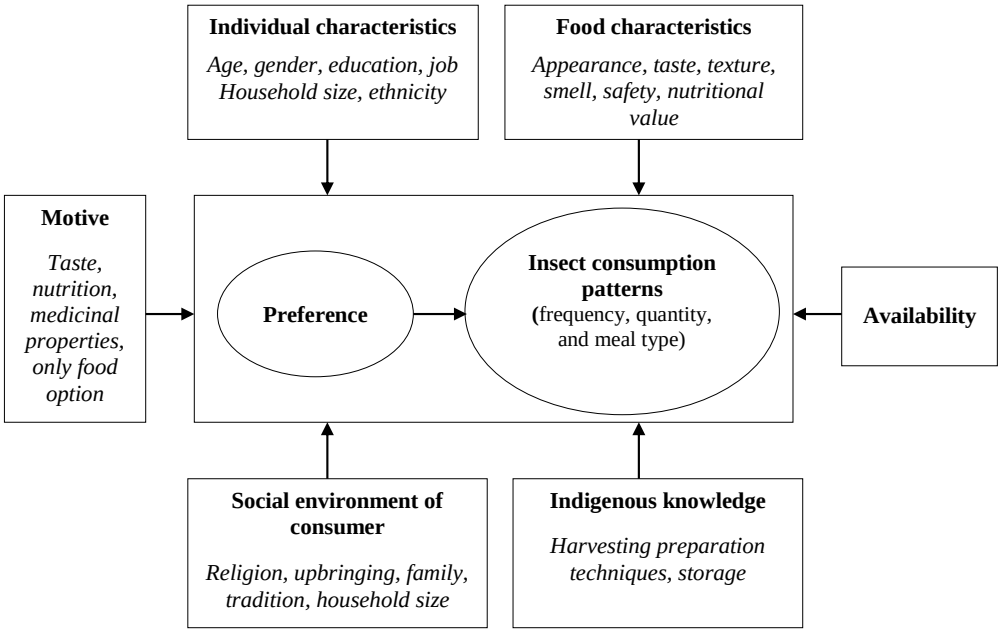

Fig 2.1 Framework for the design of the questionnaire on possible factors that can influence insect consumption patterns

2.2.2.2 Questionnaire

The questionnaire was divided into seven sections. In the first section socio-demographic information was collected. The second section contained close-ended questions related to consumption patterns (frequency of consumption, quantity, and form of consumption) and possible motives (reasons) for consuming insects. The third section contained questions about how characteristics of the consumer's social environment, including religion, individual upbringing, family habits, and edible insects' availability influenced respondents' behaviour towards entomophagy. Section 4 includes questions related to the insects' characteristics. Respondents rated the importance they assigned to the various characteristics affecting their decision to eat insects on a five point hedonic scale. Moreover, consumers had to rate appreciation of different sensory attributes (taste, texture, smell and

appearance). In the fifth section, questions on consumptions patterns were asked particularly for *Eulepida spp.* and *Henicus whellani*. Only those who actually consumed the two species completed these questions. The last two sections solicited indigenous knowledge on harvesting and traditional processing techniques of these two insect species.

2.2.3 Study area and respondents

Questionnaires were administered to 200 individuals who were randomly selected and had agreed to be interviewed at several market places and shopping centres in three urban areas: Harare (n=80), Masvingo (n=60) and Marondera (n=60). Masvingo and Marondera were purposively selected because these towns are close to rural areas where *Henicus whellani* and *Eulepida species* are commonly found. The assumption is that people living in these cities are embedded in a culture where these insects are considered a local speciality.

Questionnaires were also administered in five rural areas in the Zaka, Bikita, Mhondoro, Seke and Zvimba districts, to a total of 175 respondents. One adult per randomly selected household from a list of households provided by traditional leaders was interviewed. The rural areas were purposively selected based on where either *Henicus whellani* or *Eulepida species* were commonly found and consumed. Zaka and Bikita districts were considered to be areas in the Southern Eastern region of Zimbabwe where *Henicus whellani* is found and consumed. Mhondoro, Seke and Zvimba were selected where *Eulepida spp.* are found and consumed.

2.2.4 Data analysis

Data from the questionnaire were coded and entered into IBM SPSS Statistics (Version 22, 2013). The data of the respondents who actually consume edible insects was used for calculating the (relative) frequencies of consumption and to discover consumption patterns. A Chi-square test of independence was performed to determine if there was any significant difference between consumption patterns of rural versus urban respondents and any relationship between consumption and demographic characteristics.

2.3 Results and Discussion

2.3.1 Insect consumption patterns

A greater percentage of the rural respondents (89.7%) compared to urban respondents consumed at least one type of edible insect. However, the consumption habit was also high among urban respondents (80.0%) (χ^2 =6.736, *df*=1, p=0.009) (Table 2.1). This suggests that most respondents consumed at least one edible insect species, but not necessarily different types of insects. The results are in line with previous research, which observed that entomophagy is a common practice in

Zimbabwe (DeFoliart, 1997; Dube et al., 2013; Musundire et al., 2016). The data on consumption frequencies shows that a significantly higher percentage (63.9%) of the rural respondents consumed edible insects more than three times a week on average when it is insect harvest season compared with urban respondents (14.5%) (χ^2 =101.766, df=3, p<0.001).

The difference in frequency of consumption and quantities consumed between urban and rural respondents was expected since most of the edible insects are harvested in the wild and availability is an important factor in their consumption (Meyer-Rochow & Chakravorty, 2013). Rural communities have greater access to insects, especially to species harvested exclusively from the forests. On the other hand, urban people obtain most of their edible insects from markets. Harvesting and marketing edible insects are contributing to the improvement of livelihoods for some rural communities. van Huis (2013) highlighted that collection of insects from the wild will not sustain entomophagy and suggested a need for considering rearing of targeted species.

Table 2.1 also shows how insects are consumed (meal type) and quantities consumed. The most common way of consuming edible insects in both rural and urban areas is as a relish and/or as a snack. Relish refers to a side dish taken together with the staple food (such as thick maize porridge called sadza). In this study, a snack refers to consumption of insects as a leisure activity rather than as a meal. More rural (77.1%) than urban (61.9%) respondents consumed insects only as a relish (χ^2 =8.616, df=1, p=0.003). Most of the insect consuming respondents in the urban (70 %) and rural (79%) areas eat edible insects as snacks. Although there was no significant difference in consuming edible insects as snacks (χ^2 =3.360, df=1, p=0.067), the consumed quantities as snacks differed significantly between the rural and urban respondents (χ^2 =20.552, df=2, p<0.001). Amongst the urban and rural respondents who eat insects as a relish, 55.6% and 46.3%, respectively, eat one cup. The quantities were lower when respondents consumed insects in combination with other relishes for both urban (63.3%) and rural (72.4%) respondents. However, there was no significance difference between urban and rural for quantities consumed as a relish (χ^2 =4.325, df=2, p=0.115) and also when consumed in combination with other relishes (χ^2 =1.250, df=2, p=0.535). A cup contains between 50 and 75 g approximately of dried insects, depending on the type of insect.

Table 2.1 Frequencies (as percentage) of the edible insect consumption patterns of urban and rural during insect harvest season[a]

	Urban	Rural
Overall consumption patterns*	**n=200**	**n=175**
Consumers	80.0	89.7
Non-consumers	20.0	10.3
Frequency of consumption*	**n= 159**	**n= 155**
Greater than 3 times/week	14.5	63.9
1-2 times/ week	37.1	29.7
1-4 times a month	24.5	5.2
Less than once a month	23.9	1.3
Meal type[b]	**n=160**	**n=157**
As relish*	61.9	77.1
In combination with other relish*	31.6	48.4
Snack	70.0	79.0
Quantities consumed		
As relish	**n=99**	**n=121**
Less than 1 cup	21.2	33.9
Equivalent to 1 cup	55.6	46.3
More than one cup	23.2	19.8
As combination with other relish	**n=49**	**n=76**
Less than 1 cup	63.3	72.4
Equivalent to 1 cup	24.5	17.1
More than one cup	12.2	10.5
As snack*	**n=112**	**n=124**
Less than 1 cup	52.7	55.6
Equivalent to 1 cup	37.5	16.1
More than one cup	9.8	28.2

[a] All values represents %, [b] more than one answer possible, * significantly different between urban and rural

Tables 2.2 and 2.3 show that in both the urban and rural areas, fewer than 50% of the respondents consumed *Eulepida spp.* and *Henicus whellani. Eulepida spp.* are consumed to a greater extent in both the urban (35.5%) and rural (48%) areas than *Henicus whellani* in urban (8.5%) and rural areas (41.1%). The frequency of consumption of *Eulepida sp*ecies is 1-2 times a week for the urban (45.7%) and rural (42.0%) respondents. In the rural areas, 67.6% of the respondents eat *Henicus whellani* more than three times a week, whereas, 46.7% of the urban consumer respondents consume this insect 1-2 times a week. *Eulepida species* are mostly consumed as snacks in both urban (80.0%) and rural (83.8%) areas. A different pattern exists for *Henicus whellani,* which are mainly consumed as a relish and snack in both urban (53.3%: 53.3%) and rural (83.1%; 88.7%, respectively) areas. Consumers of *Eulepida* species mostly take quantities of less than one cup for all meal types.

Differences in the consumption of *Eulepida* species and *Henicus whellani,* as compared to general consumption, could be attributed to people preferring different insect species and ease of access. Differences in preferences and prevalence of consumption of specific species have also been attributed to availability (Niaba et al., 2012; Obopile & Seeletso, 2013; van Huis, 2013), ethnicity (Chakravorty et al., 2011; Obopile & Seeletso, 2013; Riggi et al., 2016), palatability (Chakravorty et al., 2013) and seasonality (Kinyuru et al., 2010a; Kinyuru et al., 2013). Hanboonsong et al. (2013) reported different

insect eating habits due to availability of different species in different regions. Likewise, in the current study, the higher prevalence of consumption of *Eulepida* species is probably because this species is prevalent in many regions of Zimbabwe. *Henicus whellani* is commonly present only in the South Eastern region of Zimbabwe. *Eulepida species* is therefore consumed more than *Henicus whellani* in the urban areas. Urban consumers usually obtain these insects from informal markets and as gifts from rural folk, who harvest and prepare the insects and market them dried or ready to eat.

Table 2.2 Frequencies (as percentages) of consumption patterns for *Eulepida species* of urban and rural during insect harvest season

	Urban	**Rural**
Overall consumption patterns for *Eulepida species**	**n=200**	**n=175**
Consumers	35.5	48.0
Non-consumers	64.5	52.0
Frequency of consumption*	**n=70**	**n=81**
Greater than 3 times	22.9	42.0
1-2 times a week	45.7	42.0
1-4 times a month	20.0	6.1
Less than once a month	11.4	9.9
Meal type[b]	**n=70**	**n=81**
As relish	15.7	15.0
In combination with other relish	32.9	33.8
Snack	80.0	83.8
Quantities consumed:		
As relish	**n=11**	**n=12**
Less than 1 cup	63.6	75.0
Equivalent to 1 cup	27.3	25.0
More than one cup	9.1	0
As combination with other relish	**n=23**	**n=27**
Less than 1 cup	69.6	85.2
Equivalent to 1 cup	13.0	11.1
More than one cup	17.4	3.7
As snack*	**n=56**	**n=67**
Less than 1 cup	58.9	58.2
Equivalent to 1 cup	33.9	14.9
More than one cup	17.2	26.9

* significant difference between urban and rural, [b] more than one answer possible

N.B Except in overall consumptions, n represents the number of consumer respondents in each category given

Table 2.3 Frequencies (as percentage) of consumption patterns for *Henicus whellani*of urban and rural during insect harvest season

	Urban	Rural
Overall consumption patterns for *Henicus whellani**	**n=200**	**n=175**
Consumers	8.5	41.1
Non-consumers	91.5	58.9
Frequency of consumption*	**n= 15**	**n= 71**
Greater than 3 times	26.7	67.6
1-2 times a week	46.7	25.4
1-4 times a month	13.1	4.2
Less than once a month	13.1	2.8
Meal type[b]	**n=15**	**n=71**
As relish*	53.3	83.1
In combination with other relish*	20.0	29.6
Snack*	53.3	88.7
Quantities consumed		
As relish	**n=8**	**n=59**
Less than 1 cup	25.0	39.0
Equivalent to 1 cup	37.5	40.7
More than one cup	37.5	20.3
As combination with other relish	**n=3**	**n=21**
Less than 1 cup	63.3	71.4
Equivalent to 1 cup	24.5	23.8
More than one cup	12.2	4.8
As snack*	**n=8**	**n=63**
Less than 1 cup	52.7	63.5
Equivalent to 1 cup	37.5	15.9
More than one cup	9.8	20.6

* significant difference between urban and rural, [b] more than one answer possible

N.B Except in overall consumptions, n represents the number of consumer respondents in each category given

This study revealed that overall the consumption of insects in urban and rural areas is relatively high (>80%) although consumption patterns vary with species. The significant (but small) differences in percentage of respondents consuming insects in urban and rural areas can be an indication that entomophagy is not declining in either urban or rural areas in Zimbabwe, as suggested by other authors for other countries (Obopile & Seeletso, 2013; Riggi et al., 2016). Likewise, the expectation that due to increased migration from rural to urban areas the consumption of traditional foods (under which edible insects are classified) would be abandoned, was not confirmed. Puoane et al (2006) argued that people do not completely lose their culture but adhere to old traits, despite adoption of Western diets. However, a lower frequency of consumption in urban areas could be pointing to a decline of entomophagy, a trend that may continue if not addressed.

2.3.2 Motives for consuming edible insects

Table 2.4 shows that the major motives for consuming edible insect for both urban and rural respondents are taste (74.4% and 89.2%, respectively) and nutrition (74.4% and 51.0% respectively). Various studies in other developing countries report that taste is as a major motive for insect consumption (Ayieko & Oriaro, 2008; Obopile & Seeletso, 2013). However, Obopile and Seeletso (2013) found, in their study in Botswana, that nutritional value was not the major reason for consuming insects since only 5% of their respondents indicated this motive. It is relevant to note that whilst respondents consume insects for their nutritional value, they do so because they perceive insects as high value nutritional food. They do not know exact nutritional values, rather they generalise that insects are rich in protein and health promoting components. Respondents in urban areas more often (74.4%) reported nutritional value ($\chi 2$ =18.600, df=1, $p<0.001$) and medicinal properties (28.1%) as important motives compared to respondents from the rural areas (51% and 15.3%, respectively). In the rural areas, 14.0% of the respondents consume insects because they are the only food option. This percentage is relatively low and confirms that respondents did not perceive entomophagy as necessary because of lack of food but rather as a tradition. Furthermore, in rural areas, consumption of insects is an opportunity to break the monotony of available relishes. Some respondents considered certain types of insects, such as mopane worms and termites as delicacies.

Table 2.4 Frequencies (%) of different motives for edible insect consumption in urban and rural areas of Zimbabwe (more than one answer category was possible)

Motive	Urban (n= 160)	Rural (n=157)
Taste*	74.4	89.2
Nutrition*	74.4	51.0
Medicinal properties*	28.1	15.3
Only food option*	3.1	14.0
Other reasons*	6.9	14.6

*Significance difference between urban and rural

3.3 Influence of characteristics of the consumers on consumption patterns

All socio-demographic characteristics, except gender, differed significantly between the urban and rural respondents (Table 2.5). Most of the respondents in urban (54.5%) and rural (62.3%) areas were female. In Zimbabwe, the male to female ratio is 48.1 to 51.9 both in rural and urban areas (ZIMSTAT). The respondents that consumed insects most were older than 50 years in rural areas and they were between 40 and 49 years in urban areas. The majority of the respondents in rural areas depend on subsistence farming (69.7%) with a monthly income of less than US$100 (64.0%).

For the respondents in the rural areas, no significant associations between consumption of edible insects and socio-demographic variables were found. For urban areas, there was a significant negative association between consumption and education (χ^2 =14.724, df=4, p=0.005), main source of

livelihood (χ^2 =30.966, df=7, p<0.001), and monthly income (χ^2 =24.449, df=7, p=0.001). The three characteristics were closely related. In urban areas there was a tendency for higher consumption of meat as a source of protein-rich foods with higher income (Puoane et al., 2006).

Table 2.5: Characteristics of the urban and rural respondents

	Urban : n=200 (n=160)[a]	**Rural : n= 175 (n=157)[a]**
Age group*		
≥ 18	0 (0)	0.6 (0.6)
19-29	21.5 (20)	12 (11.5)
30-39	30.5 (28.1)	21.7 (21.0)
40-49	32.0 (35.6)	13.1 (12.7)
>50	16 .0(16.3)	52.6 (54.1)
Gender		
Males	45.5 (46.3)	37.7 (40.1)
Females	54.5 (53.8)	62.3 (59.9)
Level of education*		
No education	1.0 (1.3)	8.6 (8.9)
Primary level	9.0 (7.5)	40.6 (41.4)
Secondary level	44.5 (40.0)	45.1 (44.6)
Vocational training	8.0 (7.5)	0.6 (0.6)
Tertiary	37.5 (43.8)	5.1 (4.5)
Main source of livelihood*		
Formal employment	43.5 (46.3)	7.4 (7.0)
Informal	34.0 (35.0)	12.0 (11.5)
Subsistence farming	3.5 (3.8)	69.7 (70.1)
Commercial farming	4.5 (5.6)	1.1 (1.3)
Casual labour	2.5 (2.5)	2.3 (1.9)
Remittances	1.5 (0.6)	0 (0)
Petty trade	5.0 (1.3)	1.1 (1.3)
Pension	0 (0)	1.7 (1.9)
Other	5.5 (5)	4.6 (5.1)
Monthly income (US$)*		
<100	9.5 (9.4)	64.0 (65.0)
100-199	19.5 (14.4)	20.0 (19.0)
200-350	22.5 (23.1)	9.1 (8.9)
351-450	13.5 (15)	4.0 (3.2)
451-600	13.5 (16.9)	1.7 (1.9)
601-800	9.0 (7.5)	0.6 (0.6)
801-1000	6.5 (8.1)	0.6 (0.6)
>1000	6.0 (5.6)	0 (0)

[a]The values between brackets represents characteristics of the respondents who actually consume edible insects

*Significance difference between urban and rural

2.3.4 Characteristics of the social enviroment of consumers

Religion can play an important role in preference and consumption of insects (Chakravorty et al., 2013; van Huis et al., 2013b). About 21.7% of urban and 8.7% of rural respondents are strictly forbidden from eating edible insects by their religion. On the other hand, 6.1% of the urban and 22.1% of the rural respondents' religion make them selective of the edible insects they eat. In Zimbabwe, 84% of the population are Christian (ZIMSTAT) and in this study 91.9% and 94.2% of urban and rural respondents, respectively, were Christian, although they belonged to different churches. The churches have different doctrines and differ in their views on the consumption of insects. While most traditional and pentecoastal churches are not prohibitive of insect consumption, the Apostolic churches strictly or selectively forbid consumption of insects. These sects believe that some insects are 'unclean', hence shouldn't be consumed. However, some respondents do not strictly adhere to their church's doctrines and do consume some edible insects. About 64% of the urban and 83% of the rural respondents reported that availability of edible insects influenced consumption, but most of the urban (>80%) and rural (>80%) respondents believed that their upbringing was the major influence and that eating insects was a family habit.

2.3.5 Rated importance of food characteristics of edible insects

Taste was the most important attribute of edible insects that the urban (95.6%) and rural (92.3%) respondents considered in deciding to consume them (Table 2.6) and this was true for consumers of *Eulepida* spp. and *Henicus whellani* as well, who particularly appreciated taste and texture (Table 2.7). For both insect species, salty taste and dry and crunchy texture were preferred. Moreover, urban respondents rated nutritional value (88.5%) as an important attribute. The results complement those on motives for consumption, which showed taste and nutritional value being major motives for consuming edible insects in urban areas.

Table 2.6 Frequencies (%) of respondents rating the importance of food characteristics in deciding to eat edible insects

Food characteristic	Urban	Rural
Taste	95.6	92.3
Texture	76.8	79.8
Apperance	54.9	76.8
Smell	56.6	71.8
Safety	61.5	61.3
Nutritional value	88.5	60.7

Table 2.7 Frequencies % of consumers appreciating the food characteristics of edible insects, *Eulepida* species and *Henicus whellani* when eating

Food	**Edible insects**		***Eulepida* species**		***Henicus whellani***	
Characteristic	*Urban*	*Rural*	*Urban*	*Rural*	*Urban*	*Rural*
Taste	98.8	98.7	97.1	93.1	93.8	97.1
Texture	75.6	78.7	81.4	82.7	87.5	74.3
Smell	62.0	82.6	52.9	75.0	75.0	70.0
Apperance	61.9	78.7	67.1	72.5	81.3	68.6

2.3.6 Traditional knowledge of harvesting and processing techniques for *Eulepida species* and *Henicus whellani*

About 35% of the urban and 39% of the rural respondents indicated that they had knowledge of harvesting and processing techniques for *Eulepida spp*. and 42% of rural but only 3% of urban respondents knew how to harvest and process *Henicus whellani* for consumption. The number of respondents with knowledge of insect processing was comparable with the number of consumers of the specific species. The similarity in percentage of the knowledgeable urban compared with rural respondents can be attributed to the former having a rural background and family habits of consuming such insect species. The lower percentage of respondents with knowledge of processing might be because of lack of traditional knowledge or because they were not used to consuming insects at all. Yen (2009) proposed that obtaining knowledge and support from traditional societies is important to advancing entomophagy. Knowledge of traditional systems can contribute to improvement of food and nutrition security (Alonso, 2015).

The preferred method of harvesting *Eulepida spp.* is by shaking host trees on which they are found and picking them from the ground by hand into harvesting containers. The common methods for harvesting *Henicus whellani* are collecting the insects after rains or by digging them from their burrows. In general, there is considerable variation in the processing of species before consumption. Boiling and roasting are common but there is variation in the time of boiling and amount of water used. For long storage, such as required for marketing, sun drying is the common method.

2.3.7 Conclusions and recommendations

Consumption of edible insects is still prevalent in both urban and rural areas of Zimbabwe, although consumption of particular species such as *Eulepida spp* and *Henicus whellani* is low compared to other more popular insect species such as mopane worms and termites. The most common way of eating insects is as a relish or snack. Frequency of consumption is higher in the rural than urban areas. Edible insects are not "just eaten" when it is the only food option. Taste and nutritional value are the major motives for consuming edible insects in both urban and rural areas. For respondents in urban areas, there is a significant negative association between consumption of edible insects and the socio-

demographic variables education, main source of livelihood, and monthly income. For respondents from rural areas, no significant associations were found. Differences in consumption patterns for specific insect species are likely due to individual preferences, specific availability in different geographic locations, and religious beliefs. Individual characteristics, availability, and the greater importance of nutritional value for urban consumers might explain the observed differences in the insect consumptions patterns between respondents from urban and rural areas. The environment of the consumer and indigenous knowledge of insect preparation do not seem to play important roles in the different consumption patterns.

In general, the observed high consumption of insects in rural as well as urban areas indicates that entomophagy is still dominant in Zimbabwe. To promote consumption of certain insect species with high nutritional potential, particularly in urban areas, lessons can be learnt from the mopane worm value chain. Mopane worms are now widely eaten across Southern Africa and have become a trading commodity (Stack et al., 2003). Likewise, in Thailand, insect consumption is no longer considered as food for rural or poor people, but has become common for urbanites (Hanboonsong et al., 2013). There, they commonly market both wild-harvested and farmed insects. Rearing insects is a strategy that can be used to improve availability of seasonal insects and can contribute to the development of insect value chains (Hanboonsong et al., 2013; Raubenheimer & Rothman, 2013). Such strategies could be useful in Zimbabwe because entomophagy is already common. Development of insect rearing farms and insect value chains, combined with development of attractive tasty products and communication of the nutritional value of *Eulepida spp.* and *Henicus whellani* could support the promotion of their consumption.

CHAPTER 3

Contribution of Wild Harvested Edible Insects (*Eulepida mashona* and *Henicus whellani*) to Nutrition Security in Zimbabwe.

This chapter has been published as: Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. Journal of Food Composition and Analysis, 75, 17-25. doi: 10.1016/j.jfca.2018.09.013

Abstract

Wild harvested edible insects have potential to contribute to nutrition security, yet their nutritional composition is often unknown. This study investigated the nutritional composition of wild harvested *Eulepida mashona* (EM) and *Henicus whellani* (HW) and variation in nutritional composition with respect to geographical place of harvest in Zimbabwe. Proximate and mineral composition, fatty acid, and amino acid profiles were analysed on samples of EM and HW collected at multiple locations from three and two districts in Zimbabwe, respectively. The protein content ranged between 52-56% (EM) and 59-70% (HW). High tryptophan concentration (8.68mg/g protein) in EM offers possibilities of using these insects in complementing this limiting amino acid in maize, which is the staple food in Zimbabwe. The fat content of both species was low (<10%), but differed significantly between sampling districts. The PUFA/SFA and omega 6/3 ratios of both species are recommendable for a healthy diet. The iron (24.2- 52.9mg/100g) and zinc content (10.0-20.9mg/100g) are high for both species, making them a useful mineral-containing ingredient for food enrichment. Consumption of 50g of both insect species will contribute on average to 30%, 50%, and 30% of the recommended daily protein, iron, and zinc respectively.

3.1 Introduction

Food and nutrition insecurities in the world call for the identification of sustainable sources of food. Edible insects have been identified as a potential new source for the Western world and are already a traditional one for many tropical countries (van Huis et al., 2013b). The nutritional composition of various edible insects has been studied and it is concluded that edible insects are valuable source of nutrients. Insects are usually characterized by high protein content. Some of them even meet the essential amino acids requirements (Rumpold & Schluter, 2013). They have the potential to complement limiting amino acids in other foods (van Huis et al., 2013b). Edible insects are also rich in minerals (Ajai et al., 2013; Christensen et al., 2006a), and vitamins (Alamu et al., 2013) and often contain high amounts of essential fatty acids. Sub Saharan countries experience seasonal and chronic food shortages, thus a broader utilisation of insects as alternative source of food on a wide scale could alleviate nutrition insecurity (Kinyuru et al., 2010a). The nutritional composition of many indigenous edible insects consumed in developing countries is yet unknown (Nowak et al., 2016) and the documentation of their nutritional importance to human diet is yet sparse (Jacob et al., 2013). Knowledge on these aspects could promote a broader utilisation of insects in Sub Saharan countries.
The nutritional composition of edible insects can be highly variable depending on the species, insect life stage, habitat, type of feed of the insects (van Huis, 2013; Verkerk et al., 2007), origin (Rumpold & Schluter, 2013), geographical place of harvest, seasonal and environmental factors (Zhou & Han, 2006) and the method of cooking (Yhoung- Aree et al., 1997; Zhou & Han, 2006). Also, differences in nutritional composition exist among orders (Bukkens, 1997; Kinyuru et al., 2013) and among species within the same orders (Bukkens, 1997; Rumpold & Schluter, 2013). To determine the potential of wild harvested edible insects for alleviating (local) nutritional problems, it is necessary to assess the variation in nutritional composition, particularly regarding protein and minerals, which are often the most critical in terms of nutrition deficiencies.

In Zimbabwe, just like any other Sub Saharan country, nutrition security is still a challenge. About 25% of the rural households were at risk of food insecurity in the period of 2013-2014 (ZIMVAC, 2014). According to the ZIMVAC report, meat and legumes, important protein sources, were the least consumed food groups by the rural population. Micronutrient deficiencies, particularly iron and zinc are a world-wide problem, especially in developing countries, and still remain a public health concern amongst Zimbabweans. One way of combating the deficiencies is by improving the amount of minerals absorbed from the diet and their inclusion in diets can improve iron status and prevent anaemia. According to Rumpold & Schluter (2013), consumption of edible insects could potentially reduce iron and zinc deficiency in developing countries.

A previous study on the consumption patterns of edible insects in Zimbabwe (Manditsera et al, 2018), reported that 80% of the urban and 89.7% of the rural respondents consume insects. The consumption

of *Eulepida mashona* (35.5%; 48%) and *Henicus whellani* (8.5%; 42%) significantly contributes to this figure. The major motives for insect consumption in both rural and urban areas were found to be tasty and nutritional. However, extensive knowledge on the nutritional value of these insect species is still lacking. Their proximate composition has been reported (Musundire et al, 2016), but an in-depth compositional analysis, in particular with regard to amino acid, mineral and fatty acid composition to evaluate the potential of these two insects species to human nutrition has not been performed yet. In addition, variation in nutritional composition with respect to geographical place of harvest has not been studied before. The aim of the current study is to fill this gap by investigating the nutritional composition of *Eulepida mashona* and *Henicus whellani* and how nutritional composition varies amongst different districts of harvest. Nutritional composition and potential to fulfil daily requirements are essential parameters to be considered before processing ingredients into products or dietary supplements (Ayessou et al., 2014).

3.2. Methodology

3.2.1 Sampling design and sample collection

Sampling for both insect species was done in the rainy season between November 2015 and January 2016. Sampling areas were considered based on information gathered during survey of the consumption patterns (Manditsera et al, 2018), selecting locations with prevalence of consumption for both insect species. *Eulepida mashona* was sampled from three districts and *Henicus whellani* from two districts. From each district, samples were collected from three sampling locations and each location was at least 10km away from the next. The sampling districts and locations are presented in Figure 3.1. In addition, for *Eulepida mashona*, the host trees from which the samples were harvested were noted. ZV1 and ZV2 samples were gathered from *Brachystegia spiciformis* (musasa) trees, ZV3 samples from *Piliostigma thonningii* (musekesa) trees. All samples from the district Mhondoro were collected from the trees of *Julbernadia globiflora*, (munhondo) and samples from the Seke district from *Brachystegia spiciformis*. The edible insects were collected according to local harvesting practices and transported to the laboratory within 12 hours. *Eulepida mashona* was harvested by a combination of handpicking from the host trees or shaking the host trees. For *Henicus whellani*, they were dug out from their burrows using a hoe.

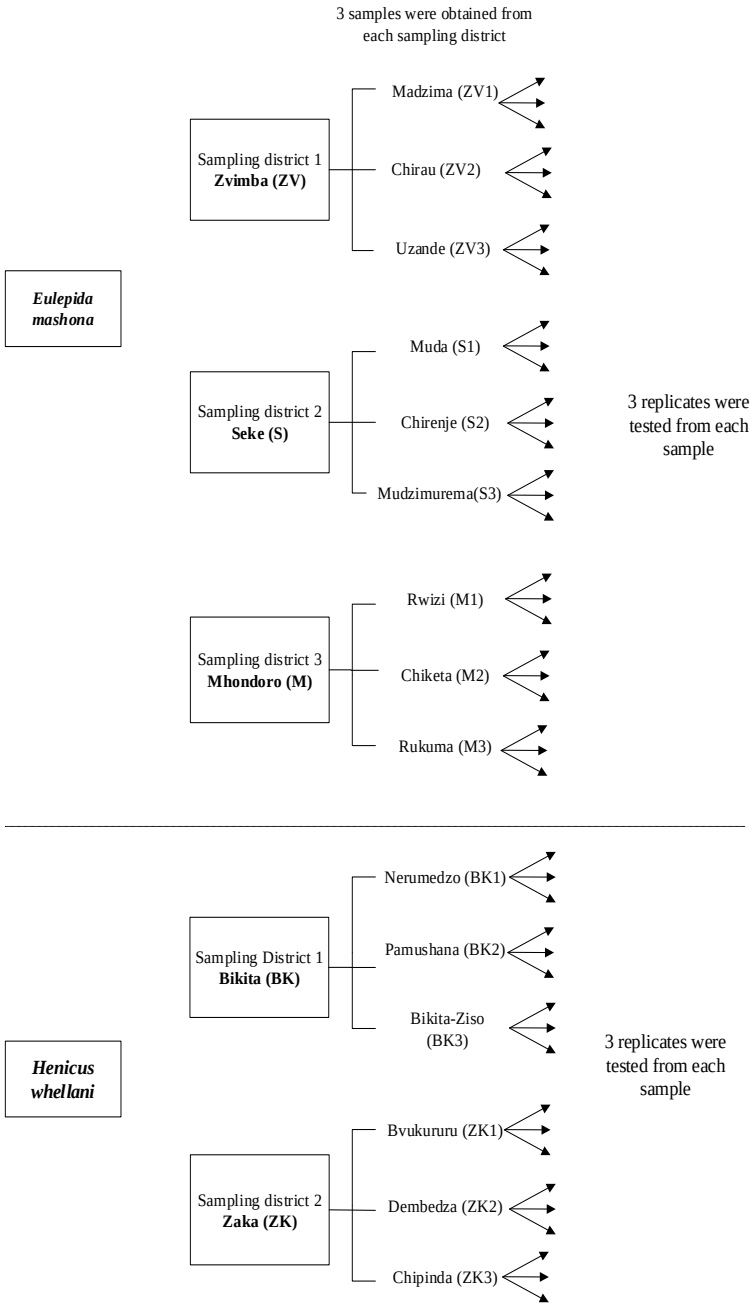

Figure 3.1: Sampling districts and locations for *Eulepida mashona* and *Henicus whellani*

3.2.2 Sample preparation and analysis

Upon arrival at the laboratory, the samples of live *Eulepida mashona* were put in sealable freezer bags and immediately frozen and kept at -21^{0}C a process, which also killed them. Directly after harvesting, the samples of *Henicus whellani*, were degutted by pulling the back of the insects and washed with clean potable water. Excess water from washing was drained. Then the insects were packed in sealable freezer bags and put in cooler boxes filled with ice for transport to the laboratory. The insects were then kept at -21^{0}C until analysis.

Frozen *Eulepida mashona* were manually de-winged following the customary local preparation procedure. Both species (100g for each replicate) were freeze-dried until they reached a constant weight. The freeze-dried samples were then ground to a homogenous sample using Pulverissete 14 rotor mill (Fritsch, Idar-Oberstein, Germany), at a speed of 6000 rpm. Further milling to a fine powder was done using a MM400 Milling machine (Retsch, Haan, Germany). Ground samples were then kept in tightly closed containers at -21^{0}C until they were used for subsequent analysis. Each sample was analysed in triplicate unless mentioned otherwise.

3.2.2.1 Proximate analysis

Nitrogen content was determined according to the Dumas method using Flash EA 1112 N analyser (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a D-methionine standard (≥98%, Sigma Aldrich). A factor of 4.76, according to Janssen et al, (2017) was used to convert the obtained nitrogen content to protein content. Fat content was determined using Soxhlet extraction method using petroleum ether for six hours. The solvent was then evaporated with rotor evaporator (R420, Buchi, Flawil, Switzerland) at 350mbars at 40^{o}C water bath. Lipid extracts were stored under nitrogen at -21^{o}C for fatty acid composition analysis. Ash content was determined by treating samples at 525^{o}C in a muffle furnace (Gallenkamp Hotspot Furnace, Gemini BV, Apeldoorn, Netherlands) for 18 hours. The remaining weight was considered as ash.

For crude chitin determination, a suspension of defatted insect powder and NaOH (11%), (1:10w/v) was incubated in a water bath at 95^{o}C for 6 hours for deproteinisation. The suspension was centrifuged afterwards for 15 minutes at 3500g to remove the upper layer. The remaining solid was washed several times with demineralised water and was incubated overnight at 70^{o}C. Then 10 mL hydrochloric acid (5%) was added to the de-proteinised sample for demineralisation and incubated in a water bath at 50^{o}C for 7 hours. Afterwards, the suspension was centrifuged at 4500g for 20 minutes, followed by washing of the residue with demineralised water and then the sample was incubated overnight at 70^{o}C. The residue was weighed and designated to be crude chitin.

3.2.2.2 Amino acid content analysis

Amino acid composition of the two insect species was determined in duplicate using ion-exchange chromatography. Samples to determine cysteine and methionine content were prepared by oxidation with performic acid, (16 hours, 0-5ºC), followed by acid hydrolysis with 6M HCl (22 hours, 105-110ºC). For tryptophan, alkaline hydrolysis with 4.2M NaOH (22 hours, 105-110ºC) was performed. Samples to determine the other amino acids were prepared by acid hydrolysis with 6M HCl (22 hours, 105-110ºC).

The hydrolysates were mixed with an internal standard solution and the mixture was injected into the analyser. Samples were then analysed by ion-exchange chromatography (30 amino acid analyser, Biochrom, Cambridge, United Kingdom), using a weak acidic cation exchange resin as stationary phase and weak acidic Li-citrate buffers as mobile phase. Detection was after post column derivatisation with ninhydrin at 570 or 440 nm. Tryptophan was analysed using a Dionex Ultimate 3000 HPLC (Thermo Fisher Scientific Inc, Waltham, MA, USA) with Phenomenex Synergy Hydro C18, 150 x 4.6 mm as stationary phase and phosphate buffer/methanol gradient mobile phase with fluorimetric detection. The mixtures were analysed versus a standard-internal standard mixture.

3.2.2.3 Mineral content analysis

Ground samples (300mg) were hydrolysed with concentrated nitric acid (65%) and hydrochloric acid (37%) by microwave digestion (MARS-X, CEM, USA). The nitrous vapours were then removed by addition of hydrogen peroxide. The selected elements were then measured with the aid of ICP-AES (iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, MA, USA). ICP-AES conformed to the guidelines of NPR-6425 and NEN 6966. The mineral analysis was performed in duplicate.

3.2.2.4 Fatty acid composition

Fatty acids were analysed as fatty acids methyl esterase (FAMEs) prepared according to the method of Metcalfe et al. (1966). Prepared samples (0.5 µL) were then analysed by a gas chromatograph (Agilent 6890N, Agilent Technologies, Inc., Washington, USA) with CP-Wax 58 (FFAP) column (length 25m; 0.25mm ID, film thickness=0.2µm) and Flame Ionisation Detector. Quantification was done using the Agilent OpenLAB software.

3.2.2.5 Statistical analysis

The data on macronutrient composition was subjected to one way ANOVA using SPSS (IBM SPSS Statistics Version 23) and significant differences were reported at 5% level of significance. Post hoc analysis to determine which samples were significantly different was done using either Tukey or Games Howell (in case of unequal variances).

3.3. Results and Discussion

3.3.1 Macronutrient composition of *Eulepida mashona* and *Henicus whellani*

Table 3.1 shows the proximate composition of *Eulepida mashona* and *Henicus whellani* sampled from different districts and locations. The protein content of *Eulepida mashona* and *Henicus whellani* ranged respectively between 52-56% and 59-70%. The difference in the protein content of *Eulepida mashona* and *Henicus whellani* amongst the sampling locations from the same district was less than 4% and between 3-10% respectively. Significant differences were found between some sampling locations in Zvimba and Mhondoro districts for *Eulepida mashona*. When assessing the differences at district level, only for *Eulepida mashona*, harvested in the Seke and Mhondoro districts, the average protein content differed significantly. For *Henicus whellani* the average content between the Bikita and the Zaka district did not differ significantly, although significant differences were found between sampling locations within both districts (Table 3.1).

The protein contents of both *Eulepida mashona* and *Henicus whellani* found in the current study were above the average concentrations as observed in another study on species of the same insect order, which were Coleoptera (40.7%) and Orthoptera (61.3%) respectively (Rumpold & Schluter, 2013). However, in their paper, Musundire and co-workers (2016) found an average protein content of 62.4% for *Eulepida mashona* and 64.3% for *Henicus whellani* which is higher than those reported here. This difference can be explained by the use of a different nitrogen to protein conversion factor used for calculating the protein content. Janssen et al. (2017) discussed that the factor 6.25 used for converting the measured nitrogen into protein content of insects can lead to an overestimation of more than 20% in insects, because of the presence of non-protein nitrogen. Therefore, in our study, we decided to calculate the protein content using 4.76 as conversion factor. On the other hand, the study from Janssen and co-authors (2017) was performed on larvae and the current work is about adult insects. It is possible that adult insects have a different specific nitrogen conversion factor due to the variable chitin content of insect species (Jonas-Levi & Martinez, 2017).

Table 3.1: The macronutrient content based on dry matter content (mean ±S.D) of *Eulepida mashona* and *Henicus whellani* sampled from different sampling locations and districts.

	Sampling district	Sampling location	% Protein	% Fat	% Ash
Eulepida mashona	Zvimba	ZV1	56.1±0.91[a]	7.21±0.86[a,b]	4.61±0.05[a]
		ZV2	54.9±0.76[a]	7.45±0.33[a]	4.70±0.08[a]
		ZV3	52.4±0.60[b]	6.46±0.34[b]	5.11±0.15[b]
			54.5±1.74[A,B]	**7.04±0.68[A]**	**4.80±0.24[A]**
	Seke	S1	53.9±0.99[a]	8.60±0.44[a]	4.64±0.12[a]
		S2	53.4±0.56[a]	6.98±0.43[b]	4.67±0.06[a]
		S3	54.2±0.22[a]	7.94±0.49[a]	4.82±0.11[a]
			53.83±0.73[A]	**7.84±0.81[B]**	**4.71±0.12[A]**
	Mhondoro	M1	56.9±0.80[a]	8.02±0.46[a]	4.64±0.06[a]
		M2	54.6±0.57[b]	9.13±0.56[b]	4.51±0.05[a]
		M3	53.9±0.44[b]	10.04±0.42[b]	4.52±0.37[a]
			55.1±1.12[B]	**9.06±0.96[C]**	**4.56±0.22[A]**
Henicus whellani	Bikita	BK1	69.3±0.41[a]	5.89±0.57[a]	4.97±0.05[a]
		BK2	65.4±0.63[b]	6.27±0.49[a]	4.86±0.14[a]
		BK3	59.9±1.57[c]	11.2±1.22[b]	5.13±0.24[a]
			64.8±4.05[A]	**7.77±2.59[A]**	**4.98±0.19[A]**
	Zaka	ZK1	70.7±1.12[a]	5.04±0.71[a]	5.33±0.25[a]
		ZK2	62.9±1.12[b]	10.35±1.60[b]	5.14±0.52[a,b]
		ZK3	66.5±0.91[c]	7.34±1.33[c]	4.51±0.23[b]
			66.7±3.40[A]	**7.58±2.54[A]**	**4.99±0.49[A]**

Values with same superscript in the same column for the same district are not significantly different from each other at 5% level of significance; Values in bold are the mean ±S.D (n=3) proximate contents of district samples; Values with the same superscript for bold values in the same column are not significantly different

The fat content of *Eulepida mashona* and *Henicus whellani* ranged respectively between 6.5-10.0% and 5.0-11.2% (Table 3.1). The difference in the fat content between the sampling locations in the same district was less than 2% for *Eulepida mashona* and was maximally 5% for *Henicus whellani*. The mean fat content of *Eulepida mashona* and *Henicus whellani* significantly differed between all the three and two districts, respectively. Furthermore, the fat content of *Henicus whellani* from all the sampling locations in ZK significantly differed. Insects collected in BK3 had the highest fat content (11.6%), which significantly differed from BK1 and BK2. In both BK and ZK, the highest fat content observed was double the lowest content. In general, adult insect species have low fat content as compared to larvae. Both insect species studied are consumed in the adult stage. The low fat content of some insects has been recommended as a benefit in replacing or supplementing food or ingredients from vertebrates (Gjerris et al., 2015). Belluco et al. (2013) reported that fat content of edible insect species ranged from 7 to 77g/100g dry matter, although contents less than 5g/100g were reported for some other species (Omotoso, 2006; Yang et al., 2006). Moreover, crude fat within species could vary because of differences in the reproductive states, season, age/life and sex (Barker et al., 1998), and diet

(Oonincx & van der Poel, 2011). Oonincx and van der Poel (2011) also found that insects with higher fat contents had lower nitrogen (protein) contents, which is a trend that is also observed for *Henicus whellani* (Table 3.1). The fat content of the species is lower than reported for *Gonimbrasia belina* (16.4%) and *Macrotermes natalensis* (41.6%), the latter are also commonly consumed edible insects in Zimbabwe (Musundire et al., 2016).

The ash content provides an indication of the mineral content of food. The mean ash content of *Eulepida mashona* (4.50-5.11%) is comparable to that of *Henicus whellani* (4.51-5.33%) as shown in Table 3.1. The mean ash content of *Eulepida mashona* was higher than the 3% reported by Musundire et al (2016) but comparable to species in the Coleoptera order (Rumpold & Schluter, 2013). The ash content of *Henicus whellani* was lower than the 16.1% reported for *Henicus whellani* before (Musundire et al., 2014a), but higher than observed in species in the Orthoptera order (Rumpold & Schluter, 2013). The ash content did not differ significantly amongst districts for both *Eulepida mashona* and *Henicus whellani*, except for two sampling locations (ZV1 and ZV3; ZK1 and ZK3). For *Eulepida mashona* harvested in Zvimba 3, the ash content differed significantly with the other two locations. Likewise, a significant difference in ash content was found for *Henicus whellani* harvested from Zaka location 1 and 3.

For chitin, our results show that the average content for *Eulepida mashona* did not differ significantly between the three districts: Zvimba (9.75±1.00); Seke (8.91±1.20) and Mhondoro (10.2±0.97). Similarly, the average chitin content of *Henicus whellani* did not differ significantly for Bikita (11.0±0.54) and Zaka (11.8±0.70). In our study, the chitin content of both species is higher than reported for field crickets (8.7%) (Wang et al., 2004), but lower than reported for *L. decemlineata* (20%) (Kaya et al., 2014). Chitin content is variable in insects because it depends on the developmental stage of the insects (Jonas-Levi & Martinez, 2017) and the type of insect. Chitin, being a component of the fibre fraction, contributes to the fibre content of insects, and it can negatively influence absorption of other nutrients such as proteins (Dossey et al., 2016; Marono et al., 2015). However, chitin has also some health benefits, such as reducing serum cholesterol (Omotoso, 2006).

3.3.2 Amino acid profiling of *Eulepida mashona* and *Henicus whellani*

The nutritional quality of a protein is determined by the amino acid composition, particularly by the concentration of the essential amino acids (Smith, 2010). Table 3.2 shows that the total amino acid content of *Eulepida mashona* and *Henicus whellani* is 629 and 636 mg/g respectively. The total essential amino acid (EAA) content in *Eulepida mashona* (288mg/g protein) meets the requirements of WHO/FAO (277 mg/g protein) for adult humans. However, leucine, lysine, methionine, and valine content do not meet the specific EAA requirements. For *Henicus whellani*, the total EAA content (274mg/g protein) was similar, but just below the WHO/FAO requirement. Both species contained

lower amounts of essential amino acids than the average of species of Coleoptera and Orthoptera orders (Rumpold & Schluter, 2013). The percentage of EAA in total amino acid and the ratio of EAA to non-EAA are used as parameters for assessing the protein quality. According to the WHO these ratios should be at least 40% and greater than 0.6 respectively (Belluco et al., 2013). Both *Eulepida mashona* (42.2% and 0.8) and *Henicus whellani* (43.4% and 0.76) met these requirements. *Eulepida mashona* and *Henicus whellani* can thus provide satisfactory amounts of the essential amino acids.

It is worth to note that not all insect species can meet the daily WHO/FAO essential amino acid requirements (Rumpold & Schluter, 2013; Zielińska et al., 2015) and often the concentration of some essential amino acids is below the recommendations. Both species studied in this paper are limited in some essential amino acids, which implies that other protein sources should complement the diet. Interestingly, the tryptophan (8.68mg/g protein) content in *Eulepida mashona* is above the WHO/FAO (6mg/g protein) requirement. Since maize, which is the staple food for Zimbabwe, has a very low tryptophan content, its combination with consumption of *Eulepida mashona* could complement the required daily intake of essential amino acids. However, the amino acid profile alone does not give a complete reflection of the protein quality. The protein digestibility is also a measure of protein quality. Musundire et al (2016) reported the presence of anti-nutrient components in both species, which may reduce the protein digestibility. Heat treatment, however, can enhance the digestibility by denaturation of proteins and reduction of anti-nutritional factors (Boye et al., 2012), but also reduction of digestibility has been found upon heat treatment (Poelaert et al., 2016). The protein digestibility is quite variable for different species (Finke & Oonincx, 2017).

Table 3.2: Amino acid profiling (mg/g protein) of *Eulepida mashona* and *Henicus whellani*

	Essential amino acids												
	Hist	Ile	Leu	Lys	Met	Thr	Trp	Val	Cys	Phe+ Try	Met+ Cys	Total EAA	Total AA
Eulepida mashona	**16.1±0.14**	**32.0±0.03**	48.5±0.10	39±0.61	*10.2*±0.08	**24.0±0.38**	**8.68±0.11**	35.2±0.22	**8.0±0.02**	**49.2±0.54**	18±0.11	**288±1.87**	629±7.35
Henicus whellani	**15.5±0.04**	28.7±0.07	51.4±0.21	33±0.96	*8.8*±0.09	**24.2±0.30**	5.2±0.15	35±0.33	5.8±0.07	**52.3±0.25**	14.7±0.16	274±1.77	636±3.61
[a]Requirements mg/g protein	**15**	**30**	**59**	**45**	**16**	**23**	**6**	**39**	**6**	**38**	**22**	**277**	
	Non-essential amino acids												
	Asp +Asn		Ser		Pro		Ala	Gly	Arg	Glu+Gln			
Eulepida mashona	56.9±069		28.2±0.52		35.2±0.32		75±1.99	48.2±0.32	32.8±0.53	82.1±1.24			
Henicus whellani	50.3±1.01		29.9±0.52		38.4±0.3		102±0.86	46.7±1.11	37.5±0.8	71.8±1.8			

Values in bold are essential amino acids meeting the requirements; [a](WHO/FAO/UNU, 2007); Italicised values represent the limiting amino acid.

3.3.3 Mineral content of *Eulepida mashona* and *Henicus whellani*

The dietary intake of minerals plays an important role in human biological systems. The presence of iron and zinc have been emphasised as a potential nutrition plus of insects over other protein sources (van Huis, 2017). Figure 3.2 shows the contents of iron and zinc found in the *Eulepida mashona* and *Henicus whellani* samples. The iron content of *Eulepida mashona* (25 to 54mg/100g) was comparable to *Henicus whellani* (30 to 55mg/100g). The zinc content in both insect species ranged from 16 to 21mg/100g and differences between samples from the same districts were less than 5mg/100g. However, the iron content of both species differed significantly between sampling locations and districts. Large variation in iron content exists for samples harvested from different sampling locations in the same district. For example, the iron content of *Eulepida mashona* samples from ZVI and ZV2 was roughly twice as high as in samples from ZV3. For *Henicus whellani*, iron content of samples from ZK3 varied from ZK2 by more than two thirds. The variation in zinc content was lower than observed for iron. The largest variation between sampling locations was 33% for *Eulepida mashona* (ZV1 and ZV3) and 16% for *Henicus whellani* (ZK1 and ZK2).

Not all minerals show the same type of variation as iron and zinc and the results are presented in Table A.1. The calcium content varied substantially. In *Henicus whellani* the calcium content varied with more than 100% between some sampling locations, whereas for *Eulepida mashona* the largest variation was just below 50%. According to various researchers, the content of some minerals, particularly Ca, K, Na and Mg could be significantly influenced by the feed of insects (Oonincx & van der Poel, 2011; Rumpold & Schlüter, 2013). This could explain differences particularly for *Eulepida mashona* from Zvimba districts, which were sampled from two different host tree species.

Potassium is the most abundant mineral in both *Eulepida mashona* (1439-1615mg/100g dry matter) and *Henicus whellani* (1110-1475mg/100g dry matter). The *Eulepida mashona* samples contained higher concentrations of phosphorous, magnesium and sulphur compared to *Henicus whellani*, whereas the latter contained higher concentrations of aluminium, sodium and calcium. The differences in mineral content composition are expected as content varies between species (Rumpold & Schluter, 2013). Apart from iron and zinc, both species can significantly contribute to required amounts of other minerals such as copper. The copper content of *Eulepida mashona* (2.33-4.7) and *Henicus whellani* was higher than the recommended 0.9mg/day.

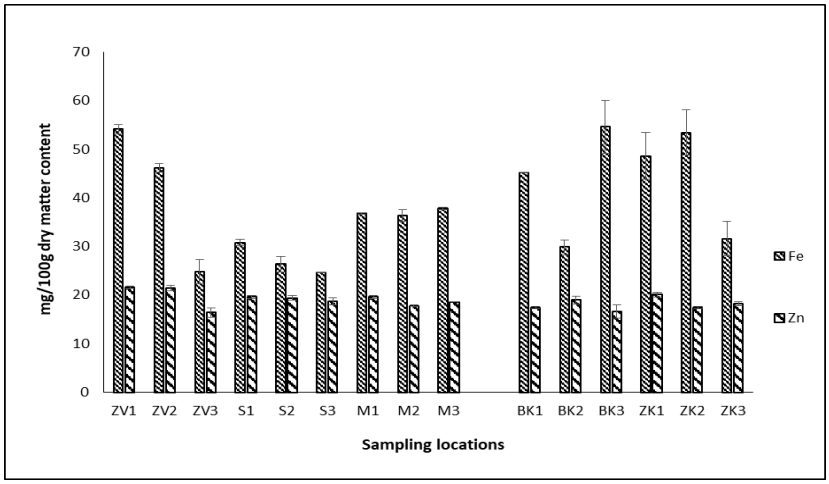

Figure 3.2: Iron and zinc contents of *Eulepida mashona* and *Henicus whellani* from different sampling locations

3.3.4 Fatty acid profiling of *Eulepida mashona* and *Henicus whellani*

The consumption of edible insects is being promoted for their richness in essential fatty acids especially the poly unsaturated fatty acids (van Huis et al., 2013b). The most abundant fatty acid in *Eulepida mashona* was palmitic acid (C16:0), which ranged from 30-35g/100g fat. For *Henicus whellani* oleic acid, (C18:1n-9) was most abundant and ranged from 38-43g/100g fat. Overall, *Henicus whellani* contained more mono-unsaturated fatty acids (42-47g/100g fat) than *Eulepida mashona* (24-35g/100g fat) (Fig. 3.3A and 3.3B). The level of polyunsaturated fatty acids in *Eulepida mashona* was comparable (20.4-34.2mg/fat) to *Henicus whellani* (20.9-27.5mg/100g fat). *Eulepida mashona* contained the highest concentration of saturated fatty acids, which ranged between 36-41mg/100g fat. As a result, the unsaturated to saturated fatty acids ratio of *Henicus whellani* (ranging 2.17-2.52) was higher than that of *Eulepida mashona* (ranging 1.33-1.65). The PUFA/SFA ratio of both *Eulepida mashona* (0.51-0.89) and *Henicus whellani* (0.67-0.95) were comparable and ratio lies within the recommended values between 0.4 and 1, for a healthy diet (Jiménez-Colmenero, 2007; Paul et al., 2017).

The omega 6 fatty acids content in *Henicus whellani* was higher (18-25g/100gfat) than that of *Eulepida mashona* (12-17g/100g fat), whereas the latter contained higher concentrations of omega 3 fatty acids (5-21mg/ 100mg fat). Due to the variation in the fat fractions, the omega 6 to 3 ratios of *Eulepida mashona* (which ranged between 0.6-2.7) was low as compared to *Henicus whellani* (ranging between 6.2-10.1). *Eulepida mashona* could meet the recommended ratio 3:1 of omega 6 to 3 fatty acids (Belluco et al., 2013). The lower ratio is desirable in reducing risk of chronic diseases, such as

cardiovascular diseases (Simopoulos, 2002). The omega 6 to 3 ratio of both species are lower as compared to reared *Tenebrio molitor* larvae (27.1) and *Acheta domesticus* (13.26) (Tzompa-Sosa et al., 2014). Paul et al. (2017) reported an even higher ratio of the omega 6/3 for *Tenebrio molito*r larvae (204.15) and *Acheta domesticus* (37.04). A high ratio is usually associated with a high concentration of omega 6 fatty acids in the diet (Paul et al., 2017). The lower omega 6/3 in the studied species could be an indication that wild harvested insects could contribute to healthier diets.

The fatty acids profiles of both insects are similar to previously reported profiles of other insects (Tabassum et al., 2016; Zielińska et al., 2015), and are comparable to those of chicken and fish (Zielińska et al., 2015). The fatty acid profiles of both species make them good sources of essential fatty acids, specifically because of the polyunsaturated fatty acids (PUFA). Variation in the fatty acid profiles were observed for both species, with the greatest variation in PUFA fractions between sampling locations for *Eulepida mashona*. Fatty acid composition is affected by the plants insects feed on (Bukkens, 1997). For example, *Eulepida mashona* gathered from *Piliostigma thonningii (*ZV3) had a fatty acid profile different from samples gathered from *Brachystegia spiciformis* (ZV1 and ZV2). The effect of the host tree on the fatty acid profile cannot be conclusive as samples of *Eulepida mashona* from the same host tree, *Brachystegia spiciformis*, showed large differences in the PUFA and omega 3 fatty acids for Zvimba and Seke districts. The insects supposedly feed from the host trees but might use other feed sources as well, which could explain the differences in fatty acid composition. Namely, the fatty acids composition of edible insects can be manipulated by the feed the insects are consuming (Finke & Oonincx, 2017). Apart from the insects' diet, fatty acid profiles can also be influenced by environmental conditions and stage of life (Paul et al., 2017). During harvesting of *Eulepida mashona* and *Henicus whellani* for consumption, there is no selection based on size or sex, hence a mixture of different stages and sexes is present, which can be a source of variation.

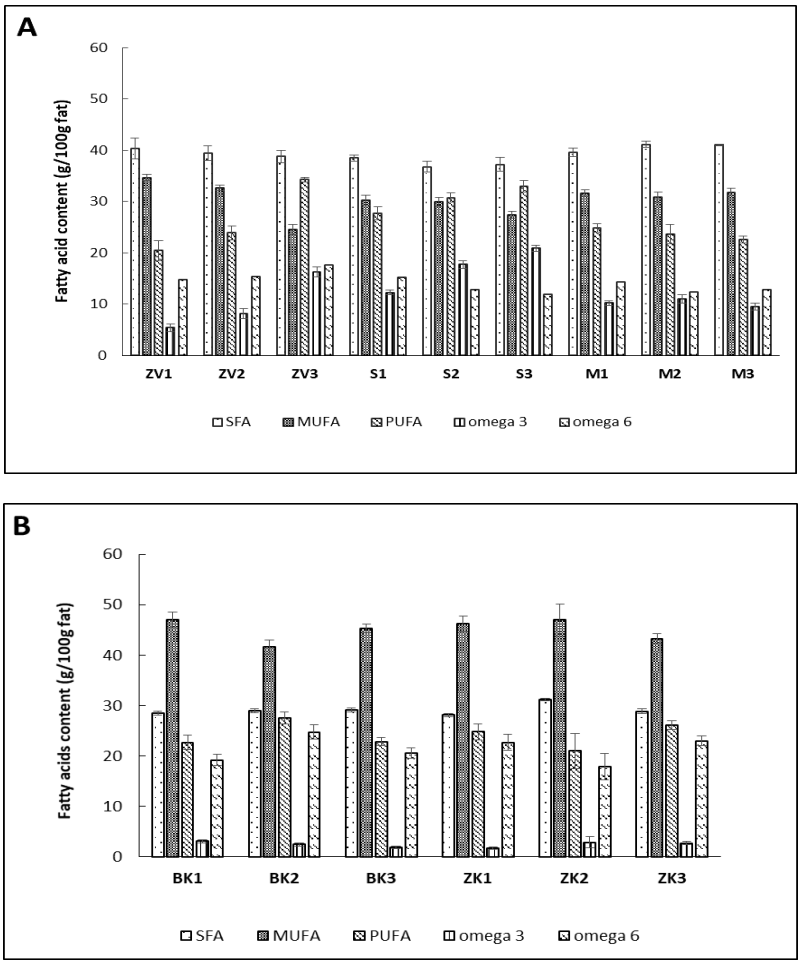

Figure 3.3: Fatty acid content of *Eulepida mashona* (A) and *Henicus whellani* (B) from different sampling locations

3.3.5 Potential contribution of the *Eulepida mashona* and *Henicus whellani* to human nutrition

The nutritional content on a fresh weight basis was considered to discuss the potential contribution of the two studied insect species to the human diet. A comparison with other common protein sources (beef, beans, eggs, wheat flour and whole milk) consumed in Zimbabwe was done. Interestingly, the protein content of both *Eulepida mashona* (16.9±0.45) and *Henicus whellani* (17.2±0.99) was lower but comparable to that of beef (23.2%) (P. G. Williams, 2007) and beans (23.4%) (USDA, 2017) and higher than that of wheat flour, milk and eggs. The iron contents found in the *Eulepida mashona* (10.9±3.04 mg/100g) and *Henicus whellani* (11.5±3.35g/100g) samples were higher than the amounts commonly found in other protein sources. However, the iron content is comparable to 10.44mg/100g of beans. The zinc contents found in both species based on fresh weight is circa 5mg/100 fresh insects,

content higher than in other protein sources. The fat contents in both species are lower than found in eggs, beef, and whole milk.

According to a previous study, the majority of consumers of the *Eulepida mashona* and *Henicus whellani* eat quantities equivalent to 50g (Manditsera et al., 2018). The protein and mineral content of *Eulepida mashona* and *Henicus whellani* on fresh weight basis, combined with the actual consumption data (average of 50g) and recommended daily intakes demonstrates that these wild harvested insects could potentially contribute to the Zimbabwean diet. Namely, consuming 50g of insects, when they are in season, provides 15% of the daily protein requirement of 50-63g per day. Whilst the edible insects are mainly consumed for their protein content, they will also contribute significantly to the recommended daily requirements of iron and zinc. Consuming 50g of the insects per day could fulfil more than half the daily recommended iron intake of 8mg/day and 3mg of the recommended 11mg/day for zinc.

3.4. Conclusion

Overall, the investigated insect species have potential to be used as directly in the diet or as an ingredient in food products based on their protein content, amino acid composition, fat composition, and Zn and Fe levels. The high protein content of *Eulepida mashona* (52-56%) and *Henicus whellani* (59-70%) makes the insects a good protein source. However, the natural variation in protein, fat, and mineral composition of wildly harvested insect species should be taken into account in the recommendations for using these insect species in the diet. Variation in protein content with geographical place of harvest is species dependent with variation of up to 10% observed in *Henicus whellani* and less than 4% for *Eulepida mashona*. The protein quality, based on the percentage of EAA in total amino acid and ratio of EAA to non-EAA, was good as both insects met the WHO requirements. Both insect species have a low fat content (<10%) and comply with the recommended PUFA/SFA ratio for healthier diets. The iron and zinc content of both species could substantially contribute to the recommended daily intake (50% and 30% respectively).To extend the nutritional benefits to communities and population at large, there is a need to explore the opportunities of commercial insect rearing, storage practices, and shelf life extensions studies as wildly harvested insects are only seasonally available.

Supplementary Material

Table A1: Mineral content (mg/100g dry matter) of *Eulepida mashona* and *Henicus whellani* from different sampling locations

Sampling location	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
					Eulepida mashona						
ZV1	1.76±0.44	65.8±2.20	4.70±0.04	54.1±0.95	1493±15	226±2.93	1.72±0.22	130±1.47	787±11.0	625±6.60	21.6±0.22
ZV2	1.91±0.22	75.6±3.64	3.90±0.11	46.2±0.87	1461±29	234±7.27	4.73±1.16	129±0.29	805±9.46	626±7.99	21.4±0.59
ZV3	3.27±0.14	98.1±1.44	2.33±0.13	24.8±2.53	1651±22	243±2.17	11.7±3.47	90.5±3.47	831±8.68	562±6.51	16.4±1.01
S1	0.65±0.07	62.4±0.00	2.38±0.08	30.8±0.65	1474±14	264±0.00	2.15±0.00	78.8±4.78	874±4.35	608.±4.34	19.6±0.29
S2	4.09±0.07	88.0±5.85	2.61±0.04	26.4±1.61	1439±29	298±6.60	22.7±0.95	83.8±1.39	840±19.0	567±5.13	19.4±0.44
S3	4.49±0.22	68.1±1.46	2.62±0.04	24.6±0.08	1460±7.29	287±5.10	10.1±0.00	62.3±1.09	861±16.1	574±6.57	18.7±0.73
M1	0.57±0.07	56.3±0.73	2.76±0.06	36.8±0.14	1473±22.0	240±0.73	1.81±0.21	94.2±3.07	841±5.11	633±0.00	19.6±0.37
M2	0.51±0.00	55.0±2.88	2.74±0.02	36.3±1.22	1461±7.20	230±2.16	1.07±0.07	95.2±1.08	816±5.04	610±4.32	17.8±0.15
M3	0.67±0.07	59.5±5.80	2.73±0.06	37.8±0.29	1507±29	234±9.42	1.75±0.15	95.9±0.66	832±21.0	616±5.80	18.6±0.00
					Henicus whellani						
BK1	138.1±16.0	50.5±7.29	2.97±0.04	45.2±0.08	1475±29	83.6±2.92	1.91±0.07	176±16.4	512±26.3	470±10.9	17.4±0.22
BK2	51.4±10.3	158.4±7.22	3.31±0.35	29.9±1.44	1390±29	95.5±0.72	1.99±0.22	196±10.1	597±2.17	494±4.33	19.0±0.80
BK3	110.5±8.6	125.4±4.12	2.44±0.16	54.6±5.47	1110±110	86.2±3.64	2.22±0.07	149±6.34	570±26.2	453±31.3	16.6±1.39
ZK1	110.4±32.4	49.3±8.71	2.68±0.11	48.6±24.8	1458±70	85.2±2.91	1.54±0.00	226±13.4	469±45.0	444±12.3	20.1±0.50
ZK2	134.0±0.07	105.8±14.5	2.64±0.06	53.4±4.65	1336±879	97.1±32.57	2.78±0.15	241±28.2	548.5±12.3	445±0.00	17.4±0.22
ZK3	75.3±9.05	107.4±20.4	3.48±0.56	31.6±3.50	1378±22	93.9±6.23	2.01±0.07	182±8.24	558±12.4	484±0.00	18.2±0.37

CHAPTER 4

Effect of Domestic Cooking Methods on Protein Digestibility and Mineral Bioaccessibility of Wild Harvested Adult Edible Insects

This chapter has been published as: Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. Food Research International, 121, 404-411. doi: https://doi.org/10.1016/j.foodres.2019.03.052

Abstract

Wild harvested edible insects are characterised by high protein and mineral contents with potential to contribute substantially to nutrition security. However, nutritional content is only beneficial when proteins are digestible and minerals bioaccessible. This study determined the effects of domestic processing on protein digestibility and mineral bioaccessibility of two wild harvested insect species: *Eulepida mashona* (beetle) and *Henicus whellani* (cricket). Samples of both insects were subjected to boiling, roasting, or combined boiling and roasting, imitating the way insects are traditionally prepared in Zimbabwe. Moreover, they were *in vitro* digested according to INFOGEST protocol. Boiling of both insects resulted in loss of protein as it leached into the boiling water. The raw insects had a higher protein *in vitro* digestibility than the boiled and roasted insects, and the maximal decrease in protein digestibility was around 25% for twice boiling of the beetles and for boiled and roasted crickets. For both insect species, boiling resulted in non-significant loss of iron and zinc. Iron was the least bioaccessible mineral in both insects, based on the concentrations of soluble mineral measured by ICP-AES. However, beetles had a much higher iron bioaccessibility (30.7%) as compared to crickets (8.11%). Interestingly, boiling resulted in about 50% decrease in iron and zinc bioaccessibility in both species while roasting did not. The reduced protein digestibility and mineral accessibility with processing can be explained by protein modification and interactions of minerals with other food components, such as chitin and phytochemicals. Because of the reduction in protein digestibility and mineral accessibility during boiling, roasting should be favoured over boiling and in any case short boiling time is recommended.

4.1 Introduction

Protein energy malnutrition and micronutrient deficiencies, particularly iron and zinc are still a persistent problem especially, in developing countries. One way of mitigating the problem is to improve the amount of nutrients absorbed from local diets. Over the past years, edible insects have gained recognition for their potential as an alternative protein source (van Huis, 2016). In general, edible insects are good sources of protein and micronutrients despite the variation that exists within different species (Manditsera et al., 2018; Rumpold & Schluter, 2013). In many tropical countries, consumption of insects is part of tradition (Chakravorty et al., 2013). The insects are consumed for their taste and nutritional value (Chakravorty et al., 2013; Dube et al., 2013; Manditsera et al., 2018). Due to their recognition as food and contribution to nutrition security, a substantial number of studies published on the nutritional composition of both wild harvested (Christensen et al., 2006; Lautenschläger et al., 2017; Manditsera et al., 2018) and reared insects (Janssen et al., 2017; Oonincx & Dierenfeld, 2012; Yi et al., 2013). The reported nutritional compositions are usually based on analysis of raw insects. However, the overall content of nutrients in a food does not provide a full picture about their nutritional quality: the bioaccessibility of the particular nutrient is a key parameter to consider (Fernandez-Garcia et al., 2009).

The bioaccessibility of nutritional compounds is directly influenced by the composition of the food matrix and the interaction with other components permitting the digested material to be available to the body (Fernandez-Garcia et al., 2009). Bioaccessibility of a nutrient is defined as the fraction that is soluble in the gastrointestinal environment and available for absorption (Cardoso et al., 2015) It is expected that bioaccessibility will vary between insect species (Latunde-Dada et al., 2016). The presence of anti-nutritional components, such as tannins and phenolic acids in some edible insects (Musundire et al., 2014a) could hinder bioaccessibility. Anti-nutritional components can bind to iron and zinc making them unavailable for absorption and use by the human body. Furthermore, chitin, which is commonly presents in insects, could negatively influence absorption of nutrients (Marono et al., 2015). On the other hand, processing of food usually leads to modifications in the food matrix, which can enhance or decrease the nutrient bio-accessibility (Fernandez-Garcia et al., 2009).

Most of the edible insects are subjected to some kind of processing before consumption (Mutungi et al., 2017). Processing of insects can improve quality, safety, taste, and shelf life (Williams et al., 2016), but can also occasionally lead to formation of anti-nutritional and or toxic components (Friedman, 1996). Boiling, steaming, frying, roasting, and drying are amongst the commonly used traditional methods for processing insects (Alamu et al., 2013; Feng et al., 2018; Kinyuru et al., 2010b; Obopile & Seeletso, 2013; Ramos- Elorduy, 1997). The processing method used varies depending on the insect species and geographical region (Mutungi et al., 2017). These traditional processing

practices are usually uncontrolled and may have detrimental effects on the nutritional content and quality of insects (Kinyuru et al., 2010b).

Several studies investigated the influence of processing on nutrient composition and bioavailability of nutrients in edible insects and reported contradictory effects depending on the insect species. Kinyuru et al. (2010b) reported a significant reduction in protein digestibility for toasted and dried grasshoppers, but there was no significant change in protein digestibility in toasted and dried termites. In contrast, Caparros Megido and co-workers (2018) reported a significant increase in protein digestibility for boiled and oven cooked mealworms. Traditional cooking and toasting methods resulted in decreased crude protein, ash, zinc content, and true dry matter digestibility for Mopane worms (Madibela et al., 2007). Lautenschläger and co-workers (2017) reported no significant influence of thermal processing on nutritional value of edible caterpillars.

Previous research demonstrated the high nutritional potential of wild harvested insects in Zimbabwe (Manditsera et al., 2019). As traditional processing differs from one household to another and little is known about the impact on actual bioaccessibility, this study aims at determining the influence of domestic processing methods on protein and minerals retention, and protein digestibility and mineral bioaccessibility of *Eulepida mashona* (a beetle) and *Henicus whellani* (a cricket).

4.2 Material and methods

4.2.1 Samples and processing treatments

Samples of both *Eulepida mashona* (hereinafter indicated as beetle) and *Henicus whellani* (hereinafter indicated as cricket) were collected between December 2016 and January 2017. The method of collection was as described by Manditsera et al. (2019). Defrosted and washed samples were subjected to treatments according to the following: a) raw, b) boiling 30 mins, c) boiling 60 mins, d) pan roasting, e) boiling 30 mins followed by pan roasting for both insect species. Additional treatment for the beetles was twice boiling for 30 mins. Each treatment was performed in triplicate. The treatments represent and imitate the common domestic cooking practices. Approximately, 100g insects were processed by boiling in water or dry roasting in a stainless pot. The boiling time refers to the total cooking time. After boiling, excess boiling water was drained off and was kept for protein and mineral analysis. Processed samples were cooled in an ice bath followed by freeze-drying until a constant weight was reached. Freeze dried samples were coarsely grinded in a Waring commercial blender (Warring Commercial, Torrington Connecticut, USA) at high speed for 30 secs. Next, the insect meal was milled to a fine powder using Retsch MM400 (Retsch, Haan, Germany) milling machine at frequency 30 for 1 min (beetle) and 30 secs (cricket). The samples were then kept at -21^{o}C until further analysis. Figure 4.1 shows the processing steps and the analyses performed for each insect.

Figure 4.1: The process flow steps of the preparation and analysis of *Eulepida mashona* (beetle) and *Henicus whellani* (cricket)

4.2.2 In vitro digestion

In vitro digestion of the freeze-dried raw and processed samples was performed in triplicate according to the INFOGEST protocol described by Minekus et al. (2014) with some modifications. Enzyme salivary amylase was excluded in the simulated oral phase. Briefly, 2.5 g of sample was mixed with 2.5 ml of Milli Q water to make a paste like consistency followed by adding 4 ml simulated salivary fluid (SSF), 25 µl $CaCl_2$ and 975 µl water. The mixture was incubated for 2 mins at 37ºC. The gastric and intestinal phase were performed as described in the protocol. Porcine pepsin, pancreatin and bile extract were purchased from Sigma Aldrich (St Louis, MO, USA). Blank sample was prepared with

2.5 ml of MilliQ water. At the end of the simulated intestinal phase, the mixture was cooled in an ice bath for 15 mins, followed by centrifugation at 4500 rpm for 15 mins. The sample pellet and supernatant were snap frozen with liquid nitrogen. The frozen supernatant and pellet were then stored at -21ºC until further analysis for soluble mineral and protein.

4.2.3 Protein content determination

Samples of freeze-dried raw and processed insects were analysed in duplicate for nitrogen content by Dumas method using Flash EA 1112 N analyser (Thermo Fisher Scientific Inc., Waltham, MA, USA). D-methionine (ACROS Organics, 99% purity) was used as a standard and cellulose (Aldrich, microcrystalline powder, 20 µm) as a blank. A factor of 4.76 according to Janssen et al. (2017a) was used to convert the obtained nitrogen content to protein content. The boiling water was freeze dried first in order to determine the protein content loss during boiling.

4.2. 4 In-vitro protein digestibility

Protein digestibility was determined based on measuring the amount of free α-amino groups quantified by o-phthalaldehyde (OPA) method based on Schasteen et al. (2007). The free α- amino groups in the *in vitro* digested and total acid hydrolysed samples were determined by the o-phthalaldehyde (OPA) analysis following the method of Nielsen et al. (2001). Necessary dilutions were made before analysis. A calibration curve was made from L-leucine (Reagent Plus, >99%), Sigma Aldrich) in concentrations ranging from 0.078mM to 10mM. O- phthaladehyde and sodium tertraborate were purchased from Merck. Sodium dodecyl sulphate and dithiothreithol used in OPA analysis were purchased from Sigma Aldrich. Absorbance was measured at 340nm with Cary 50 Bio UV-visible spectrophotometer (Varian Agielent Technologies, Amstelveen, Netherlands) producer, city country). Total hydrolysis was performed by adding 5 ml of 6N HCl to approximately 200mg of undigested sample and heating at 110^0C for 24 hours. The hydrolysate was filtered, followed by measuring the total free α-amino groups by OPA analysis. Whey protein isolate, a well-known, highly digestible protein source was also *invitro* digested to compare the protein digestibility of the two insects using the same method. The protein digestibility was determined according to Equation 4.1.

$$\%\ protein\ digestibilty = \frac{conc.\ of\ free\ \alpha-amino\ groups\ in\ digested\ samples}{conc.\ of\ free\ \alpha-amino\ groups\ in\ total\ acid\ hydrolysed\ sample} * 100$$

(Equation 4.1)

As no peptidase was added in this protocol, dipeptide is the minimum size of the product formed. This implies that the maximum theoretical digestibility is 50%.

4.2.5 Mineral analysis and *in vitro* mineral bio-accessibility

The mineral content of freeze-dried undigested samples, supernatant and boiling water was analysed by ICP-AES using method described by Manditsera et al. (2019). Mineral bioaccessibility, regarded as the soluble mineral fraction in the supernatant was calculated using Equation 4.2.

$$\%\ mineral\ bioaccessibility = \frac{The\ amount\ of\ mineral\ in\ the\ digested\ sample}{Total\ mineral\ content\ in\ undigested\ sample} * 100$$

(Equation 4.2)

4.2.6 Statistical analysis

Results were analysed and expressed as means ± standard deviation of the replicates. Analysis of variance (ANOVA) was performed to determine significance differences between treatments. Post hoc analysis (Tukey and Games Howell) were performed to determine significantly different treatments at 5% level of significance ($p<0.05$). Analysis was done using IBM SPSS Statistics 23 software (SPSS Inc, Chicago, IL, USA)

4.3. Results and Discussion

4.3.1. Effect of processing on the protein content

Table 4.1 shows the protein content of the beetle and cricket and the protein measured in the boiling water after processing. Protein content of the insects decreased with boiling time, while it increased in the boiling water, indicating a protein leach into water. Twice boiling of beetles for 30 mins resulted in a significant decrease in protein content compared to all other treatments. Twice boiling of beetles and boiling for 60 mins for crickets resulted in maximal loss of protein. The absolute protein loss in the crickets (2.25-2.68 g) was higher as compared to beetles (0.98-1.81 g). The higher loss of protein in crickets could be due to the pre-treatment before cooking. Degutting the crickets could have increased the migration of proteins into water as the inner part of the insects' body expose to water. As expected, the roasting process did not affect the protein content.

The observed protein decrease with boiling is in line with previous findings in edible insects and other food products. A decrease in protein content was also reported in studies with Mopane caterpillar (Madibela et al., 2007), mealworms (Caparros Megido et al., 2018), and rabbit meat (Zhang et al., 2014). According to Zhang et al. (2014), the loss in protein content after boiling is likely due to the hydrolysis of parts of connective tissue and other proteins, whereas Caparros Megido et al. (2018) suggested that soluble proteins migrate into the insect exudate. Lautenschläger et al. (2017), however, reported no significant change in nutrient composition of cooked caterpillars.

Table 4.1: The protein content (g/100g) on fresh weight basis of *Eulepida mashona* (beetle) and *Henicus whellani* (cricket) and protein (g) lost into boiling water after processing

	***Eulepida mashona* (beetle)**		***Henicus whellani* (cricket)**	
	Protein in the insect (g/100g)	% loss	Protein in the insect (g/100g)	% loss
Raw	16.23^{a} ±0.21 (NA)	NA	20.90^{a} ±0.35 (NA)	NA
Boiled 30 mins	16.04^{a} ±0.23 (0.98)	1.17	18.92^{b} ±0.23 (2.25)	9.47
Boiled 60 mins	14.76^{b} ±0.42 (1.76)	9.06	18.79^{b} ±0.48 (2.68)	10.09
Boiled 30 mins twice	13.85^{c} ±0.28 (1.81)	14.66	NA	NA
Boiled roasted	15.42^{ab} ±0.36 (1.02)	0.81	19.30^{b} ±0.22 (2.37)	
Roasted	16.54^{a} ±0.26 (NA)	NA	20.96^{a} ±0.45 (NA)	NA

The data represents mean ± standard deviation (n=3). The values in brackets represent the amount of protein (g/100g of fresh sample) leached into boiling water. Same letter in a column for the same insect species represent no significance difference at 5% significance level.

4.3.2 Effects of processing on *in vitro* protein digestibility

Figure 4.2 shows the total protein digestibility of the various insect samples. Overall, the digestibility of raw beetles (30.6%) is comparable to that of crickets (29.7%). Boiling twice for 30 mins reduced significantly the protein digestibility for beetles (raw, 30.6% and boiled twice, 23%). Interestingly, roasting did not have any significant effect on protein digestibility of beetles, while for crickets, both boiling and roasting reduced significantly the protein digestibility (24.2 and 24.7% respectively). However, there was no significant difference in digestibility between boiled and roasted crickets. Based on the method used, which only measures the free amino groups, the maximum expected theoretical digestibility is 50%. No peptidase was added to the supernatant to further break down any dipeptides or oligopeptides that could have been formed.

Other studies have also reported a decrease in protein digestibility after processing edible insects. For example, a decrease in protein digestibility was reported for boiled and fried Sudanese tree locusts (El Hassan et al., 2008), for fried and roasted grasshoppers and termites (Kinyuru et al., 2010b), and for oven cooked mealworms and house crickets (Poelaert et al., 2016). Interestingly, (Caparros Megido et al., 2018) reported an increase in protein digestibility with boiling and oven cooking of mealworms. The variable findings on effects of processing on protein digestibility could be due to multiple differences related to species variability. For instance, the presence of anti-nutrients, which can bind to proteins, has been reported to affect protein digestion (Caparros Megido et al., 2018).

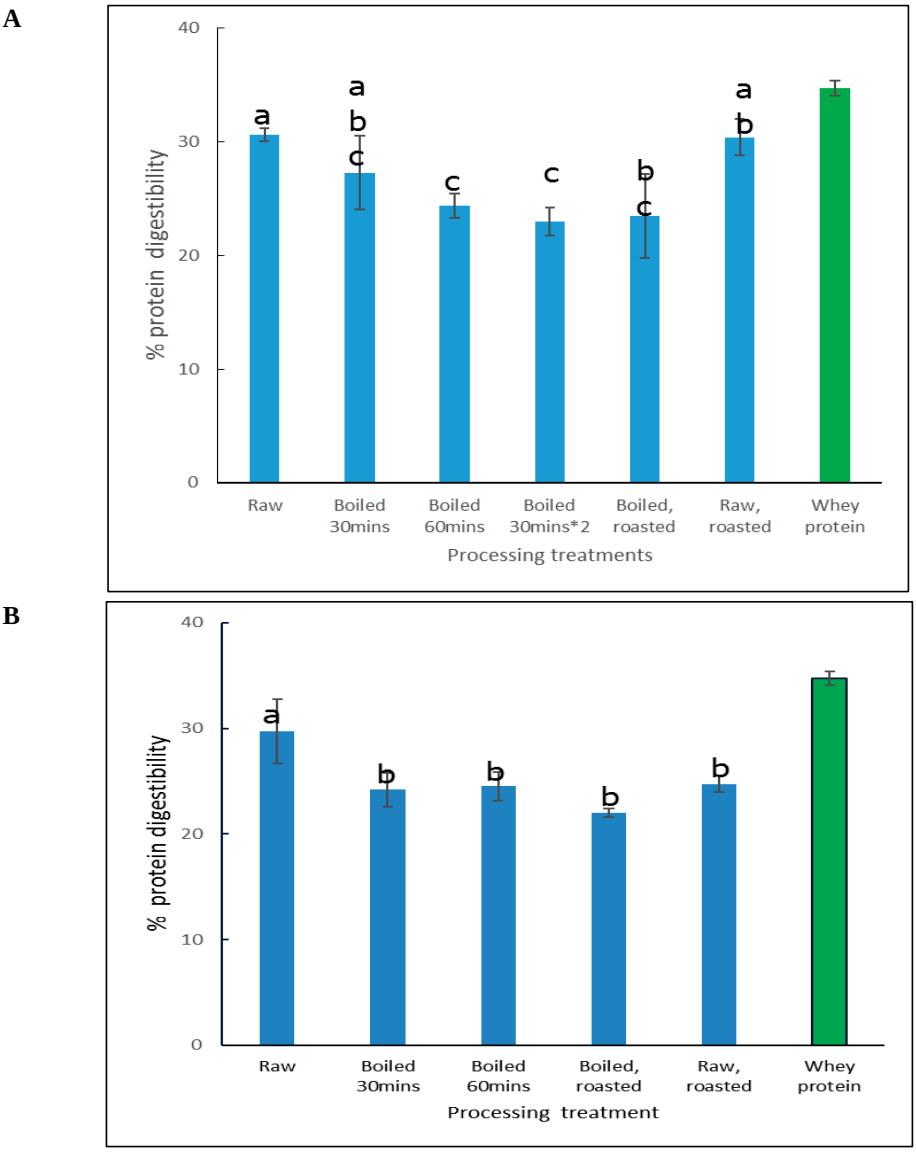

Figure 4.2: Percentage protein digestibility of *Eulepida mashona* (beetle) (A) and *Henicus whellani* (cricket) (B) processed differently. Note: the maximum possible theoretical protein digestibility is 50% (see methods section 4.2.4).

In our study, the absolute values of protein digestibility were lower than for other reported raw insect species. In the review of Mutungi et al., (2017), they indicated that, edible insects in general have a high protein digestibility that can exceed 90%. However, Akullo et al. (2018) observed lower protein digestibility values (less than 50%) for three raw insect species compared to other species. Marono et al. (2015) reported a crude protein digestibility of 65-69% for *Tenebrio molitor* and *Hermetia illucens*. Moreover, several studies have reported varying values for protein digestibility of mealworms

(Caparros Megido et al., 2018; Marono et al., 2015; Yi et al 2016). These differences are likely due to the methodologies used in determining the *in vitro* protein digestibility. The experimental protocols of digestion differ in terms of enzymes used, duration of digestion, and method used to calculate the digestibility. In our study, we did not add peptidase therefore the minimum size of the product formed was dipeptide. For this reason, the maximum theoretical digestibility in our experiments is 50%. Although, the different values of digestibility may complicate appropriate comparison between studies, the focus in our study was on the differences among the various treatments. The digestion of whey protein was taken as control to provide an indication of the absolute digestion efficacy.

Another reason for differences in protein digestibility observed among insect species, could be differences in types of proteins (Bosch et al., 2014). Jonas-Levi and Martinez (2017) reported that some insect proteins are not digestible for humans. Moreover, chitin could interfere with the digestibility of proteins. In their study on mealworms, Marono et al. (2015) reported that protein digestibility is negatively correlated with chitin content. The insect species in our study are consumed at adult stage and contain a considerable amount of chitin (Manditsera et al. 2019), which could explain the relatively low absolute digestibility as compared to that of whey proteins (35%). However, a maximal difference of 5% between protein digestibility of raw insects and whey proteins suggests that insects' proteins are highly digestible.

Studies in food products other than insects showed that heat treatment can be associated with either a decrease or an increase in protein digestibility (Boye et al., 2012). Protein digestibility after heat treatment depends on how proteins transform during the process. For example, the formation of disulphide links in the protein matrix reduces protein digestibility (Mutungi et al., 2017). A previous study showed that the beetles and the crickets contain sulphur containing amino acids (Manditsera et al., 2019), which could have resulted in formation of disulphide bonds. Reduced protein digestibility is also associated with interaction of proteins and phytochemicals, which form stable complexes that make proteins unavailable for enzymes (Boye et al., 2012). Formation of such complexes reduces accessibility of proteins for proteolytic enzymes, which can be also the case for protein aggregation induced by heating (Kaur et al., 2014).

4.3.3 Protein profiles of digested, non-digested and supernatants

SDS–PAGE was done to check how processing and *in vitro* digestion influenced protein profiles. Figure 4.3 shows the protein profiles of the undigested samples, pellet and supernatant from digestion of raw, boiled and roasted insects. Pellet refers to the insoluble material remaining after *in vitro* digestion. For both insect species, the protein profiles of raw and boiled samples are clearly different. For boiled samples, there was disappearance of some high molecular weight proteins. However, the protein profile of raw was more or less similar to that of roasted insects. There was a decrease in the

number and intensity of the bands in pellets. Less protein profiles in pellets is an indication that insect protein was digestible. Supernatant of all samples for both insect species had only one visible protein band of less than 53kDa. The band could be undigested soluble protein. Protein bands in the range of 46-65kDa have been previously identified in *Tenebrio molitor* (Bubler et al., 2016).

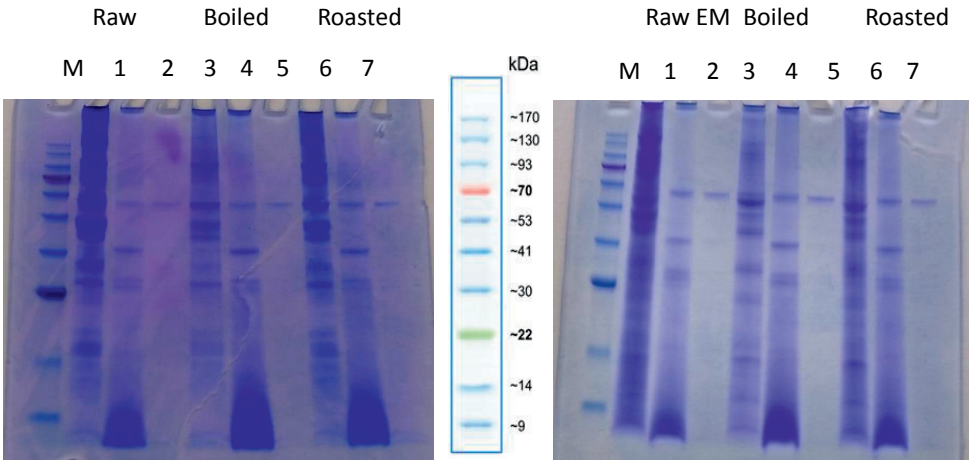

Figure 4.3: SDS PAGE protein profiles of raw, boiled and roasted *Eulepida mashona* (beetles) and *Henicus whellani* (crickets) before and after protein digestion (on two separate fractions).
EM- *Eulepida mashona*; HW- *Henicus whellani*
M-marker; 1-non-digested; 2- pellet; 3- supernatant; 4-non-digested; 5- pellet; 6- supernatant; 7-non-digested; 8-pellet; 9- supernatant

4.3.4 Effect of processing on mineral content

Table 4.2 and Table 4A1 respectively show the iron and zinc content and other minerals (Ca, Cu, K, Mg, Mn, Na, P and S) content of raw and processed samples of the beetles and crickets. The loss of mineral into boiling water during boiling is reported in the tables. Table 4.2 shows that the change in the iron and zinc content for both beetles and crickets between the different treatments was not significantly different. The amount of iron and zinc lost into water was higher for beetles (maximal 0.31mg; 0.21mg) than for crickets (maximal 0.10mg; 0.06mg. Boiling had no significant effect on the copper and manganese content of beetles and Ca, Cu and Mn content of crickets. For all the other minerals, boiling significantly decreased the mineral content while roasting as expected did not (Table 4A1).

Table 4.2: The iron and zinc content (mg/100g) on fresh weight basis of *Eulepida mashona* and *Henicus whellani* after processing and amount (mg) measured in remaining boiling water.

	Eulepida mashona			
	Iron (mg/100g)	% loss	Zinc (mg/ 100g)	% loss
Raw	6.40[a] ±0.41 (NA)	NA	4.30[a] ±0.57 (NA)	NA
Boiled 30 mins	6.59[a] ±0.38 (0.17)	0.03	4.24[a]±0.24 (0.13)	1.40
Boiled 60 mins	5.65[a] ±0.33 (0.25)	11.7	3.87[a]±0.28 (0.19)	10.0
Boiled 30mins twice	5.99[a] ±0.63 (0.31)	6.41	3.78[a]±0.13 (0.21)	12.1
Boiled roasted	6.04[a] ±0.63 (0.29)	5.63	3.86[a]±0.23 (0.10)	10.2
Roasted	6.49[a] ±0.40 (NA)	NA	4.19[a] ±0.25 (NA)	NA
	Henicus whellani			
	Iron (mg/100g)	% loss	Zinc (mg/ 100g)	% loss
Raw	15.25[a] ±0.86 (NA)	NA	5.07[a]±0.22 (NA)	NA
Boiled 30 mins	16.19[a] ±2.26 (0.06)	6.16	4.61[a]±0.66 (0.04)	9.07
Boiled 60 mins	16.44[a] ±0.20 (0.10)	7.80	4.58[a]±0.35 (0.05)	9.66
Boiled roasted	15.58 [a] ±1.68 (0.07)	2.23	4.85[a]±0.23 (0.06)	4.34
Roasted	16.91 [a] ±5.83 (NA)	NA	4.89[a]±0.01 (NA)	NA

The values in brackets represent the amount of mineral (mg/100g of fresh sample) measured in remaining water after boiling. Same letter in a column for the same species represent treatments not significantly different

In general, during boiling of foods, minerals are lost into the boiling water (da Silva et al., 2017), which results in a certain decrease in the mineral content. The extent of loss is dependent on the mineral, the boiling duration, the food matrix, and the chemical form of mineral in food. Moreover, the differences in mineral loss with boiling between the two species and between minerals could be explained by the type of interactions the minerals have with the food matrix. In line with our results, El Hassan et al. (2008), found that iron and zinc content remained stable after boiling tree locusts. The differences could be due to how the mineral exist in the food matrix.

4.3.5 Effects of processing on bioaccessible mineral content and *in vitro* mineral bioaccessibility

The mineral solubility (bioaccessible mineral content) gives an indication of the amount of the mineral that can be bioavailable to the human body (Wienk et al., 1999). Table 4.3 shows the bioaccessible iron and zinc content after *in vitro* digestion of the beetles and crickets. Additionally, Table 4A2 shows the results of other minerals measured. Bioaccessible iron content was higher in raw beetles

(7.41mg/100g dry matter) than in crickets (4.21mg/100mg dry matter). Boiling significantly reduces the bioaccessible Fe, Mg, P, S and Zn content of beetles, but not of Ca, Cu, Mn, and Na. Roasting had no significant effect on the bioaccessible content for all the minerals except for sulphur in beetles. Boiling and roasting had no significant effect on bioaccessible iron content of crickets.

Table 4.3: Bioaccessible iron and zinc content (mg/100g dry matter) of *Eulepida mashona* (beetles) and *Henicus whellani* (crickets)

	***Eulepida mashona* (beetles)**		***Henicus whellani* (crickets)**	
	Bioaccessible iron (mg/100g)	Bioaccessible zinc (mg/100g)	Bioaccessible iron (mg/100g)	Bioaccessible zinc (mg/100g)
Raw	7.41^{a}±0.45	6.75^{a}±0.17	4.21^{a}±0.42	5.90^{a}±0.68
Boiled 30 mins	4.41^{b}±0.25	4.70^{bc}±0.11	2.95^{ab}±0.49	2.33^{b}±0.11
Boiled 60 mins	4.69^{b}±0.36	3.75^{c}±0.42	2.92^{ab}±0.29	2.49^{b}±0.69
Boiled 30 mins twice	5.40^{ab}±0.61	3.66^{c}±0.06	NA	NA
Boiled and roasted	4.97^{b}±0.67	4.21^{bc}±0.52	2.30^{b}±0.04	2.19^{b}±0.26
Roasted	6.27^{ab}±0.88	5.55^{ab}±0.65	3.13^{ab}±0.32	3.73^{b}±0.02

The data represents mean ± standard deviation (n=2). Same letter in a column for the same insect species represent no significance difference at 5% significance level.

There are limited studies on mineral bioaccessibility of edible insects. Soluble iron contents in raw and processed beetles was higher than reported for raw grasshoppers, crickets, mealworms and sirloin beef (Latunde-Dada et al., 2016). Furthermore, they reported a lower zinc solubility for their studied insects than *Eulepida mashona* and *Henicus whellani*. However, in our study, the iron solubility of cricket was lower than that reported for crickets by (Latunde-Dada et al., 2016). Our findings of significant difference in the mineral solubility between the beetle and cricket are in line with Latunde-Dada et al. (2016) who also reported a significant difference between crickets, grasshoppers, and mealworms. They also reported the lowest bioavailability for crickets, which also had the highest iron content. In the present study, the cricket had a higher iron content than the beetle before and after processing.

Figure 4.4 shows the percentage of mineral bioaccessibility of the beetle and cricket before and after processing. Phosphorous had the highest bioaccessibility for both raw beetles (88%) and crickets (82%). Iron had the lowest bioaccessibility for both species. The iron bioaccessibility was higher for raw beetles (30.7%) than for crickets (8.11%). Similarly, zinc bioaccessibility was higher for raw beetles (41.5%) than for crickets (34.2%). In general, the absorption of heme iron ranges from 15-35% whilst non-heme can range from 2-15% (Zielińska-Dawidziak, 2015). Insects store most of their iron bound in ferritin (Nichol et al., 2002) and it has been stated that ferritin improves non-haem iron

bioavailability (Mwangi et al., 2018; Zielińska-Dawidziak, 2015). The differences in mineral bioaccessibility between insect species could be related to the insect matrix. Iron and zinc are complexed with other food constituents and type of bonds associated could be different for iron and zinc.

Boiling beetles significantly reduced the percentage mineral bioaccessibility of iron, sulphur and zinc. Boiling and roasting had no significant effect on the percentage mineral bioaccessibility of K, Mg, Mn and P of beetles. For both insect species, roasted samples had a lower significantly different zinc bioaccessibility compared to raw samples. For all minerals, bioaccessibility of boiled crickets were significantly lower than of raw samples.

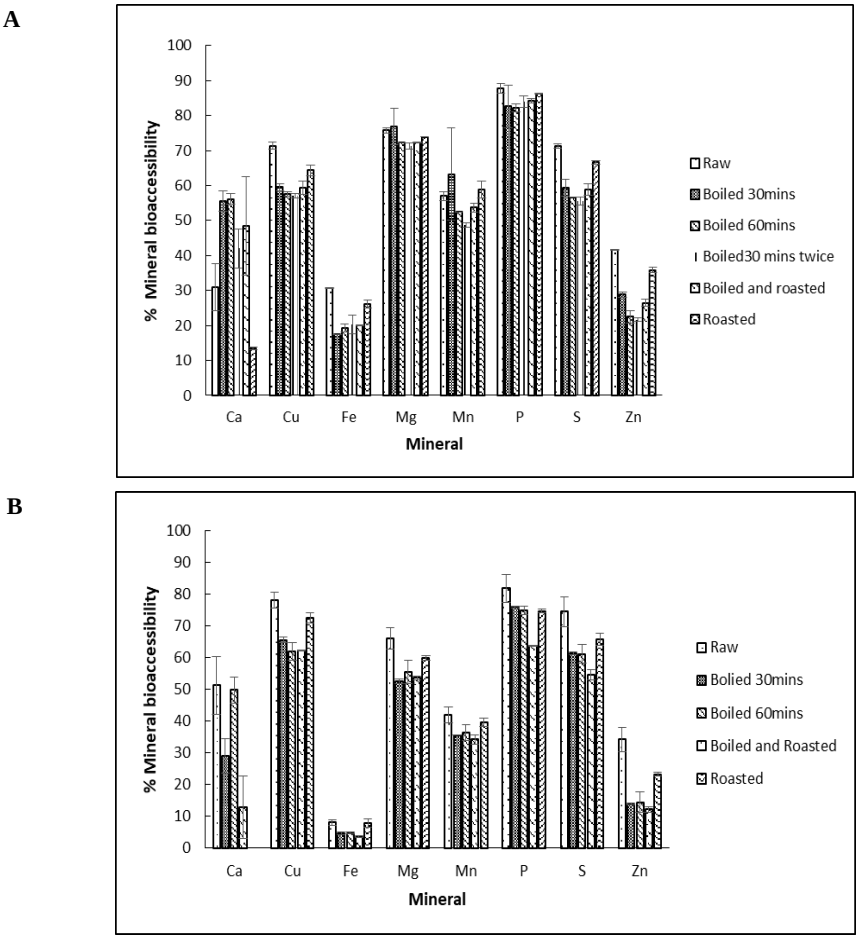

Figure 4.4: Percent mineral bioaccessibility of differently processed *Eulepida mashona* (beetle) (A) and *Henicus whellani* (cricket) (B)

Other studies have also reported a decrease in mineral bioaccessibility with heat treatment (de Oliveira et al., 2018; Menezes et al., 2018). Iron bioaccessibility of beetles was higher than reported for different processed beef, chicken and pork by (Menezes et al., 2018). In contrast to our findings, Singh et al. (2016) reported higher bioavailability for iron as compared to zinc.

During processing, bivalent minerals form interactions with other food constituents such as protein and carbohydrates during processing and thus decrease the bioaccessibility (Gharibzahedi and Jafari, 2017). Heat induced reactions form compounds that can bind mineral tightly and the formed products are usually more resistant to digestion such that their mineral binding characteristics remain the same. Both beetles and crickets have high protein content and this could explain the reduced bioaccessibility that occurred during processing. Insoluble dietary fibre binds iron and zinc thereby reducing their bioaccessibility. Previous studies showed that crickets had a higher chitin content than beetles (Manditsera et al. 2019) and presence of phytochemicals (Musundire et al, 2016) for both insect species, which are likely to influence mineral bioaccessibility.

4.4. Conclusion

In our study, the insect species are a good source of digestible protein as shown by the comparable value of digestibility with that of whey. This study further demonstrated that traditional processing has an influence on the protein content, protein digestibility and mineral bioaccessibility of edible insects. Boiling resulted in protein loss for both insects while roasting did not. Boiling resulted in a reduced protein digestibility in both insects, whilst roasting had no significant difference for beetles. However, boiling duration had no significant influence on protein digestibility for both species. Boiling and roasting did not have any significant influence on the iron and zinc content for both insects. The high iron and zinc content could contribute significantly to the daily requirements. Beetles are a better source for bioaccessible iron than crickets, an indication that mineral bioaccessibility is insect species dependent. Therefore, concerning promoting edible insects for nutritional security because of their high mineral content, each species needs individual evaluation for bioaccessibility. Overall, protein, iron, and zinc in the wild harvested insects in this study can substantially contribute to human nutrition. How specific factors of an insect matrix can influence digestibility and mineral bioaccessibility requires further investigation.

Supplimentary

Table 4A1: Mineral content (mg/100g) on fresh weight basis of *Eulepida mashona* and *Henicus whellani* after processing and amount (mg) measured in remaining boiling water

	Ca	Cu	K	Mg	Mn	Na	P	S
				***Eulepida mashona* (beetles)**				
Raw	17.7^{a}±0.22 (N.A)	1.05^{a}±0.52 (N.A)	388^{a}±1.51 (N.A)	64.6^{a}±2.06 (N.A)	1.55^{a}±0.18 (N.A)	24.0^{a}±0.11 (N.A)	221^{a}±2.31 (N.A)	149^{a}±4.12 (N.A)
Boiled 30mins	22.1^{ab}±0.10 (3.19)	0.72^{a}±0.05 (0.05)	294^{b}±12.4 (61.4)	57.7^{bc}±3.95 (4.22)	1.13^{a}±0.08 (0.01)	18.4^{b}±0.11 (4.61)	186^{b}±8.78 (24.8)	144^{ab}±3.62 (10.4)
Boiled 60mins	26.9^{bc}±0.13 (7.07)	0.64^{a}±0.08 (0.09)	213^{c}±11.0 (94.0)	49.8^{c}±2.35 (6.92)	1.47^{a}±0.21 (0.01)	14.7^{cd}±0.89 (7.96)	144^{cd}±8.63 (38.6)	124^{c}±7.59 (15.44)
Boiled 30mins twice	29.2^{c}±1.47 (7.06)	0.68^{a}±0.06 (0.11)	191^{c}±12.5 (101)	48.4^{bc}±1.29 (6.99)	2.00^{a}±0.91 (0.025)	13.1^{d}±0.54 (8.37)	127^{d}±3.25 (42.6)	118^{c}±0.64 (17.1)
Boiled and roasted	21.9^{ab}±2.67 (3.13)	0.61^{a}±0.01 (0.03)	265^{b}±12.5 (54.9)	56.2^{c}±1.39 (4.02)	1.80^{a}±0.86 (0.01)	15.6^{c}±0.36 (4.07)	166^{bc}±8.22 (22.6)	129^{bc}±2.72 (8.69)
Roasted	18.3±1.23 (N.A)	0.60^{a}±0.02 (N.A)	389^{a}±8.06 (N.A)	68.1^{a}±3.19 (N.A)	1.70^{a}±0.67 (N.A)	22.6^{a}±0.05 (N.A)	222^{a}±8.46 (N.A)	152^{a}±4.45 (N.A)
				***Henicus whellani* (crickets)**				
Raw	13.6^{a}±2.06 (N.A)	0.80^{a}±0.10 (N.A)	305^{a}±10.2 (N.A)	21.3^{a}±0.37 (N.A)	0.50^{a}±0.03 (N.A)	42.9^{a}±5.49 (N.A)	124^{a}±3.31 (N.A)	126^{a}±0.94 (N.A)
Boiled 30mins	13.1^{a}±1.63 (1.56)	0.76^{a}±0.05 (0.01)	185^{b}±9.74 (65.1)	15.5^{b}±0.52 (3.05)	0.45^{a}±0.03 (0.02)	21.9^{b}±1.45 (9.89)	84.7^{b}±3.92 (24.9)	111^{b}±0.79 (5.89)
Boiled 60mins	10.9^{a}±0.54 (2.10)	0.75^{a}±0.02 (0.01)	164^{b}±12.7 (93.6)	13.8^{b}±0.65 (4.15)	0.44^{a}±0.05 (0.02)	17.6^{b}±2.59 (13.74)	75.6^{b}±4.14 (27.7)	108^{b}±0.46 (7.62)
Boiled and roasted	10.5^{a}±1.43 (2.48)	0.75^{a}±0.02 (0.01)	174^{b}±3.55 (75.7)	14.4^{b}±0.33 (3.93)	0.41^{a}±0.04 (0.02)	21.9^{b}±2.28 (12.8)	77.7^{b}±4.35 (25.7)	110^{b}±1.43 (6.93)
Roasted	13.9^{a}±2.21 (N.A)	0.71^{a}±0.04(N.A)	318^{a}±23.4 (N.A)	21.6^{a}±1.06 (N.A)	0.51^{a}±0.01 (N.A)	45.4^{a}±3.92 (N.A)	133^{a}±8.17 (N.A)	125±3.27 (N.A)

The values in brackets represent the amount of mineral (mg) measured in remaining water after boiling. Same letter in a column for the same species represent treatments not significantly different

Table 4A2: Bioaccessible mineral content (mg/100g dry matter) in differently processed *Eulepida mashona* (beetles) and *Henicus whellani* (crickets)

	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
					***Eulepida mashona* (beetles)**					
Raw	20.8^{a}±4.88	2.81^{a}±1.33	7.41^{a}±0.45	1640^{a}±449	185^{a}±5.90	3.34^{a}±0.43	1185^{a}±152	732^{a}±9.75	402^{a}±4.24	6.75^{a}±0.17
Boiled 30mins	47.2^{bc}±2.45	1.64^{a}±0.13	4.41^{b}±0.25	1751^{a}±42	170^{ab}±0.71	2.78^{a}±0.80	1197^{a}±47.8	589^{b}±17.93	328^{b}±6.89	4.7^{bc}±0.11
Boiled 60mins	65.1^{c}±1.41	1.58^{a}±0.14	4.69^{b}±0.36	1399^{a}±165	155^{b}±0.36	3.33^{a}±0.64	1205^{a}±11.8	510^{c}±0.61	303^{c}±5.77	3.75^{c}±0.42
Boiled 30mins twice	54.6^{bc}±4.14	1.72^{a}±0.16	5.40^{ab}±0.61	1244^{a}±30	154^{b}±7.52	4.38^{a}±2.09	1153^{a}±17.2	475^{c}±7.31	294^{c}±1.20	3.66^{c}±0.06
Boiled and roasted	43.1^{b}±8.56	1.48^{a}±0.10	4.97^{b}±0.67	1475^{a}±19	167^{b}±0.58	3.97^{a}±1.88	1136^{a}±65.9	573^{b}±7.87	311^{bc}±10.78	4.21^{bc}±0.52
Roasted	5.09±5.65	1.41^{a}±0.04	6.27^{ab}±0.88	1792^{a}±15	186^{a}±2.23	3.68^{a}±1.48	1198^{a}±15.7	706^{a}±2.93	374^{d}±5.05	5.55^{ab}±0.65
					***Henicus whellani* (crickets)**					
Raw	24.3±8.95	2.12±0.09	4.21±0.42	924±35	47.9±3.84	0.71±0.04	1190±41.5	345±25.45	321±37.4	5.90±0.68
Boiled 30mins	13.6±1.12	1.80±0.05	2.95±0.49	583±19	29.7±0.76	0.59±0.03	1086±5.4	234±5.25	248±1.79	2.33±0.11
Boiled 60mins	20.4±0.24	1.74±0.10	2.92±0.29	535±34	28.9±2.68	0.61±0.12	1140±127	213±11.26	249±8.37	2.49±0.69
Boiled and roasted	5.30±4.62	1.73±0.06	2.30±0.04	556±12	28.7±0.06	0.51±0.03	1191±224	183±8.94	224±5.94	2.19±0.26
Roasted	0±0.00	1.68±0.09	3.13±0.32	910±61	42.3±0.72	0.66±0.05	1183±27.3	325±16.77	269±9.88	3.73±0.02

Same letter in a column for the same species represent treatments not significantly different

CHAPTER 5

Lipid Oxidation during Storage of Edible Insects as Affected By Grinding, Drying, and Storage-Temperature

This chapter is to be submitted as: Faith A. Manditsera, Catriona M.M. Lakemond, Vincenzo Fogliano and Pieternel, A. Luning. (2019). Lipid oxidation during storage of edible insects as affected by grinding, drying-, and storage-temperature.

Abstract

Seasonality and geographical availability can limit the potential contribution of wild harvested insects to food and nutrition security. Therefore, it is useful to extend shelf life of harvested insects by drying to improve availability and thereby valorisation of insects. Lipid oxidation is an important determinant for shelf life of fat-rich dried products. This study investigated the influence of grinding, drying, and storage conditions on lipid oxidation, particularly the formation of secondary lipid oxidations compounds, in two wild harvested edible insects (*Henicus whellani* and *Eulepida mashona*). Boiled samples were dried at 40ºC and 100ºC to imitate respectively sun drying and oven drying of insects. Thereafter, half of the dried insects were treated as whole insects and other half as grinded insects. All samples were packed into polyethylene re-sealable bags thereafter stored at 4ºC and 50ºC for 8 weeks. Every second week, a sample was taken from the packages and analysed for water activity, moisture content, malondialdehyde content (TBARS analysis), and volatile compounds (GC-MS by HS-SPME). Drying of *Henicus whellani* and *Eulepida mashona* at 40ºC resulted in water activity of respectively 0.35 and 0.43; while drying at 100ºC resulted in a water activity of respectively 0.14 and 0.16. The malondialdehyde concentration was higher for insects dried at 100ºC compared to 40ºC, which indicated for both insect an increase in lipid oxidation. In total 62 volatile compounds were identified in both insect species. Only 22 volatiles were present in raw insects, the other volatiles developed during drying or storage. The concentration of volatile lipid oxidation products (VLOPs) gradually increased during storage at 4ºC and 50ºC for both insect species. The concentration of VLOPs peaked at 4 weeks storage for insects dried at 40ºC, and at 2 weeks storage for insects dried at 100ºC. Drying insects at moderate temperature (40ºC) resulted in water activity levels that could favour lipid oxidation during storage, which is less when dried at 100ºC. VLOPs provide useful information on the drying and storage conditions however, they are not clear indicator of the amount of lipid oxidation.

5.1. Introduction

Edible insects play an important role in the diets of the people who consume insects. Despite some reported cases of decline in practice, entomophagy is still prevalent in developing countries especially in rural areas (Ayieko et al., 2010; Chakravorty et al., 2011, 2013; Manditsera et al., 2018; Payne, 2015). Edible insects are mainly consumed for their taste, contrary to the belief that insects, are consumed when no other food options are available (Manditsera et al., 2018). Moreover, they contribute to dietary diversity of the rural households, where edible insects are abundant (Ayieko & Oriaro, 2008). In Africa, most of the edible insects, at different morphological stage, are seasonally harvested from the natural environment (Kinyuru et al., 2013; Murefu et al., 2019; Mutungi et al., 2017). Therefore, the potential contribution of wild harvested insect species to food and nutrition security can be limited by seasonality (Ayieko et al., 2010; van Huis, 2016) and geographical availability (Raheem et al., 2018). Due to their biological nature, harvested insects are susceptible to spoilage and deterioration in quality (Ayieko et al., 2010; Klunder et al., 2012; Stoops et al., 2016). Therefore, shelf life extension of harvested insects is important for improving availability and valorisation of insects for consumption.

Whilst boiling and roasting are short-term methods for preserving quality of harvested insects (Manditsera et al., 2019), drying can be used to extend the shelf life of edible insects. Traditionally, surplus insects are sun-dried before storage (Ayieko et al., 2010) for later consumption. Drying can be followed by grinding insects into an insect meal/ powder before storage (Mutungi et al., 2017). Such insect powders can be used as an ingredient in other food products to increase nutritional value or functionality (Ayieko et al., 2010; Münke-Svendsen et al., 2017; Williams et al., 2016). However, the presence of unsaturated fatty acids in edible insects (Berezina, 2017; Manditsera et al., 2018) in combination with low water activity of dried insects can increase their susceptibility to lipid oxidation, which may limit shelf life.

Lipid oxidation is one of the major causes of product degradation in storage of dried products (Chudy et al., 2015; Sun et al., 2002), which can lead to formation of off-flavours and or affect food safety (Raitio et al., 2012). The volatile secondary products, such as aldehydes and ketones, can cause a rancid smell (Hu, 2016). Oxidation rates are highest below a_w 0.2 and above a_w 0.3 in general (Hu, 2016), but actual rates are product specific (Raitio et al., 2012). Storage conditions and environmental factors, such as unlimited oxygen supply, high temperatures, and daylight can also increase lipid oxidation (Alamprese et al., 2017; Hu, 2016; Sun et al., 2002; Tazi et al., 2009). These are exactly the external factors limiting the shelf life of dried edible insects in rural Africa.

To the best of our knowledge, there are only a few studies on lipid oxidation of insects and they are focused on extracted insect oil (Jeon et al., 2016; Tiencheu et al., 2013). These studies showed that

heat treatment before oil extraction influence oxidative stability of oils. The food matrix, however, can also affect the lipid oxidation process (Hu, 2016). Therefore, the process may be different in insect oil compared to dried insects as whole or powder. As there is a need to enlarge the availability of insect-based foods in developing countries e.g. (Ayieko et al., 2016; Mutungi et al., 2017), a further understanding of shelf stability of traditionally dried insects would be useful. The aim of this study is to investigate the influence of drying and storage conditions on lipid oxidation, particularly on the formation of secondary lipid oxidations compounds. Two wild harvested edible insects, a beetle (*Eulepida mashona)* and a cricket (*Henicus whellani)*, with high nutritional potential (Manditsera et al., 2019) were used as case study. The two insect species are only available in the rainy season and currently their consumption rarely goes beyond the seasons (Manditsera et al., 2019) as there is yet restricted insight in shelf life limiting processes.

5.2 Materials and methods

5.2.1 Sampling, sample preparations and experimental design

Samples of *Eulepida mashona* and *Henicus whellani* were respectively sampled from Mhondoro and Bikita districts during the November/December rainy season of 2017/18. Sampling was done according to the methodology described by Manditsera et al. (2019). Samples were then kept at -21^{o}C until further treatment and analysis. Insect samples were washed and boiled for 15 minutes followed by drying in an oven. Two temperatures were employed for drying. Moderate temperature of 40^{o}C to imitate the sun drying and 100^{o}C as a high temperature that can be used for oven drying insects. For both temperatures, the samples were dried until they reached the constant weight. Thereafter half of the dried insects were ground at low speed into coarse grinded insect with a Waring laboratory blender (Torrington, CT, USA). The other half of the dried insects was treated further as whole insects. All samples were then packed into polyethylene re-sealable bags and kept in storage at 4^{o}C and 50^{o}C for 8 weeks. Every second week, samples were drawn from the packages and analysed for water activity, moisture content, malondialdehyde content and volatile compounds. Figure 5.1 summarises the experimental design. The experiments were performed in duplicate. The experimental results are presented as mean values with standard deviations (mean ± standard deviation).

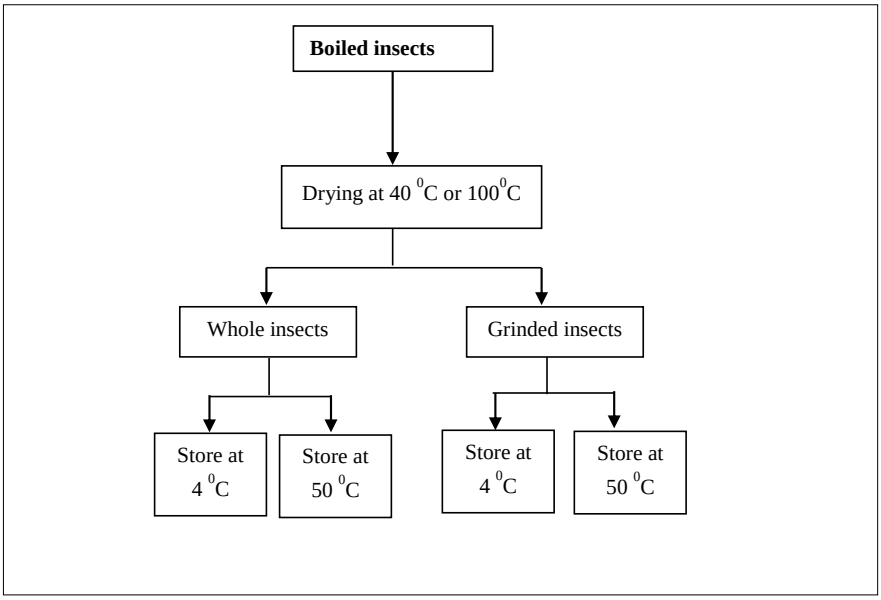

Figure 5.1: Schematic overview of the experimental design

5.2.2 Dry matter content and water activity determination

The dry matter content of the insects was measured by using oven drying at 105°C for at least 18 hours. The water activity (a_w) was measured with a Novasina LabMaster-aw water activity meter (Lachen, Switzerland) set at 25°C.

5.2.3 Thiobarbituric Acid Reactive Substances (TBARS) test

The TBARS test was performed to get an indication of the amount of the secondary non-volatile oxidation product malondialdehyde. The method from de las Heras et al. (2003) was adapted and used to determine the malondialdehyde concentration. Whole insects were first ground using a mortar and pestle. A suspension was made using approximately 1 gram of ground insect sample and 20 mL of MilliQ followed by mixing for 10 minutes with a Multi Reax shaker (Heidolph, Germany). Sample suspensions were then centrifuged for 5 minutes at 2000g, followed by filtering on a 5951/2 Whatman paper filter (Maidstone, UK). Equal volumes (5ml) of the filtrate and ice cold trichloroacetic (TCA, 15%), were mixed followed by filtering again on a 0.45 µm 25mm CA filter (Phenomenx, Torrance, US). Then equal volumes (2ml) of filtrate and thiobarbituric acid (TBA) solution were mixed and further incubated for 35 minutes at 100°C. Afterwards the solution was cooled down to room temperature (10 minutes on ice), followed by measuring absorbance at 532nm and 600 nm using a Carry 50 UV-Visible spectrophotometer (Varian Agielent Technologies, Amstelveen, Netherlands). The measured values obtained at 600nm were subtracted from those obtained at 532 nm to correct for

sample turbidity. A calibration curve was made using dilutions of malondialdehyde in 15.0% TCA solution and was used to calculate the malondialdehyde concentration in the samples.

5.2.4 Extraction and analysis of the volatiles by headspace SPME-GC/MS

The volatiles of the insect samples were measured using gas chromatography/ mass spectrometry (GC/MS) (Thermo Fisher Scientific Inc., Waltham, MA, US) by headspace Solid Phase Micro Extraction (HS-SPME). The method was based on a method of (Goodridge et al., 2003) with some modifications. At least 0.5 gram of the ground insect sample withdrawn from the packages (whole insects were first grinded) was put in a 20mm headspace vials. Vials were tightly closed followed by placing the vials in the Triplus auto-sampler (Thermo Fisher Scientific, Waltham, MA, USA). The sample was incubated for 15 minutes at 40°C before extraction of the volatiles. An SPME fibre assembly coated with 50/30µm thickness DVB/CAR/PDMS (divinylbenzene/ carboxen/polydimethylsiloxane) stableflex 2cm (Supelco, Bellefonte, PA, USA) was inserted into a fibre holder for auto sampling. Extraction of the headspace volatiles was performed at 40ºC for 30 minutes. The extraction procedure was carried out by a programmed Tri-Plus Auto sampler controlled by Xcalibur system. For volatile analysis, an aliquot of headspace gas was injected into the GC with a stabilwax DA capillary column (30 m x 0.25 mm IDx 0.25 µm, Restek Corporation, Benner Circle, Bellefonte, USA). The desorption time in the splitless injection port of the GC was 10 minutes Oven temperature was set at 40°C for 2 minutes and was then increased to 200 °C at a rate of 10 °C/min and held for 5 minutes. Helium was used as carrier gas with a flow rate of 1 ml per minute. The volatiles were identified with the Chromeleon 7 software (Thermo Fisher Scientific). The mass spectra from the samples were compared to volatiles from the NIST database.

5.3 Results and Discussion

5.3.1 Changes in water activity during storage

Figure 5.2 shows the effect of the initial drying temperature (40^0 C and 100^0 C) and subsequent storage at 4^0 C and 50ºC on the water activity of *Henicus whellani*. Insects dried at 40ºC showed a sharp decrease in water activity in the first two weeks of storage at 50ºC. On the contrary, insects stored at 4ºC showed a slight increase in water activity after 2 weeks. Drying at 100ºC resulted in an initial lower water activity, which gradually increased at both 4ºC and 50ºC storage. At both drying temperatures, the water activities of insects stored at 4ºC were higher than at 50ºC. Whole insects had similar water activities as grinded insects, when dried at both 40ºC and 100ºC and after 2 weeks of storage, except for drying at 100ºC dried and storage at 50ºC. The data on effect of the initial drying temperature (40^0 C and 100^0 C) and subsequent storage at 4^0 C and 50ºC of *Eulepida mashona* are reported in supplementary materials S1. The comparison of the data from both insects dried at 40ºC and 100ºC, revealed that *Eulepida mashona* had a higher water activity (respectively 0.43 and 0.16)

than *Henicus whellani* (respectively 0.35 and 0.14). For *Eulepida mashona*, similar trends as for *Henicus whellani* were observed for water activities in subsequent storage after drying.

The observed differences in water activity during storage at 4°C and 50°C could be explained by the fact that the resulting water activity is temperature dependent (Kamau et al., 2018; Lipasek et al., 2013). The increase in water activity of dried insects stored at 4ºC could be a result of rehydration. At lower temperature, proteins have more water binding capacity (Kamau et al., 2018), which can result in adsorbing moisture from the external environment. In addition, in this study polyethylene was used to store insects. Polyethylene is moderately permeable to water (Kamau et al., 2018) and moisture from the external environment could thus have permeated into the package. The difference in water activity between the two insect species could be explained by the difference in the insect matrix. *Eulepida mashona* is a beetle with a rubbery skin that could hinder migration of water, which may explain the higher remaining water activity after drying while *Henicus whellani* is a cricket with a thin exoskeleton.

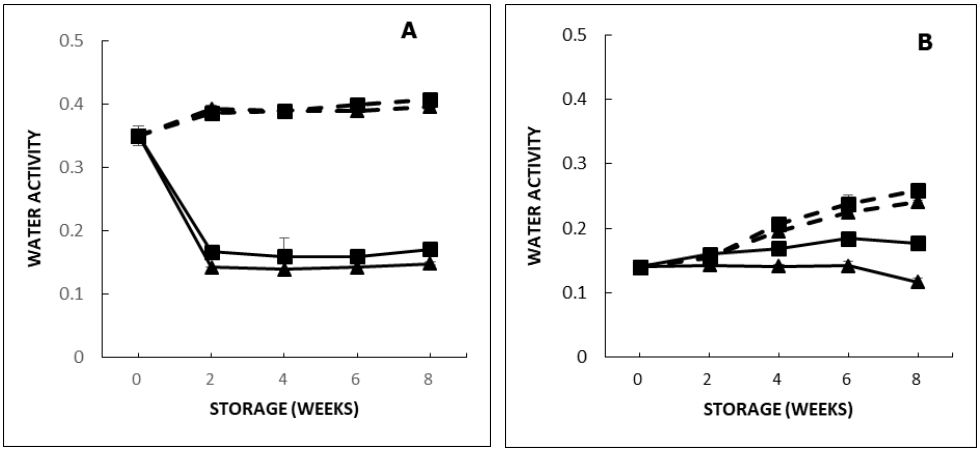

Figure 5.2: Water activity of whole and grinded *Henicus whellani* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line).
Key: ■ =whole insects; ▲ = grinded insects

5.3.2 Changes in the secondary non-volatile lipid oxidation product during storage

To investigate the extent of lipid oxidation in dried insects during storage, the malondialdehyde (secondary non-lipid oxidation product) was analysed using the TBARS method. Figure 5.3 shows the changes in malondialdehyde concentration of *Henicus whellani* dried at 40ºC and 100ºC and subsequently stored at 4ºC and 50ºC (data of *Eulepida mashona* shown in supplementary materials S2). The concentration of malondialdehyde, at the start of storage, was higher for both insect species dried at 100ºC than those dried at 40ºC. Higher TBARS values were indeed expected in samples dried

at 100ºC opposed to 40ºC as the extend of lipid oxidation increases with temperature (Sun et al., 2002). Subsequent storage at 4ºC and 50ºC resulted in variable concentrations of malondialdehyde, which was less obvious in *Henicus whellani* compared to *Eulepida mashona.* The concentration of malondialdehyde of *Henicus whellani* dried at 40ºC decreased in the first 4 weeks both at 4ºC and 50ºC storage, and subsequently increased after 4weeks. For all *Henicus whellani* samples dried at 100ºC, except for whole insects stored at 50ºC, the concentration of malondialdehyde increased in the first two weeks and thereafter decreased. *Eulepida mashona* samples dried at 40ºC showed an increase in the malondialdehyde concentration in respectively the first 2 and 4 of weeks for samples stored at 4ºC and 50ºC. In contrast, for *Eulepida mashona* dried at 100ºC, the concentration decreased in the first 4 weeks and thereafter increased. Several studies have shown either an increase e.g. (Bak et al., 1999; Mielnik et al., 2006) or decrease (Munekata et al., 2017) in TBARS value over time for various food products. TBARs can fluctuate with various stages of lipid oxidation and during storage (Lee et al., 2014). Whilst an increase in malondialdehyde concentration indicates an increase in lipid oxidation, decrease in TBARS values could be due to instability of malondialdehyde. Malondialdehyde is susceptible to oxidation yielding organic alcohols and acids, which are not measured by the TBARS assay (Fernández et al., 1997).

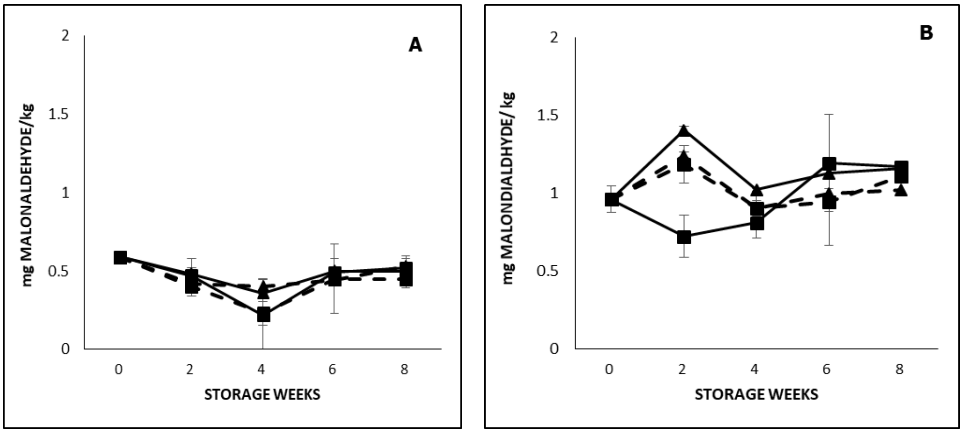

Figure 5.3: Malondialdehyde concentration of whole and grinded *Henicus whellani* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line).

Key: ■ =whole insects; ▲ = grinded insects

The TBARS assay could also measure irrelevant components not originating from lipid peroxidation e.g. amino acids or carbohydrates, which can react with the TBA and cause colour formation (Moore & Roberts, 1998). Despite *Eulepida mashona* has higher TBARS values, it cannot be concluded that lipid oxidation was higher in the beetles than crickets, as the TBARS method lacks specificity. Furthermore, it was expected that grinded insects would be more susceptible to lipid oxidation due to

the large surface area and thus larger contact area with oxygen (Chudy et al. 2015). However, TBARS values were not significantly different between grinded and whole insects dried at 40^{o}C. Only, insects dried at 100^{o}C and stored at 50^{o}C showed significant differences at two weeks storage, which levelled after four weeks storage.

5.3.3 Identified volatile compounds in insects

Table 5.1 shows the volatiles components identified in both insect species and typical odour characteristics of some volatiles based on information from the literature. In addition, the table shows which volatiles are typical lipid oxidation products. In total, 62 volatile components, belonging to 11 functional groups, were identified. The main groups, based on the number of volatiles detected were hydrocarbons, alcohols, carboxylic acids, aldehydes, and nitrogen containing groups. Many (22/62) of the identified compounds belong to the hydrocarbons group. Raw insects contained the least number (22) of volatile compared to the dried insects, which contained 35 volatiles. Acetoin, which has a butter cream odour characteristic, was the major volatile component (based on peak area) in raw insects for both *Henicus whellani* and *Eulepida mashona*. The concentration of acetoin decreased during boiling, drying, and storage. Moreover, boiling decreased the total concentration of volatiles belonging to multiple functional groups. However, some volatiles were only found in boiled insects for example, 2- methyl phenol. Although, three typical lipid oxidation volatiles (heptanoic, hexanoic, and heptanoic acids) were identified in raw insects, the largest number of such volatiles (like nonanoic acid, hexanal, octanoic acid) was identified in the dried and subsequently stored insects. Typical odour characteristics of these volatiles include fatty, rancid, cheese, which may contribute to an unsatisfactory smell that can limit the shelf life of dried insects

Table 5.1: Volatile compounds identified in the raw, boiled, and dried *Eulepida mashona* and *Henicus whellani*, and some typical odour characteristics

Retention index	Component	Raw	Dried	Volatile lipid oxidation products (VLOPs)	Odour characteristic	References
	Alcohols (5)					
625	2-methyl-1-Propanol		+			
736	3-methyl -1-butanol	+	+		Whiskey, malt, burnt	(Acree & Arn, 2004; Arsa & Theerakulkait, 2018)
1277	2-butyl-1-octanol		+			
1166	Phenylethyl alcohol					
1407	2,4,7,9-Tetramethyl-5-decyn-4,7-diol					
	Aldehydes (5)					
800	Hexanal		+	++	Green, grassy, fruity; grass, tallow, fat	(Arsa & Theerakulkait, 2018; Caporaso et al., 2018)
901	Heptanal			++	Fat, citrus, rancid	(Acree & Arn, 2004)
1104	Nonanal		+	++	Tallow and fruity; fatty, citrus, green	(Arsa & Theerakulkait, 2018; Lee et al., 2014)
1263	2-Decenal, (Z)-					
962	Benzaldehyde	+			Almond, burnt sugar	(Arsa & Theerakulkait, 2018)
	Aromatic Hydrocarbons (1)					
893	Styrene		+			
	Carboxylic acids (9)					
610	Acetic acid	+	+		Pungent, vinegar; sour	(Arsa & Theerakulkait, 2018; Caporaso et al., 2018)
700	Propanoic acid	+	+		Pungent, acidic, cheesy, vinegar; pungent, rancid soy	(Acree & Arn, 2004; Caporaso et al., 2018)
772	2-methylpropanoic acid	+	+			
805	Butanoic acid	+	+			
863	3-methyl butanoic acid	+	+			
990	Hexanoic acid	+	+	++	sweaty	(Arsa & Theerakulkait, 2018)

1078	Heptanoic acid	+	+	++	Sour-sweat-like	(Arsa & Theerakulkait, 2018)
1180	Octanoic acid		+	++	Sweat, cheese	(Acree & Arn, 2004; Arsa & Theerakulkait, 2018)
1273	Nonanoic acid		+	++	Green, fat	(Arsa & Theerakulkait, 2018)
	Diverse functional groups (3)					
661	1-methoxy-2-propanol,					
713	Acetoin	+	+		Butter, cream	(Acree & Arn, 2004)
985	4-(acetyloxy)- 2-Butanone,	+				
	Esters (1)					
861	2-Propenoic acid, butyl ester					
	Hydrocarbons (22)					
863	4-methyl-octane		+			
836	2,4-Dimethylhept-1-ene	+	+			
991	2,2,4,6,6-pentamethyl-heptane,		+			
-	2,2,4,4-Tetramethyloctane		+			
1100	Undecane		+			
1060	4-methyl-decane,		+			
1054	1,4-dimethyl-, cis-cyclooctane					
1152	6-methyl-undecane					
1200	Dodecane		+		alkane	(Acree & Arn, 2004)
1125	3,7-dimethyl-decane					
1170	3-methyl-undecane,		+			
-	2,7,10-trimethyl-dodecane					
1210	2,5-dimethyl-undecane					
1030	Limonene	+			Lemon, orange,	(Acree & Arn, 2004)
1207	4,7-dimethyl-Undecane	+				

870	2,4,6-trimethyl-heptane,	+			
-	2-Undecene, 3-methyl-, (E)-				
1400	Tetradecane				
1346	Heptylcyclohexane				
1346	Cyclotetradecane				
1600	Hexadecane				
	2,3,4-trimethyl-Hexane		+		
	Ketones (3)				
1193	2-Decanone				
986	6-methyl-5-Hepten-2-one	+	+		
1073	(E,E)-3,5-Octadien-2-one,				
	Nitrogen-containing (7)				
917	2,5-dimethylpyrazine		+		
1004	Trimethylpyrazine	+	+		
1089	Tetramethyl Pyrazine	+	+		
742	Acetamide	+	+		
-	6-Undecylamine				
1174	2-Piperidinone				
	Formamide		+		
	Phenols (4)				
1054	2-methyl-Phenol,				
980	Phenol	+			
1075	3-methyl-Phenol,	+	+		
1077	p-Cresol				
	Sulphur-containing(2)				
824	Dimethyl Sulfoxide	+	+		
922	Dimethyl sulfone	+	+	Sulfur, burnt	(Acree & Arn, 2004)

Key: + represents the presence of a component in either raw or dried insects; ++ indicates that the component is a typical lipid oxidation volatile.

Figure 5.4 shows the changes in total concentration of volatiles in *Henicus whellani* dried at 40ºC and 100ºC in storage at 4ºC and 50ºC (data of *Eulepida mashona* are shown in the supplementary material. For both species, insects dried at 100ºC had a lower total concentration of volatiles at the beginning of storage. The lower concentration can be attributed to the loss of volatiles during drying at the high temperatures. Acetoin, a typical component found in raw insects as a pheromone (Rochat et al., 2002), is an example of volatile that decreased in concentration. The total volatiles concentration of dried insects (40ºC and 100ºC) increased in first 2-4 weeks at both 4ºC and 50ºC storage for both insects. Our observation of the decrease in volatiles with drying is similar to a decrease in volatiles as found in herbs by (Chaliha et al., 2013). The decrease in concentration could be attributed to loss of volatiles due to the high temperatures. Drying insects resulted in carboxylic acid group volatiles dominating the present volatile groups and there was an increase in the concentration. In a study by (Yeo et al., 2013), acids made the largest proportion of the volatiles in raw *P. brevitarsis* larvae. An increase in the volatiles concentration during storage has also been reported by Chaliha et al. (2013) for the studied herbs.

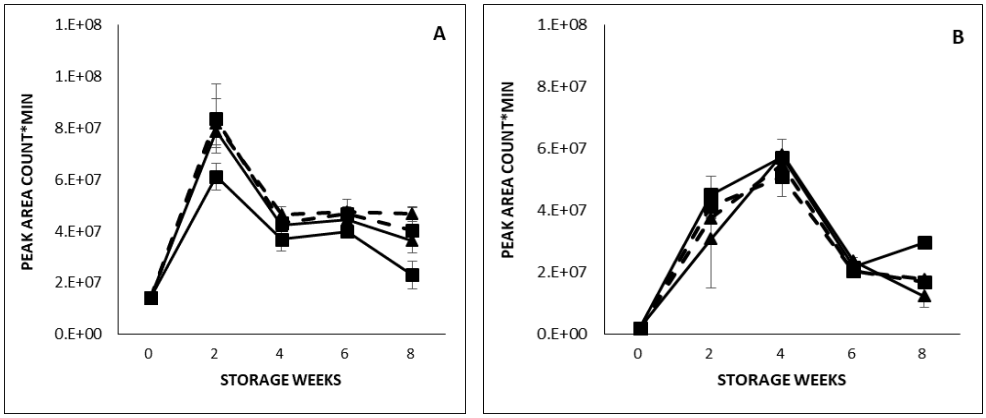

Figure 5.4: Total concentrations of volatiles measured in whole and grinded *Henicus whellani* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line)
Key: ■ =whole insects; ▲ = grinded insects

5.3.5 Changes in total lipid oxidation volatiles products during drying and storage

Volatile lipid oxidation products (VLOPs) identified from the typical products of oxidising oleic, linoleic, and linolenic acids according to Schaich (2005) were summed to indicate the extent of lipid oxidation during storage. The components referred to as the VLOPs (in this study) are hexanal, heptanal, nonanal, hexanoic acid, octatonic acid, and nonanoic acid. Figure 5.5 shows the concentration of the VLOPs measured in the raw, boiled, and dried insects. The concentration of VLOPs was higher in raw *Eulepida mashona* than in raw *Henicus whellani*. Boiling increased the total concentration of the VLOPs for both insect species, with highest concentrations found for *Henicus*

whellani. Similar concentration of VLOPs were observed for *Eulepida mashona* dried at 40ºC and 100ºC whilst *Henicus whellani* dried at 100ºC showed a lower concentration than for 40ºC. The results show that beetles VLOPs are not influenced by processing while in cricket both boiling and drying at low temperature generated high amount of VLOPs. It should be noted that other volatile lipid oxidation components could have been have formed from degradation of intermediate components, but these are not included in the VLOPs. Qiu et al. (2019) reported that higher temperature might quickly remove moisture from the product surface and form an outer layer that can protect the inner lipids from oxidation.

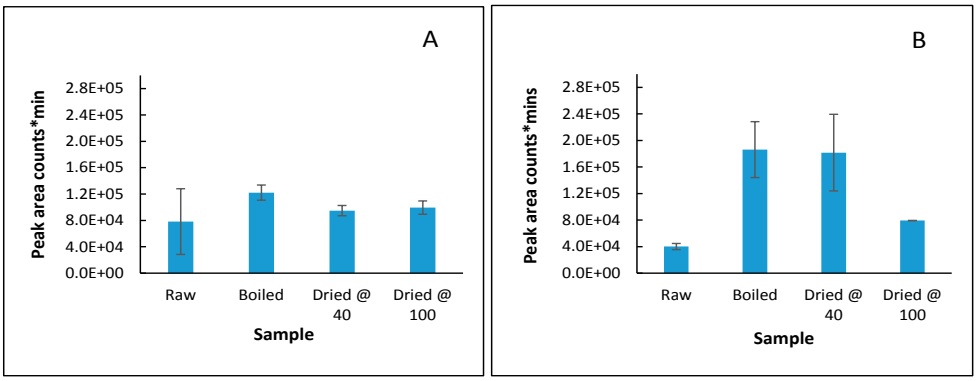

Figure 5.5: Total concentration of VLOPs in raw, boiled, and dried *Eulepida mashona* (A) and *Henicus whellani* (B). Components belonging to the VLOP's are hexanal, heptanal, nonanal, hexanoic acid, octatonic acid, and nonanoic acid.

Figure 5.6 shows the changes in the VLOPs of dried *Henicus whellani* during storage at 4ºC and 50ºC (data of *Eulepida mashona* is shown in supplementary materials S4). There was no clear difference in the initial concentration of VLOPs for both insect species dried at 40ºC and 100ºC. Overall, the concentration of VLOPs gradually increased the first 2-4 weeks during storage at both storage temperature for both insect species. The concentration of VLOPs was highest in insect samples dried at 40ºC and stored for 4 weeks at 4ºC. For samples dried at 100ºC the maximum amount of VLOPs was present after 2 weeks storage. Overall, the concentration of VLOPS was higher in *Henicus whellani* samples (dried at both temperatures) stored at 4ºC compared to those stored at 50ºC. However, this trend was not observed for *Eulepida mashona* dried at 40ºC, as the VLOPs concentration of all samples except grinded insects stored at 4ºC, gradual increased during the 8weeks storage. No clear differences in the VLOPs concentration were observed for whole and grinded *Henicus whellani* dried at 40ºC but for samples dried at 100ºC and stored at 50ºC, which had higher values for the whole insects.

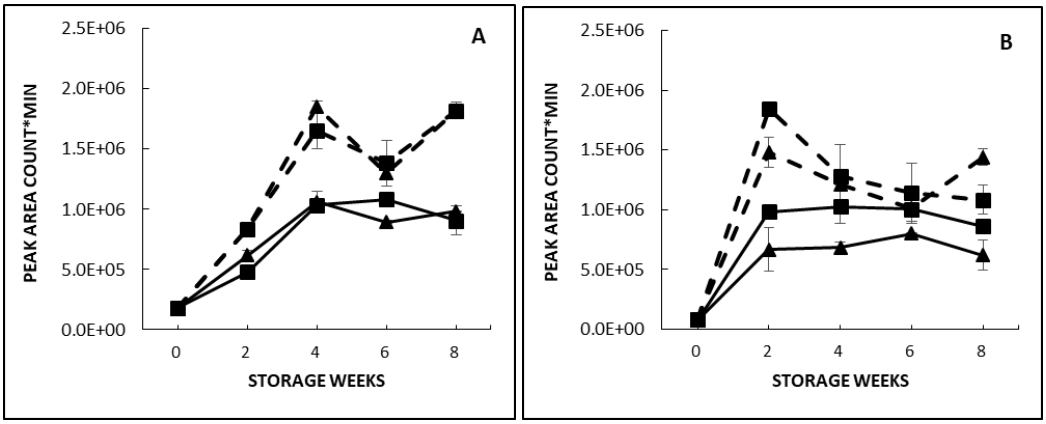

Figure 5.6: Total sum concentration VLOPs of whole and grinded *Henicus whellani* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line).
Key: ■ =whole insects; ▲ = grinded insects

Data showed lipid oxidation is favoured at high water activity. The higher concentration of VLOPs observed in dried samples, which were stored at 4ºC might be explained by their water activity. Insect samples stored at 4ºC showed higher water activities (0.40) than of samples stored at 50ºC (a_w=0.26). According to Fu and Labuza (1997), the rate of lipid oxidation is highest below a_w 0.2 and above a_w 0.3. The decrease in the VLOPs after 2 and 4 weeks in storage could be attributed to the lipid co-oxidation that occurs in dried foods. Due to the high protein content in both insect species, it could be possible that lipid co-oxidation with proteins took place. The secondary oxidation products, such as the aldehydes, ketones, and free radicals can react with proteins and amino acids resulting in a decrease in the hydroperoxides and the volatile oxidation products. The aldehydes may also oxidise further to their carboxylic acid form (Hu, 2016; Schaich, 2005). This could explain the presence of butanoic acid and propanoic acid, which are oxidised aldehydes (butanal and propanal).

The initial concentration of the VLOPs in *Henicus whellani* was higher than in *Eulepida mashona*. The difference in the concentration of VLOPs between the two insect species is in line with their fatty acid profiles. Data on monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in *Eulepida mashona* showed concentrations of 30.4 and 26.9 g/100g fat respectively whilst *Henicus whellani* MUFA and PUFA content is 45.1 and 24.2 g/100g fat respectively (Manditsera et al., 2019).

Conclusion and recommendation

This study demonstrated that lipid oxidation occurs in wild harvested edible insects during drying and subsequent storage, which will limit the shelf life of insects. Measuring of the volatile compounds provided more accurate insight in the pathways of lipid oxidation than the TBARS method (non-volatile products). Drying and storage temperatures are important factors that influenced the concentration of the selected volatile lipid oxidation products (VLOPS) of the studied insects. The drying temperature is critical because drying at 100^{o}C compared to 40^{o}C promoted lipid oxidation, which resulted in higher initial concentrations at the start of storage. However, the low drying temperature (40^{o}C) resulted in relatively higher water activities, which further increased during storage at 4^{o}C. At this storage temperature, the highest concentration of volatile lipid oxidation products was observed after 4 weeks. It is therefore important to dry insects at higher temperature to attain very low water activities, which will slow down further lipid oxidation during storage. Dried insects can be stored as whole insects or grinded insects since no obvious differences were observed for both volatile and non-volatile lipid oxidation products. Multiple factors can influence the dynamics of the lipid oxidation process, such as formation and degradation of volatile and non-volatile products and diffusion of volatile components through packaging. Further research is required to identify the optimal packaging material to improve dried insect shelf life: however, moisture barrier is likely more relevant than oxygen barrier property.

Supplementary material

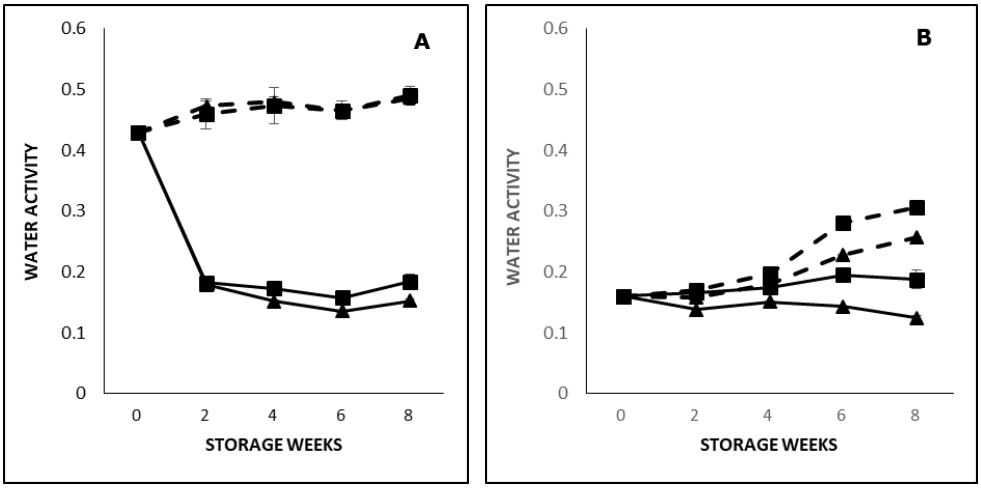

Figure S1: Water activity of whole and grinded *Eulepida mashona* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line)

Key: ■ =whole insects; ▲ = grinded insects

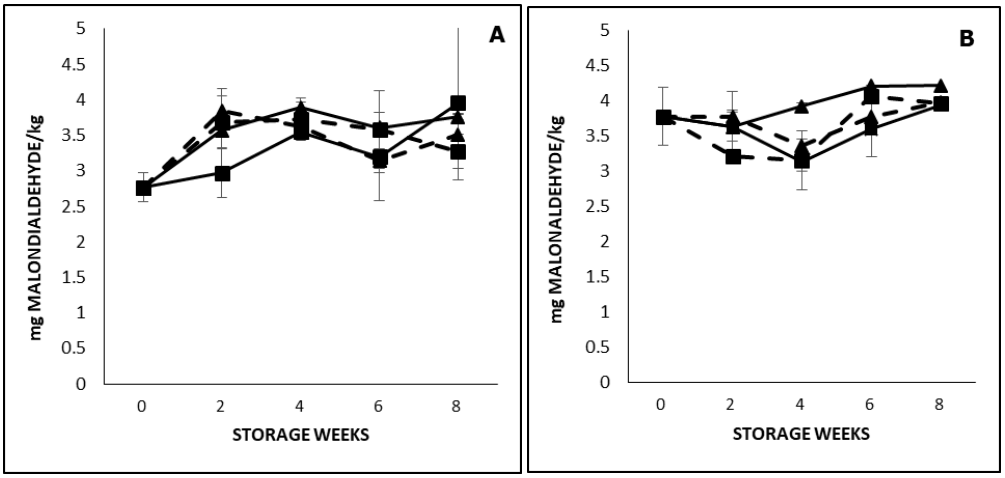

Figure S2: Malondialdehyde concentration of whole and grinded *Eulepida mashona* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line)

Key: ■ =whole insects; ▲ = grinded insects

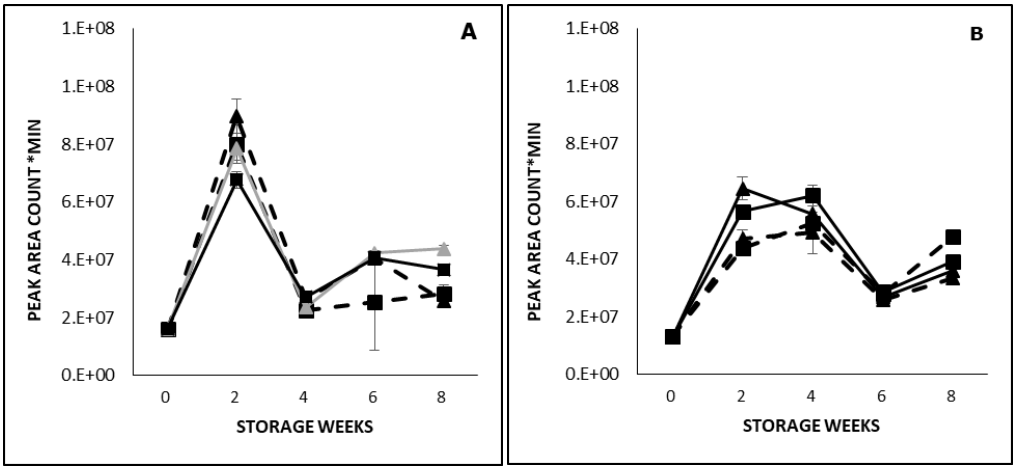

Figure S3: Sum of total concentration volatiles of whole and grinded *Eulepida mashona* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line)
Key: ■ =whole insects; ▲ = grinded insects

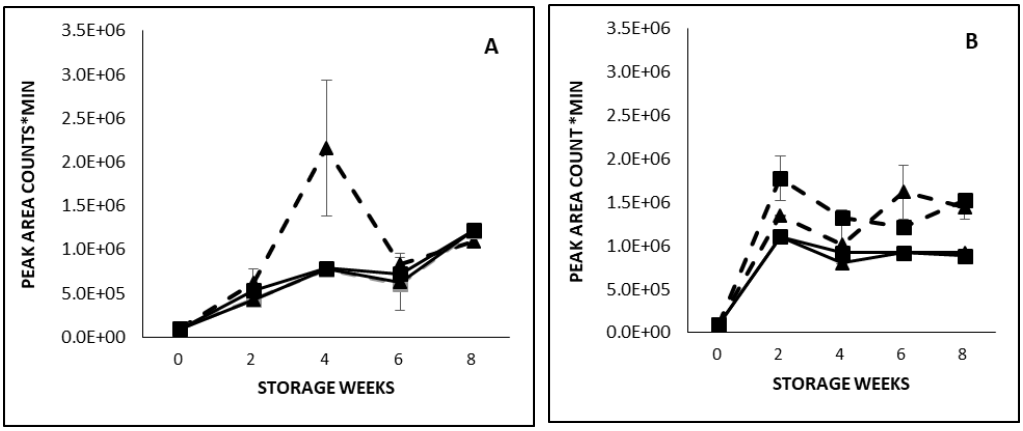

Figure S4: Total sum concentration VLOPs of whole and grinded *Eulepida mashona* dried at 40ºC (A) and 100ºC (B) during storage at 4ºC (broken line) and 50ºC (continuous line)
Key: ■ =whole insects; ▲ = grinded insects

CHAPTER 6

General Discussion

6.1 Background of research

Since time immemorial, wild edible insects have played an important role in the diets of people of the tropics (van Huis, 2003) where climatic conditions promote their abundance. Over 2000 edible insect species are documented to be consumed worldwide (Jongema, 2017), with most being collected from the natural environment. With the world facing food and nutrition insecurity, there has been a huge advocacy for the consumption of edible insects for food and nutrition security (FAO, 2012). However, for most developing countries the insect value chain is mainly traditional and limited to household level. This is despite the potential of wild edible insects to contribute towards food and nutrition security. The traditional insect value chain is characterised by dependence on wild harvested edible insects.

Knowledge on consumer preferences, composition variability, processing conditions, nutrient availability, and shelf stability could strengthen the scientific basis for expanding the use of wild harvested edible insects and for reinforcing nutrition security. This knowledge could give insights that can enable the development of a commercial chain and may ignite interest of various actors (e.g. government and food industry) to support development of such an edible insects' value chain. An understanding of the current practices in the traditional value chain is essential in development of a commercial insect value chain that could help in strengthening the sustainable contribution of edible insects to nutrition security. The main objective of this thesis was therefore to investigate how wild harvested edible insects can contribute to nutrition security in developing countries, with Zimbabwe as a case study. This chapter summarises the major findings and discusses how the research findings could contribute to the effective use of wild edible insects for nutrition security.

6.2 Main research findings

Understanding the current consumption patterns of edible insects, particularly the difference between urban and rural consumers and identification of factors influencing the consumption of edible insects is important for promoting consumption of insects. Insights into the consumer preferences are important in developing targeted strategies to promote consumption of edible insects to certain groups. In **Chapter 2**, a survey was carried out to determine insect consumption patterns among rural and urban populations and identify factors that influence these patterns. The results indicated that consumption of insects was prevalent in both urban (80%) and rural (89.7%) in Zimbabwe. As expected, the rural people (63.9% of insect consumers) consumed edible insects more frequent and in larger quantities than the urban people (14.5% of insect consumers), explained by the fact that rural people have better access to wild harvested edible insects than urban people have. Importantly, the study revealed that in both urban and rural areas, edible insects are not "just eaten" because it is the only food option but are mainly consumed for their taste. In addition, urban people also appreciated the nutritional value and medicinal properties of insects for consumption more than the rural people

do. Insights from this study triggered the interest to gain more knowledge on the nutritional value of wild harvested edible insects, as studied in **Chapter 3**.

Wild harvested edible insects naturally occur without control of environmental factors or their feed. Thus, before proposing general recommendation to use wild harvested insects, the extent to which the nutrient content is similar for the same insect species but harvested from different geographical locations should be established. Therefore**,** in **Chapter 3,** we investigated the nutritional composition and variability in nutritional composition of *Eulepida mashona* (beetle) and *Henicus whellani* (cricket) harvested in different geographical locations (districts) in Zimbabwe**.** Overall, the results indicate that both the beetle and cricket are high in protein (52-57%; 60-71% respectively), iron (24.2-52.9mg/100g) and zinc (10.0-20.9mg/100g). However, some species dependent variations, in protein, fat, and mineral content, exist with geographical place of harvest. The largest variation was found for fat and mineral content. The results revealed that fat and mineral content could be double for insects harvested from different geographical location. It is interesting to note that both insect species were low in fat content (<10%) and comply with the recommended PUFA/SFA ratio for healthier diets, which makes them suitable for low fat diets. Further, the amino acid profiles of both insect species meet the WHO essential amino acid requirements guidelines. Overall, both the beetle and cricket have potential to be used directly in the diet or as an ingredient in food products based on their protein and mineral content levels.

Research on nutritional potential of edible insects is usually focused on the nutritional composition of raw insects. Whilst some insects' species such as termites can be consumed raw, crickets and beetles require some form of heat treatment to improve palatability. However, studies on the effect of processing on the nutritional composition of edible insects are limited. Therefore, in **Chapter 4** we studied the influence of domestic processing methods on protein and mineral retention, protein digestibility, and mineral bioaccessibility of *Eulepida mashona* and *Henicus whellani*. The study revealed that raw (16.23g/100g FW) and roasted (16.54g/100FW) insects had high protein content as compared to boiled (14.76g/100FW) insects indicating that protein was partly lost into boiling water. A maximal decrease in protein digestibility around 25% for boiled beetles and for boiled and roasted crickets was found. The results showed that *Eulepida mashona* (beetles) are a better source for bioaccessible iron than *Henicus whellani* (crickets), as raw beetles had a much higher iron bioaccessibility (30.7%) as compared to raw crickets (8.11%), an indication that mineral bioaccessibility is insect species dependent. Overall, the results on iron content and bioaccessible iron content of the two insect species showed that high a nutrient content does not translate into high bioaccessibility. This should be a matter of concern as many insect species have been reported to potentially curb iron and zinc deficiency based on the mineral contents. Interestingly, boiling resulted in about 50% decrease in iron and zinc bioaccessibility in both species, while roasting did not.

However, despite the negative influence of processing on the nutritional composition and availability, both insect species, based on the recommended daily allowances (RDAs) remain a valuable source of protein and minerals, indicating their great potential to contribute towards nutrition security.

Seasonality of wild harvested edible insects limits their consumption throughout the year. Traditionally, edible insects are sun dried to prolong shelf life, and lipid oxidation could limit the shelf life of dried insects. Therefore, in **Chapter 5**, we examined the effects of processing conditions (drying temperature, grinding, and storage temperature) on lipid oxidation of dried insects. The results showed that drying insects at 40ºC (same as in sun drying) resulted in relatively higher water activities than for insects dried at 100ºC. The water activities respectively increased or decreased in subsequent storage at 4ºC and 50ºC. Insects dried at 100ºC showed a higher concentration of non-volatile lipid oxidation products than insects dried at 40ºC, an indication that high drying temperature promoted lipid oxidation. Volatile lipid oxidation products (VLOPs) increased the first 2-4 weeks during storage at both storage temperature for both insect species. Lipid oxidation was highest in insects dried at 40ºC and stored at 4ºC. Dried insects can be stored as either grinded or whole insects, as no clear differences in the lipid oxidation volatiles components were observed. The key findings of the research presented in this thesis are summarised in Table 6.1.

Table 6.1: Summary of the research key findings

Chapter	Objective	Main findings
2	Determine insect consumption patterns among rural and urban populations and identify factors that influence these patterns	▪ Edible insect consumption prevalent in both rural (89.7%) and urban (80%) Zimbabwe ▪ Rural people consume insects more frequently and in quantity than urban population ▪ Taste is the main motive for consumption ▪ Nutritional value and medicinal properties important for urban population
3	Investigate the variability in nutritional composition of *Eulepida mashona* and *Henicus whellani* amongst different districts of harvest	▪ Protein content of *Eulepida mashona* and *Henicus whellani* is comparable to that of animal sources. ▪ Both insects are good sources of iron and zinc ▪ Variation exist in the protein, fat and mineral with geographical location ▪ Larger variation in fat and mineral content than in protein content ▪ Both insects meet WHO essential amino acid requirement
4	Study the influence of domestic processing methods on protein and mineral retention, protein digestibility and mineral bioaccessibility of *Eulepida mashona* and *Henicus whellani.*	▪ *Eulepida mashona* and *Henicus whellani* percent protein loss was 15% and 10% respectively due to boiling ▪ Percent loss of iron was 12% and 8% for boiled *Eulepida mashona* and *Henicus whellani* ▪ Protein digestibility was lower in boiled insects than in raw and roasted insects. ▪ Raw insects had a higher bioaccessible iron and zinc content than boiled insects ▪ *Eulepida mashona* had higher iron bioaccessibility than *Henicus whellani* ▪ Boiling resulted in about 50% decrease in iron and zinc bioaccessibility in both species
5	Examine effects of processing conditions (grinding, drying and storage temperature) on shelf stability	▪ Drying at 40^{o}C and 100^{o}C, resulted in *Eulepida mashona* having a higher water activity (0.43 and 0.16) than *Henicus whellani* (0.35 and 0.14). ▪ Drying at 100^{o}C promotes more lipid oxidation than drying at 40^{o}C. ▪ Insects dried at 100^{o}C had a higher concentration of malondialdehyde at the beginning of storage than insects dried at 40^{o}C ▪ Concentration of VLOPs increased in the first 2-4weeks at 4^{o}C and 50^{o}C storage ▪ VLOPs concentration was higher for insects stored at 4^{o}C than at 50^{o}C ▪ No notable influence of grinding on the lipid oxidation of dried insects

6.3 Overall discussion

A food-based approach to nutrition security is considered as one of the best ways to tackle malnutrition and micronutrient deficiencies (Allen, 2008), with dietary diversification as a preferred strategy that works best in developing countries (Gibson, 2011). Edible insects or other wild harvested products are nutritious foods that can contribute to dietary diversity and improved nutrition (Kennedy et al., 2017). However, substantial and sustained consumption of nutrient rich foods is needed to attain nutrition security (Maestre et al., 2017). The actual contribution of wild harvested products to nutrition is dependent on the availability, the number of people using the products, the frequency of use, and the quantities consumed relative to other foods (Powell et al., 2015). Therefore, this thesis considered the insect value chain as a whole, which ranges from insects as a raw material, processing to consumption. This research focused on the traditional insect value chain, which is functional at household level (Figure 6.1), and identified the keys aspects that are necessary to promote increased consumption of edible insects. Knowledge on the key aspects i.e. on composition variability, processing conditions, nutrient availability, shelf stability, and consumer preferences of the traditional insect value chain, could be used for transforming the traditional value chain into a commercial insect value chain (Figure 6.1). A justification of insect consumption based on the traditional chain is necessary before recommending change into a commercial chain. Knowledge on these key aspects should result in increased consumption of edible insects through both types of chains thus contributing indirectly to improved nutrition security.

The shortfalls of the traditional insect value chain (discussed later in this section) necessitate improving the functioning and sustainability of the traditional insect chain. In this section, we therefore discuss the implications of the thesis findings for nutritional security and how the knowledge on insect consumption patterns, natural variation in wild harvested insects, and domestic processing conditions in traditional insect value chain can be used for improvement of the traditional chain and development of commercial chains.

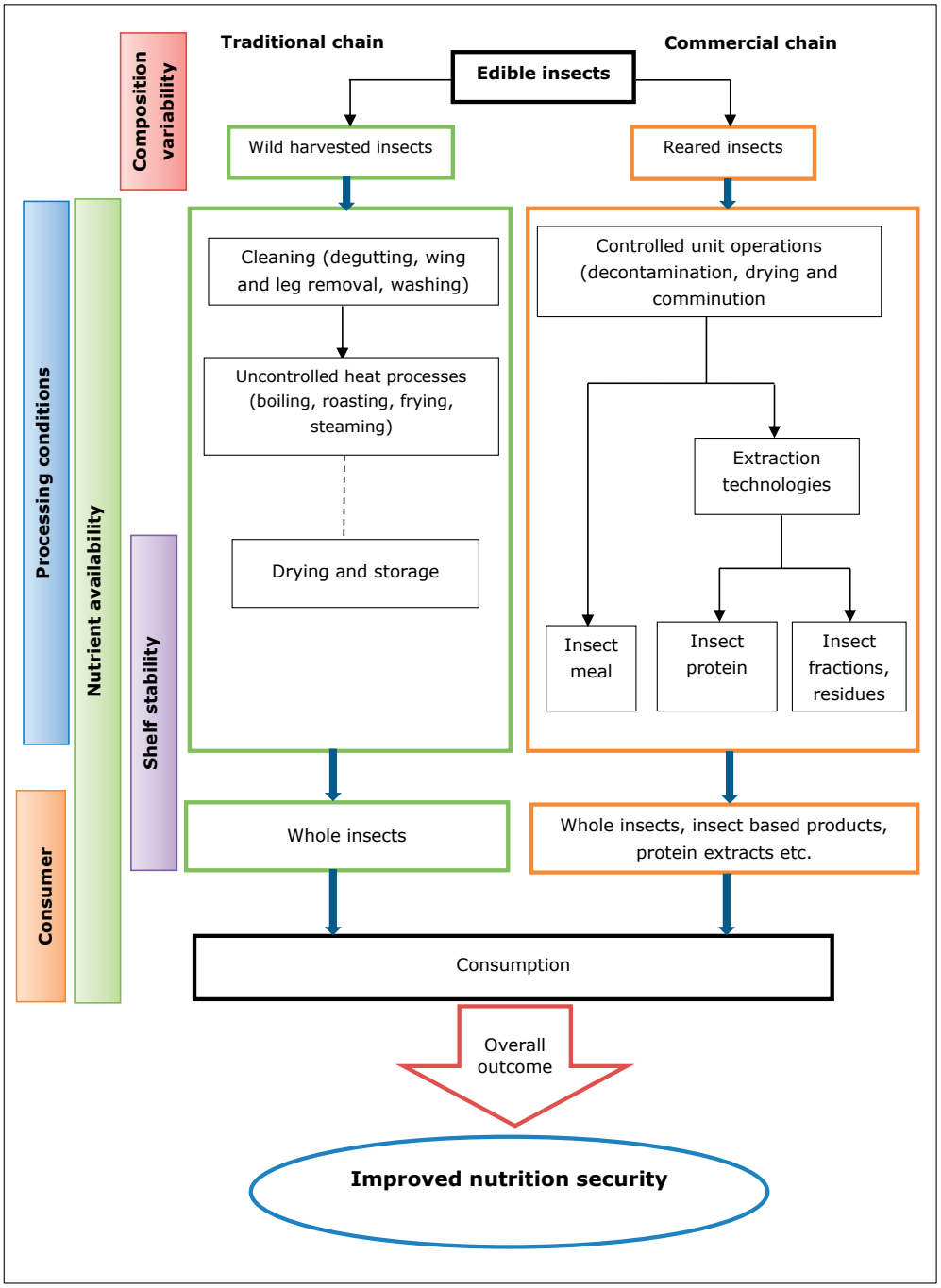

Figure 6.1: The traditional and commercial insect value chains showing the keys aspects that are essential for improving and transforming the traditional insect value chain to commercial value chain

6.3.1 Implications of findings for nutrition security in traditional insect value chain

Edible insects have been identified as one of the future foods, whose essential macro and micro nutrients make them better alternatives for animal than plant based food sources (Parodi et al., 2018). Knowledge on the nutritional content of foods is important for selection and promoting consumption of nutrient dense food. In **Chapter 2**, the consumer survey demonstrated that nutritional value of the edible insects was considered as one of the major motives for consumption of edible insects. According to Powell et al. (2015), lack of data on nutrient composition of wild foods limits studies to quantify the contribution of wild foods to nutrient intake. The current study added data to the body of data on nutritional composition of wild foods and confirmed that edible insects can be potential nutrient dense foods, although differences exist based on geographical locations (Manditsera et al., 2019). Given the diversity of wild harvested edible insects and variability in nutritional composition, this thesis pointed to the need of more nutritional data with respect to geographical location.

Consumption of wild harvested edible insects could contribute substantially to the recommended daily allowances (RDA) of proteins. The research in **Chapter 3** showed that the studied species had a high protein content as compared with other wild harvested and reared insects species (Rumpold & Schlüter, 2013a), even though we used a lower nitrogen conversion factor (4.76 compared to the usual 6.25) for calculating protein content. Addressing malnutrition requires sufficient intake of relevant nutrients from the diet. Knowledge on protein content and/or quality of wild harvested insects helps consumers make informed decisions on what insect species they could consume, to acquire the recommended amounts of nutrients. In **Chapter 2**, we showed that a person could consume at least 50g of insects on average per day. Table 6.2 shows that a portion of 50g of boiled and roasted insects provided between 7.71g and 9.65g of protein that could contribute to 15 to 19 % of the RDA of proteins. Boiled and roasted insects results were used as it is the form the insects are commonly consumed. Table 6.2 further shows a comparison with other protein rich sources being beef, beans and eggs. The observed values for protein are within the range of values for beef and beans.

The diets of many sub Saharan Africa countries are characterised by cereals e.g. maize as a staple. Limiting amino acids in cereals requires that other proteins should be included in the diets. The current thesis also demonstrated that *Eulepida mashona* could complement tryptophan, a limiting amino acid in maize, whereas *Henicus whellani* could not (**Chapter 3**). This indicated that not all edible insects are able to complement the limiting amino acids in staple cereals (Manditsera et al., 2019; van Huis et al., 2013a). Therefore, consumers should be encouraged to consume a variety of insects to increase overall dietary diversity. Parodi et al. (2018) emphasised that future foods should be consumed as part of a diverse diet to ensure that specific nutrient requirements are fulfilled or not exceeded.

Table 6.2: Protein, iron, and zinc content and percentage contribution to RDA of 50g boiled and roasted *Eulepida mashona* and *Henicus whellani* and raw common protein sources

	Protein (g)	Iron (mg)	Zinc (mg)	%RDA protein	%RDA iron	%RDA zinc
Eulepida mashona	7.71	3.02	1.43	15.4	37.8	13.0
Henicus whellani	9.65	7.79	2.43	19.3	97.4	22.1
Beef	11.6	0.9	2.3	23.2	11.3	20.9
Beans	11.7	5.2	1.85	23.4	65.0	16.8
Eggs	3.0	0.9	0.65	6.0	11.3	5.91

Furthermore, developing countries are now challenged with a double burden of malnutrition i.e., undernutrition co-existing with increasing rates of non-communicable diseases. This problem calls for consumption of healthy foods, which are rich in micronutrients and low in fat content. The study in **Chapter 3** showed that wild harvested insects could be a healthy choice, because of their richness in minerals, polyunsaturated fatty acids, and they have a desirable SFA/PUFA ratio (Manditsera et al., 2019). Communities consuming wild harvested edible insects can benefit from the high mineral content of the wild harvested edible insects (**Chapter 3**). However, the high variability in the mineral content of insects harvested from different geographical region can result in consumers not getting the same intake of minerals, depending on from which district the insects are harvested. Table 6.2 shows that a portion of 50g of boiled and roasted insects provided between 3.02mg and 7.79mg of iron and between 1.43mg and 2.43mg zinc that could contribute to up to 97.4% and 22.1% of the RDA of iron and zinc respectively. The observed % RDA values for insects are within or above the values for beef, beans and eggs indicating that insects for iron and zinc are comparable to other protein sources. To improve the mineral intake, consumption of a variety of insect species is encouraged. This message could be communicated to people already consuming the insects through nutrition education.

The results of the high variability in mineral content (**Chapter 3)** and low iron and zinc bioaccessibility **(Chapter 4)** in edible insects, especially for crickets in this study, necessitates the need to rethink that all edible insects can alleviate micronutrient deficiency, particularly iron and zinc deficiencies. Various researches have concluded that edible insects can possibly combat iron and zinc deficiency (Christensen et al., 2006; Mwangi et al., 2018; van Huis et al., 2013a). Since nutrition security is beyond nutrient content, results reported in **Chapter 4** showed that iron bioaccessibility is low especially in crickets. Therefore, we concluded that not all edible insects could contribute substantially in alleviating micronutrient deficiencies. In **Chapter 4**, we reported that *Henicus whellani* (crickets) have a lower iron bioaccessibility (8.11%) than (*Eulepida mashona*) beetles (30.7%) despite the higher iron content in crickets (15.25mg/100g FW) than beetles (6.40mg/100g

FW). A study by Latunde-Dada et al. (2016) also confirmed these findings. The available wild harvested edible insects therefore need to be screened based on the bioavailability of the minerals rather than the mineral content. Moreover, there is a need to look into the different possibilities to improve on the bioaccessibility. Furthermore, lessons can be learnt from iron-rich cereals for which micronutrient bioavailability has been improved by certain household food preparation and processing practices. Germination, fermentation, dehulling, and soaking are examples of techniques used to improve the mineral bioaccessibility of cereals. The strategy can either induce hydrolysis of anti-nutrients or cause passive diffusion of anti-nutrients from the cereals. However, different strategies might be required for edible insects since the matrix differs from that of cereals.

The common domestic boiling process has a negative influence on the contribution of wild harvested edible insects to nutrition security. In **Chapter 4**, we showed that domestic boiling reduced the protein digestibility and mineral bioaccessibility of both beetles and crickets. Longer boiling of the insects resulted in an increased loss of proteins and mineral into the boiling water. In practice, the remaining water is not used, which decreases the possible intake of the nutrients. Nutritional deficiencies occur when physiological requirements cannot be met by the absorption from the diet (Zimmerman and Hurrell, 2007). Even a gram of protein or mineral lost during cooking could make a difference to a person who is malnourished and in need of proteins or a particular mineral. It is therefore necessary to communicate the results to practising communities to adopt minimal boiling and roasting in order to ensure minimal loss of nutrients. However, local people usually believe that longer boiling of insects improves the safety and softens the insects. The safety risks of edible insects inherent to the shorter boiling and roasting need to be confirmed before the advice can be widely communicated. In addition, communities could be encouraged to explore other preparation technique that minimise loss of nutrients.

6.3.2 Transformation from the traditional to the commercial insect value chain

The current traditional insect value chain, which solely depends on wild harvesting, is characterised by variability in the nutritional composition, uncontrolled processing conditions, and limited insect products. In general, wild harvesting will be unsustainable because of growing demand for insect products and rapid population growth (Kelemu et al., 2015). To increase the potential of contributing to nutrition security, a transformation from traditional to commercial insect value chain would be necessary. Commercial value chain requires standardised raw materials in terms of nutritional composition. The findings in **Chapter 3,** about the variability in protein, fat and mineral content of wild harvested edible insects from different geographical place of harvest showed that the protein content variation in the investigated insects was rather low, which makes them a good constant raw material source. However, the seasonal availability of the insects may limit its possibilities for supply into commercial chains. Rearing insects is an important prerequisite to avail insects in large quantities

(Kelemu et al., 2015). However, commercialisation of wild harvested insects just like any other non-wood forest products can result in ecological consequences including resource depletion, changes in biodiversity, and resource quality (Baiyegunhi & Oppong, 2016). Therefore, mass rearing would be necessary to realise a commercial insect value chain that can supply to a large population. Through mass rearing, the variability in nutritional composition of insects could be controlled and adjusted. Through selection of species, consumer preferences can be acknowledged. Over the past years, in the western countries, there has been a rise on captive rearing of edible insects and efforts have also started in Africa (Ayieko et al., 2016; Dobermann et al., 2017). Rearing of currently, consumed insects should be encouraged as it increases the chance of acceptance.

The knowledge gained on the domestic processing method (**Chapter 4**) and shelf life stability (**Chapter 5**) can be useful in designing processing conditions for insects in the commercial value chain. We demonstrated that boiling insects for 30 minutes or longer resulted in 25% reduction in protein digestibility and about 50% reduction in iron and zinc bioaccessibility. It is therefore necessary to optimise conditions or explore other processing methods to minimise loss of nutrients. Sun drying of edible insect (investigated by drying at 40^{o}C in **Chapter 5**) limited the shelf life of dried insects due to high remaining water activity and occurrence of lipid oxidation. Any processing technique applied to food products should balance between palatability, nutritional quality, and safety. Safety is a key aspect that needs to be further investigated to realise the full potential of nutritious foods (Maestre et al., 2017). In a commercial insect value chain, maintaining eating and keeping qualities is essential. Thus, a need to design processing techniques that limit lipid oxidation in stored dried insects.

The protein content of edible insects is comparable to that of animal based and other emerging new protein sources such as algal proteins (Loveday, 2019) (**Chapter 3)**, presenting a possibility to be used as a raw material for protein-based products. In developing countries, the impact of edible insects to nutrition security in urban areas can be limited by the lack of availability of insects (Ayieko et al., 2010; Manditsera et al., 2018; Obopile & Seeletso, 2013). In the survey in **Chapter 2**, we found differences in insect consumption patterns and preferences between rural and urban consumers. Urban consumers appreciated the nutritional value and medicinal properties of insects more compared to rural consumers. As emphasised by Kelemu et al., (2015), people expand their food choices to fit their culture and change in lifestyles. The insect-based food products should fit with consumer lifestyles of different populations. Moreover, the success of insect-based foods can be also affected by appropriate communication of valuable quality attributes that fit consumer demands (Alemu et al. (2017). It is imperative that insect products are designed to cater for the needs and preferences of different groups. Thus, an opportunity exists for a commercial insect value chain to design and develop a wide range of edible insect products. As shown in Fig 6.1, the commercial value chain could go beyond whole insects as the sole final product but consider other products and extraction of major components for

use into other foods. Through the development of a commercial insect value chain, a diversification of insect products could increase the uptake of insects.

6.4 Methodological considerations

This thesis is based on a case study of Zimbabwe and generalisations are made on the use of edible insects for nutrition security in developing countries. Each country has its own food consumptions patterns and more so differences can exist for different regions in same country. Availability and accessibility of edible insects are not the same for all countries. Edible insects are traditional food whose consumption could be linked to tradition and beliefs of people in a country. Given that diverse beliefs exist, their impact on the extent of prevalence of insect consumption could be different. However, similarities in insect consumptions exist (van Huis, et al., 2013)

The use of 6.25 factor has been reported to overestimate the protein content of insects. For the purpose of this thesis, 4.76 nitrogen to protein conversion factor was used to calculate the protein content (Janssen et al., 2017). However, the protein content could still be lower than reported. We calculated conversion factors based on the amino acid profiling and nitrogen content, and found 4.65 and 3.83 for *Eulepida mashona* and *Henicus whellani*, respectively. However, because of the variation that exists in protein content of wild harvested insects, we could not use these conversion factors.

For this thesis, we used the *in-vitro* method to estimate the nutrient availability of the insects. The harmonised INFOGEST protocol used in this thesis is well recommended for *in-vitro* studies. Whilst the protocol resembles what happens during human digestion, it lacks chitinase, an enzyme that exist in the human system of insect eating consumers. Furthermore, the subsequent methods for measuring nutrient availability after digestion have limitations in getting the true picture of nutrient bioavailability. In this study, the *in-vitro* methods only determined the release of the component from the matrix in which it is present because of gastrointestinal digestion but did not evaluate absorption of the released nutrient. The true nutrient bioavailability is beyond the release of nutrients as it has a physiological or metabolic endpoint (Etcheverry et al., 2012) and is further influenced by host factors such as age, genotype, etc. (Etcheverry et al., 2012).

Iron bioaccessibility based on solubility is sometimes not a reliable indicator of bioavailability (Etcheverry et al., 2012). The method served well the purpose of determining the relative differences that could exist in the insect species and processing methods. *In vitro* mineral bioaccessibility methods are simple, rapid and inexpensive used as an alternative to human and animal *in-vivo* studies (Cilla et al., 2018) method of screening foods for bioaccessibility (Fernandez-Garcia et al., 2009) and influence of processing on mineral bioaccessibility (Etcheverry et al., 2012). Validation of the in vitro

results using in- *vivo* studies is required to get the true nutrient bioavailability. In vivo studies are expensive and very time consuming and is out of the scope of this thesis work.

Furthermore, composition of meals is of importance in bioavailability studies. In this study, we did not consider the influence of meal composition on nutrient availability. In Zimbabwe, the survey results (**Chapter 2)** showed that 77% of the insect consumers eat insects in combination with the staple maize meal (Manditsera et al., 2018). Presence of anti- nutrient factors such as phytic acid, tannins, and phenolics in cereal-based products could negatively influence bioaccessibility.

In this thesis, the in-vitro protein digestibility was based the degree of hydrolysis using O-phthaldialdehyde (OPA) method. The method is limited in that it only quantifies primary amino groups and it is not possible to know the resulting essential amino acids in the supernatant. In **Chapter 3**, we reported that both raw insects met the FAO essential amino acid requirements. The amino acid profiling was not performed after processing and digestion in **Chapter 4**, and it is possible that some essential amino acids are lost during processing and could affect negatively on nutrition security.

For the purpose of this thesis, we considered lipid oxidation as the limiting factor of shelf life of edible insects. In **Chapter 5**, we monitored lipid oxidation through measuring volatiles lipid oxidation products. Quantification of the volatiles was based on peak areas thus could not necessarily link the concentrations to the smell. We thus recommend quantification of the volatiles so that they can be linked to the threshold values. Moreover, a sensorial study should follow to get insights into how people perceive the changes in the aroma of insects during storage.

6.5 Recommendation for research

The following are recommendations for further research based on the outcomes of the PhD research:

a) In this study, we were able to show that boiling and roasting influence mineral bioaccessibility negatively. This thesis did not investigate the mechanisms behind the reported mineral bioaccessibility and protein digestibility. The following are still unknown: a) the state in which minerals exist in the insects b) mineral interaction with other food components c) how insect matrix affect protein digestibility. Therefore, we recommend the investigation of the mechanisms of mineral bioaccessibility and protein digestibility to be undertaken. Moreover, insights into how different processing techniques modify the insect matrix are required to understand mechanisms that affect protein digestibility and mineral bioaccessibility.

b) This study focused on lipid oxidation as one of the possible limiting factors of shelf life of edible insects. However, other factors could have an influence on the physical, chemical, and biological shelf stability of edible insects as well. Therefore, further investigations could elaborate in more detail the main shelf life limiting factors and underlying mechanisms to optimise storage and packaging conditions. More specifically, regarding the lipid oxidation study, we focussed on drying and storage temperatures, whereas packaging could play a role in maintaining the quality properties of edible insects, which needs further investigation.

c) This thesis explored consumptions patterns of insects (what, how, when and how much) but to develop insect value chain for sustainable consumption, further research is needed to gain insight in factors determining consumption dynamics of fresh and processed insects and insect-based products.

6.6 Conclusion

The studies in this thesis have shown that wild harvested insects have a potential to contribute to the nutrition security of people who are currently consuming insects. First, it became clear that traditional insect consumption is an existing practise for the majority of the population of rural and urban areas in Zimbabwe, although the lack of availability in urban areas is a factor that limits the actual intake/ frequency of consumption. Data on the nutritional composition and nutritional quality of *Eulepida mashona* and *Henicus whellani* as a case study justified the current traditional insect value chain, also when the variability in composition with harvesting location is taken into account. However, to increase benefits from the nutritional composition, the current traditional processing practices need modifications to ensure optimal nutritional. In addition, there is a need to dry insects at high temperatures and identify optimal packaging material to improve shelf life of dried insects.The thesis also provides a scientific knowledge basis for expanding the understanding of mineral and protein bioavailability in edible insects. Insights provided by this thesis, suggested that wild harvested edible insects can be treated just like any other food material that can have a formal supply chain, whereby quality of primary products, processing/ storage conditions, and compliance to consumer preferences are of importance. As such, the traditional insect value chain can co-exist with and/or could develop into a commercial insect value chain, whereby rearing will improve the availability of edible insects especially for the urban population. The knowledge on the key aspects related to insect consumption i.e. composition variability, processing conditions, nutrient availability, shelf stability, and consumer preferences could be used to support transforming traditional value chains into a commercial ones.

REFERENCES

Acree, T., & Arn, H. (2004). Flavornet and human odor space Retrieved 25 March 2019, 2019, from http://www.flavornet.org/flavornet.html

Ajai, A. I., Bankole, M., Jacob, J. O., & Audu, U. A. (2013). Determination of some essential minerals in selected selected edible insects. *African Journal of Pure And Applied Chemistry, 7*(5), 4.

Akullo, J., Agea, J. G., Obaa, B. B., Okwee-Acai, J., & Nakimbugwe, D. (2018). Nutrient composition of commonly consumed edible insects in the Lango sub-region of northern Uganda. *International Food Research Journal, 25*(1), 159-166.

Alamprese, C., Cappa, C., Ratti, S., Limbo, S., Signorelli, M., Fessas, D., & Lucisano, M. (2017). Shelf life extension of whole-wheat breadsticks: Formulation and packaging strategies. *Food Chemistry, 230*, 532-539.

Alamu, O. T., Amao, A. O., Nwokedi, C. I., Oke, O. A., & Lawa, I. O. (2013). Diversity and nutritional status of edible insects in Nigeria: A review. *International Journal of Biodiversity and Conservation, 5*(4), 8.

Alemu, M. H., Olsen, S. B., Vedel, S. E., Kinyuru, J. N., & Pambo, K. O. (2017). Can insects increase food security in developing countries? An analysis of Kenyan consumer preferences and demand for cricket flour buns. *Food Security : The Science, Sociology and Economics of Food Production and Access to Food, 9*(3), 471-484.

Allen, L. H. (2008). To what extent can food-based approaches improve micronutrient status? . *Asia Pacific Journal of Clinical Nutrition, 17* (1), 103-105.

Arsa, S., & Theerakulkait, C. (2018). Preparation, aroma characteristics and volatile compounds of flavorings from enzymatic hydrolyzed rice bran protein concentrate. *Journal of the Science of Food and Agriculture, 98*(12), 4479-4487.

Ayessou, N. C., Ndiaye, C., Cissé, M., Gueye, M., Sakho, M., & Dornier, M. (2014). Nutrient composition and nutritional potential of wild fruit Dialium guineense. *Journal of Food Composition and Analysis, 34*(2), 186-191.

Ayieko, M., Oriaro, V., & Nyambuga, I. A. (2010). Processed products of termites and lake flies: improving entomophagy for food security within the lake victoria region. *African Journal of Food, Agriculture, Nutrition and Development, 10*(2).

Ayieko, M. A., Ogola, H. J., & Ayieko, I. A. (2016). Introducing rearing crickets (gryllids) at household levels: adoption, processing and nutritional values. *Journal of Insects as Food and Feed, 2*(3), 203-211.

Ayieko, M. A., & Oriaro, V. (2008). Consumption, indegenous knowledge and cultural values of the lakefly within Lake Victoria region. *African Journal of Environmental Science and Technology, 2*(10), 5.

Baiyegunhi, L. J. S., & Oppong, B. B. (2016). Commercialisation of mopane worm (Imbrasia belina) in rural households in Limpopo Province, South Africa. *Forest Policy and Economics, 62*, 141-148.

Bak, L. S., Andersen, A. B., Andersen, E. M., & Bertelsen, G. (1999). Effect of modified atmosphere packaging on oxidative changes in frozen stored cold water shrimp (Pandalus borealis). *Food Chemistry, 64*(2), 169-175.

Barker, D., Fitzpatrick, M. P., & Dierenfeld, E. S. (1998). Nutrient Composition of Selected Whole Inverterbrates. *Zoo Biology, 17*, 123–134.

Becker, T., Benner, E., & Glitsch, K. (2000). Consumer perception of fresh meat quality in Germany. *British Food Journal, 102*(3), 246-266.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., & Ricci, A. (2013). Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety, 12*(3), 296-313.

Berezina, N. (2017). Insects: novel source of lipids for a fan of applications. *Oilseeds and Fat crops and Lopids, 24*(4).

Blásquez, J. R.-E., Moreno, J. M. P., & Camacho, V. H. M. (2012). Could Grasshoppers Be a Nutritive Meal? *Food and Nutrition Sciences, 03*(02), 164-175.

Bosch, G., Zhang, S., Oonincx, D. G., & Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *J Nutr Sci, 3*, e29.

Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition, 108* (Suppl 2), S183-211.

Bukkens, S. G. F. (1997). The nutritional value of edible insects. *Ecology of Food and Nutrition, 36*(2-4), 287-319.

Caparros Megido, R., Poelaert, C., Ernens, M., Liotta, M., Blecker, C., Danthine, S., Tyteca, E., Haubruge, E., Alabi, T., Bindelle, J., & Francis, F. (2018). Effect of household cooking techniques on the microbiological load and the nutritional quality of mealworms (Tenebrio molitor L. 1758). *Food Res Int, 106*, 503-508.

Caporaso, N., Whitworth, M. B., Cui, C., & Fisk, I. D. (2018). Variability of single bean coffee volatile compounds of Arabica and robusta roasted coffees analysed by SPME-GC-MS. *Food Res Int, 108*, 628-640.

Cardoso, C., Afonso, C., Lourenço, H., Costa, S., & Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk–benefit evaluation of food. *Trends in Food Science & Technology, 41*(1), 5-23.

Chakravorty, J., Ghosh, S., & Meyer-Rochow, V. B. (2011). Practices of entomophagy and entomotherapy by members of the Nyishi and Galo tribes, two ethnic groups of the state of Arunachal Pradesh (North-East India). *J Ethnobiol Ethnomed, 7*(5), 14.

Chakravorty, J., Ghosh, S., & Meyer-Rochow, V. B. (2013). Comparative Survey of Entomophagy and entotherapeutic among six tribes of eastern india. *Journal of ethnomedicine, 9*(50), 12.

Chaliha, M., Cusack, A., Currie, M., Sultanbawa, Y., & Smyth, H. (2013). Effect of Packaging Materials and Storage on Major Volatile Compounds in Three Australian Native Herbs. *Journal of Agricultural and Food Chemistry, 61*(24), 5738-5745.

Chavunduka, D. M. (1975). Insects as a source of protein to the Africans. The Rhodesian Science News, 9 (7), 4.

Chegere, M. J. (2018). Post-harvest losses reduction by small-scale maize farmers: The role of handling practices. *Food Policy, 77*, 103-115.

Chen, X., Feng, Y., & Chen, Z. (2009). Common edible insects and their utilization in China. *Entomological Research, 39*(5), 299-303.

Christensen, D. L., Orech, F. O., Mungai, M. N., Larsen, T., Friis, H., & Aagaard-Hansen, J. (2006). Entomophagy among the Luo of Kenya: a potential mineral source? *International Journal of Food Science and Nutrition, 57*(3-4), 198-203.

Chudy, S., Pikul, J., & Rudzinska, M. (2015). Effects of storage on lipid oxidation in milk and egg mixed powder. *Journal of Food and Nutrition Research, 54*(1), 31-40.

Cilla, A., López-García, G., & Barberá, R. (2018). In vitro bioavailability of iron and calcium in cereals and derivatives: A review. *Food Reviews International, 34*(1), 1-33.

Costa-Neto, E. M. (2016). Edible insects in Latin America: old challenges, new opportunities. *Journal of Insects as Food and Feed, 2*, 1-2.

da Silva, F. L. F., de Lima, J. P. S., Melo, L. S., da Silva, Y. S. M., Gouveia, S. T., Lopes, G. S., & Matos, W. O. (2017). Comparison between boiling and vacuum cooking (sous-vide) in the bioaccessibility of minerals in bovine liver samples. *Food Research International, 100*(Pt 1), 566-571.

de las Heras, A., Schoch, A., Gibis, M., & Fischer, A. (2003). Comparison of methods for determining malondialdehyde in dry sausage by HPLC and the classic TBA test. *European Food Research and Technology, 217*(2), 180-184.

DeFoliart, G. (1992). Insect as human food; Gene DeFoliart discusses some nutritional and economic aspects. *Crop Protection, 11*.

DeFoliart, G. R. (1997). An overview of the role of edible insects in preserving biodiversity. *Ecology of Food and Nutrition, 36*(2-4), 109-132.

Dobermann, D., Swift, J. A., & Field, L. M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin, 42*(4), 293-308.

Dossey, A. T., Tatum, J. T., & McGill, W. L. (2016). Modern Insect-Based Food Industry: Current Status, Insect Processing Technology, and Recommendations Moving Forward *Insects as Sustainable Food Ingredients* (pp. 113-152).

Dube, S., Dlamini, N. R., Mafunga, A., Mukai, M., & Dhlamini, Z. (2013). A survey of entomophagy prevalence in Zimbabwe. *African Journal of Agriculture, Nutrition and Development, 13*(1), 12.

El Hassan, N. M., Hamed, S. Y., Hassan, A. B., Eltayeb, M. M., & Babiker, E. E. (2008). Nutritional Evaluation and Physicochemiacla Properties of Boiled and Fried Tree Locust. *Pakistan Journal of Nutrition, 7*(2), 325-329.

Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B(6), B(12), D, and E. *Front Physiol, 3*, 317.

FAO, IFAD, UNICEF, WFP, & WHO. (2018). The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome: FAO.

FAO, IFAD and WFP. (2015). The State of food insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome, FAO

Feng, Y., Chen, X. M., Zhao, M., He, Z., Sun, L., Wang, C. Y., & Ding, W. F. (2018). Edible insects in China: Utilization and prospects. *Insect Science, 25*(2), 184-198.

Fernandez-Garcia, E., Carvajal-Lerida, I., & Perez-Galvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutr Res, 29*(11), 10.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*(3), 345-353.

Finke, M., & Oonincx, D. G. A. B. (2017). Nutrient content of foods. In A. van Huis & J. K. Tomberlin (Eds.), *Insects as food and feed: from production to consumption* (pp. 291-316). Netherlands: Wageningen Academic Publishers.

Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo Biology, 26*(2), 11.

Finke, M. D., & Oonincx, D. G. A. B. (2017). Nutrient content of insects. In A. van Huis & J. K. Tomberlin (Eds.), *Insects as Food and Feed. From Production to Consumption* (pp. 447). The Netherlands: Wageningen Academic Publishers.

Fontaneto, D., Tommaseo-Ponzetta, M., Galli, C., Rise, P., Glew, R. H., & Paoletti, M. G. (2011). Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. *Ecology of Food and Nutrition, 50*(4), 351-367.

Friedman, M. (1996). Nutritional Value of Proteins from Different Food Sources. A Review. *Journal of Agriculture and Food Chemistry, 44*(1), 6-29.

Frimpong, S. (2013). Urbanisation and pattern of food consumption in Ashanti region, Ghana: Implications for food security. *Journal of Economics and Sustainable Development, 4*(9), 104-111.

Gahukar, R. T. (2011). Entomophagy and human food security. *International Journal of Tropical Insect Science, 31*(3), 129-144.

Gardiner, A. J., & Gardiner, E. M. (2003). Edible insects, Part 1. Preparation of species from MushumbiPools Zimbabwe. *African Entomology, 11*(1), 125-127.

Gerbens-Leenes, W., & Nonhebel, S. (2005). Food and land use. The influence of consumption patterns on the use of agricultural resources. *Appetite, 45*(1), 24-31.

Ghaly, A. E. (2009). The use of insects as human food in Zambia. *Journal of Biological Sciences, 9*(4), 12.

Gibson, R. S. (2011). Strategies for preventing multi-micronutrient deficiencies: A review of experiences with Food-based Approaches in developing countries. In B. Thompson & L. Amoroso (Eds.), *Combating Micronutrient Defiencies: Food based approaches* FAO.

Gjerris, M., Gamborg, C., & Rocklinsberg, H. (2015). Entomophagy – why should it bug you? The ethics of insect production for food and feed *Know* (pp. 345-352).

Goodridge, C. F., Baeaudry, R. M., Pestka, J. J., & Smith, D. M. (2003). Solid phase Microextaction-Gas Chrpomatography for Quantifiying Headspace Hexanal baove Freeze dried Chicken Myofibrils. *Journal of Food Agricultural and Chemistry, 51*, 4185-4190.

Han, E., & Powell, L. M. (2013). Consumption patterns of sugar-sweetened beverages in the United States. *J Acad Nutr Diet, 113*(1), 11.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. (2013). *Six-legged livestock : edible insect farming, collection and marketing in Thailand*: RAP Publications.

Hu, M. (2016). Oxidative Stability and Shelf Life of Low-Moisture Foods *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats* (pp. 313-371).

Huis, A., & Vantomme, P. (2014). Conference report: insects to feed the world. *Food Chain., 4*.

Hwalla, N., El Labban, S., & Bahn, R. A. (2016). Nutrition security is an integral component of food security. *Frontiers in Life Science, 9*(3), 167-172.

Jacob, A. A., Emenike, A. F., Kayode, A., Olusegun, O., Uzoma, A., & Rukayat, K. Q. (2013). Entomophagy: A Panacea for Protein-Deficient-Malnutrition and Food Insecurity in Nigeria. *Journal of Agricultural Science, 5*(6).

Janssen, R. H., Vincken, J. P., Van Den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. *Journal of Agricultural and Food Chemistry, 65*(11), 2275-2278.

Jeon, Y. H., Son, Y. J., Kim, S. H., Yun, E. Y., Kang, H. J., & Hwang, I. K. (2016). Physicochemical properties and oxidative stabilities of mealworm (Tenebrio molitor) oils under different roasting conditions. *Food Science and Biotechnology, 25*(1), 105-110.

Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends in Food Science & Technology, 18*(11), 567-578.

Jonas-Levi, A., & Martinez, J.-J. I. (2017). The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis, 62*, 184-188.

Jongema, Y. (2017). List of edible insects of the world Retrieved 20-01-09, from 2017 https://www.wur.nl/upload_mm/8/a/6/0fdfc700-3929-4a74-8b69-f02fd35a1696_Worldwide%20list%20of%20edible%20insects%202017.pdf

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. M. (2018). Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket Acheta domesticus (L.) and black soldier fly larvae Hermetia illucens (L.). *Food Research International, 106*, 420-427.

Kaya, M., Baran, T., Erdogan, S., Mentes, A., Ozusaglam, M. A., & Cakmak, Y. S. (2014). Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata). *Material Science and Engineering C, 45*, 72-81.

Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonnang, H., Maniania, N. K., & Ekesi, S. (2015). African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed, 1*(2), 103-119.

Kennedy, G., Stoian, D., Hunter, D., Kikulwe, E., & Termote, C. (2017). Food biodiversity for healthy, diverse diets. In B. Bailey (Ed.), *Mainstreaming Agrobiodiversity in Sustainable Food Systems* Rome, Italy: Bioversity International.

Kinyuru, J. N., Kenji, G. M., Muhoho, S. N., & Ayieko, M. (2010a). Nutritional potential of longhorn grasshopper (Ruspolia Differens) consumed in Siaya District , Kenya. *Journal of Agriculture, Science and Technology, 1*,15

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2010b). Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (Macrotermes subhylanus) and grasshopper (Ruspolia differens). *Food and Bioprocess Technology, 3*(5), 778-782.

Kinyuru, J. N., Konyole, S. O., Roos, N., Onyango, C. A., Owino, V. O., Owuor, B. O., Estambale, B. B., Friis, H., Aagaard-Hansen, J., & Kenji, G. M. (2013). Nutrient composition of four species of winged termites consumed in western Kenya. *Journal of Food Composition and Analysis, 30*(2), 120-124.

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food Control, 26*(2), 628-631.

Komprda, T., Zorníková, G., Rozíková, V., Borkovcová, M., & Przywarová, A. (2013). The effect of dietary Salvia hispanica seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. *Journal of Food Composition and Analysis, 32*(1), 36-43.

Kouřimská, L., & Adámková, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal, 4*, 22-26.

Kumar, D., & Kalita, P. (2017). Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *Foods, 6*(1).

Latunde-Dada, G. O., Yang, W., & Vera Aviles, M. (2016). In Vitro Iron Availability from Insects and Sirloin Beef. *Journal of Agricultural and Food Chemistry, 64*(44), 8420-8424.

Lautenschläger, T., Neinhuis, C., Kikongo, E., Henle, T., & Förster, A. (2017). Impact of different preparations on the nutritional value of the edible caterpillar Imbrasia epimethea from northern Angola. *European Food Research and Technology, 243*(5), 769-778.

Lee, J., Xiao, L., Zhang, G., Ebeler, S. E., & Mitchell, A. E. (2014). Influence of storage on volatile profiles in roasted almonds (Prunus dulcis). *J Agric Food Chem, 62*(46), 11236-11245.

Lipasek, R. A., Li, N., Schmidt, S. J., Taylor, L. S., & Mauer, L. J. (2013). Effect of Temperature on the Deliquescence Properties of Food Ingredients and Blends. *Journal of Agricultural and Food Chemistry, 61*(38), 9241-9250.

Looy, H., Dunkel, F. V., & Wood, J. R. (2013). How then shall we eat? Insect-eating attitudes and sustainable foodways. *Agriculture and Human Values, 31*(1), 131-141.

Loveday, S. M. (2019). Food Proteins: Technological, Nutritional, and Sustainability Attributes of Traditional and Emerging Proteins. *Annu Rev Food Sci Technol, 10*(1), 311-339.

Madibela, O. R., Seitiso, T. K., Thema, T. F., & Letso, M. (2007). Effect of traditional processing methods on chemical composition and in vitro true dry matter digestibility of the Mophane worm (Imbrasia belina). *Journal of Arid Environments, 68*(3), 492-500.

Maestre, M., Poole, N., & Henson, S. (2017). Assessing food value chain pathways, linkages and impacts for better nutrition of vulnerable groups. *Food Policy, 68*, 31-39.

Manditsera, F. A., Lakemond, C. M. M., Fogliano, V., Zvidzai, C. J., & Luning, P. A. (2018). Consumption patterns of edible insects in rural and urban areas of Zimbabwe: taste, nutritional value and availability are key elements for keeping the insect eating habit. *Food Security, 10*(3), 561-570.

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). The contribution of wild harvested edible insects (Eulepida mashona and Henicus whellani) to nutrition security in Zimbabwe. *Journal of Food Composition and Analysis, 75*, 17-25.

Marono, S., Piccolo, G., Loponte, R., Di Meo, C., Attia, Y. A., Nizza, A., & Bovera, F. (2015). In vitro crude protein digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with chemical composition traits. *Italian Journal of Animal Science, 14*(3), 338-343.

McNamara, P., & Tata, J. (2015). Principles of Designing and Implementing Agricultural Extension Programs for Reducing Post-harvest Loss. *Agriculture, 5*(4), 1035-1046.

Menezes, E. A., Oliveira, A. F., Franca, C. J., Souza, G. B., & Nogueira, A. R. A. (2018). Bioaccessibility of Ca, Cu, Fe, Mg, Zn, and crude protein in beef, pork and chicken after thermal processing. *Food Chemistry, 240*, 75-83.

Meyer-Rochow, V. B., & Chakravorty, J. (2013). Notes on entomophagy and entomotherapy generally and information on the situation in India in particular. *Applied Entomology and Zoology, 48*(2), 105-112.

Mielnik, M. B., Olsen, E., Vogt, G., Adeline, D., & Skrede, G. (2006). Grape seed extract as antioxidant in cooked, cold stored turkey meat. *LWT - Food Science and Technology, 39*(3), 191-198.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Menard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food Function, 5*(6), 1113-1124.

Mlcek, J., Rop, O., Borkovcova, M., & Bednarova, M. (2014). A Comprehensive Look at the Possibilities of Edible Insects as Food in Europe – a Review. *Polish Journal of Food and Nutrition Sciences, 64*(3), 11.

Moore, K., & Roberts, L. J. (1998). Measurement of Lipid Peroxidation. *Free Radical Research, 28*(6), 659-671.

Munekata, P. E., Domínguez, R. n., Campagnol, P. C., Franco, D., Trindade, M. A., & Lorenzo, J. M. (2017). Effect of natural antioxidants on physicochemical properties and lipid stability of pork liver pâté manufactured with healthy oils during refrigerated storage. *Journal of Food Science And Technology -Mysore-, 54*(13), 4324-4334.

Münke-Svendsen, C., Flore, R., Halloran, A., Ayieko, M., & Roos, N. (2017). Edible insect gastronomy in Kenya.

Murefu, T. R., Macheka, L., Musundire, R., & Manditsera, F. A. (2019). Safety of wild harvested and reared edible insects: A review. *Food Control, 101*, 209-224.

Musundire, R., Zvidzai, C. J., Chidewe, C., Samende, B. K., & Chemura, A. (2016). Habitats and nutritional composition of selected edible insects in Zimbabwe. *Journal of Insects as Food and Feed, 2*(3), 189-198.

Musundire, R., Zvidzai, C. J., Chidewe, C., Samende, B. K., & Manditsera, F. A. (2014a). Nutrient and anti-nutrient composition of Henicus whellani (Orthoptera: Stenopelmatidae), an edible ground cricket, in south-eastern Zimbabwe. *International Journal of Tropical Insect Science, 34*(4), 223-231.

Musundire, R., Zvidzai, J. C., & Chidewe, C. (2014b). Bio-Active Compounds Composition in Edible Stinkbugs Consumed in South-Eastern Districts of Zimbabwe. *International Journal of Biology, 6*(3).

Mutungi, C., Irungu, F. G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., Ekesi, S., & Fiaboe, K. K. M. (2017). Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. *Critical Reviews in Food Science and Nutition*, 1-23.

Mwangi, M. N., Oonincx, D., Stouten, T., Veenenbos, M., Melse-Boonstra, A., Dicke, M., & van Loon, J. J. A. (2018). Insects as sources of iron and zinc in human nutrition. *Nutrition research reviews, 31*(2), 248-255.

Niaba, K. P. V., Atchibri, O. L., Gbasi, G. K., Beugre, A. G., Adou, M., Anon, A. B., & D., G. (2012). Consumption survey of edible winged termites in Cote d'Ivoire. *International Journal of Agricultural and Food Science, 2*(4), 149-152.

Nichol, H., Law, J. H., & Winzerling, J. J. (2002). Iron Metabolism In Insects. *Annual Reviews Entomology, 47*, 535-559.

Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved Method for Determining Food Protein Degree of Hydrolysis. *Journal of Food Science, 66*(5), 642-646.

Nowak, V., Persijn, D., Rittenschober, D., & Charrondiere, U. R. (2016). Review of food composition data for edible insects. *Food Chemistry, 193*, 39-46.

Obopile, M., & Seeletso, T. G. (2013). Eat or not eat: An analysis of the status of entomophagy in Botswana. *Food Security, 5*(6), 817-824.

Omotoso, O. T. (2006). Nutritional quality, functional properties and anti-nutrient compositions of the larva of Cirina forda (Westwood) (Lepidoptera: Saturniidae). *Journal of Zhejiang University Science B, 7*(1), 51-55.

Onigbinde, A. O., & Adamolekun, B. (1998). The nutrient value of Imbrasia belina Lepidoptera: Saturnidae (madora). *Central African Journal of Medicine, 44*(5), 3.

Oonincx, D. G., & Dierenfeld, E. S. (2012). An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biology, 31*(1), 40-54.

Oonincx, D. G., & van der Poel, A. F. (2011). Effects of diet on the chemical composition of migratory locusts (Locusta migratoria). *Zoo Biology, 30*(1), 9-16.

Oonincx, D. G. A. B., Itterbeeck, J., Heetkamp, M. J. W., Brand, H., Loon, J. J. A., & Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE., 5*.

Parodi, A., Leip, A., De Boer, I. J. M., Slegers, P. M., Ziegler, F., Temme, E. H. M., Herrero, M., Tuomisto, H., Valin, H., Van Middelaar, C. E., Van Loon, J. J. A., & Van Zanten, H. H. E. (2018). The potential of future foods for sustainable and healthy diets. *Nature Sustainability, 1*(12), 782-789.

Paul, A., Frederich, M., Megido, R. C., Alabi, T., Malik, P., Uyttenbroeck, R., Francis, F., Blecker, C., Haubruge, E., Lognay, G., & Danthine, S. (2017). Insect fatty acids: A comparison of lipids

from three Orthopterans and Tenebrio molitor L. larvae. *Journal of Asia-Pacific Entomology, 20*(2), 337-340.

Payne, C. L. R. (2015). Wild harvesting declines as pesticides and imports rise: The collection and consumption of insects in contemporary rural Japan. *Journal of Insects as Food and Feed, 1*(1), 57-65.

Poelaert, C., Beckers, Y., Despret, X., Portetelle, D., Francis, F., & Bindelle, J. (2016). In vitro evaluation of fermentation characteristics of two types of insects as potential novel protein feeds for pigs. *Jounal of Animal Science, 94*, 198-201.

Powell, B., Thilsted, S. H., Ickowitz, A., Termote, C., Sunderland, T., & Herforth, A. (2015). Improving diets with wild and cultivated biodiversity from across the landscape. *Food Security, 7*(3), 535-554.

Puoane, T., Matwa, P., Bradley, H., & Hughes, G. (2006). Socio-cultural Factors Influencing Food Consumption Patterns in the Black African Population in an Urban Township in South Africa. *Human Ecology Special Issue, 14*, 89-93.

Qiu, X., Chen, S., & Lin, H. (2019). Oxidative Stability of Dried Seafood Products during Processing and Storage: A Review. *Journal of Aquatic Food Product Technology, 28*(3), 329-340.

Raheem, D., Carrascosa, C., Oluwole, O. B., Nieuwland, M., Saraiva, A., Millan, R., & Raposo, A. (2018). Traditional consumption of and rearing edible insects in Africa, Asia and Europe. *Critical Review in Food Science and Nutrition*, 1-20.

Raitio, R., Orlien, V., & Skibsted, L. H. (2012). Effects of palm oil quality and packaging on the storage stability of dry vegetable bouillon paste. *Food Chemistry, 132*(3), 1324-1332.

Ramos-Elorduy, J. (2002). Edible insects of chiapas, Mexico. *Ecology of Food and Nutrition, 41*(4), 271-299.

Ramos-Elorduy, J. (2006). Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. *Journal of Ethnobiology and Ethnomedicine, 2*.

Ramos-Elorduy, J. (1997). Insects: A sustainable source of food? *Ecology of Food and Nutrition, 36*(2-4), 247-276.

Raubenheimer, D., & Rothman, J. M. (2013). Nutritional ecology of entomophagy in humans and other primates. *Annual Review of Entomology, 58*, 24. doi: 10.1146/annurev-ento-120710-100713

Riggi, L. G., Veronesi, M., Goergen, G., MacFarlane, C., & Verspoor, R. L. (2016). Observations of entomophagy across Benin – practices and potentials. *Food Security, 8*(1), 139-149.

Rochat, D., Morin, J.-P., Kakul, T., Beaudoin-Ollivier, L., Prior, R., Renou, M., Malosse, I., Stathers, T., Embupa, S., & Laup, S. (2002). Activity of Male Pheromone of Melanesian Rhinoceros Beetle Scapanes australis. *Journal of Chemical Ecology, 28*(3), 479-500.

Rumpold, B. A., Bubler, S., Jager, H., & Schluter, O. (2017). Insect processing. In A. van Huis & J. K. Tomberlin (Eds.), *Insects as food and feed: From production to consumption* (pp. 320-342). Netherlands: WageningenAcademic Publishers.

Rumpold, B. A., & Schlüter, O. K. (2013a). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research, 57*(5), 802-823.

Rumpold, B. A., & Schlüter, O. K. (2013b). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies, 17*, 1-11.

Sarwar Gilani, G., Wu Xiao, C., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition, 108 Suppl 2*, S315-332.

Schaich, K. M. (2005). Lipid oxidation: Theoretical aspects. In F. Shahidi (Ed.), *Bailey's Industrial Oil and Fat Products* (6th ed., Vol. 6, pp. 269-355): John Wiley & Sons Inc.

Schasteen, C. S., Wu, J., Schulz, M. G., & Parsons, C. M. (2007). Correlation of an Immobilized Digestive Enzyme Assay with Poultry True Amino Acid Digestibility for Soybean Meal. *Poultry Science, 86*, 343-348.

Sijtsema, S., Linnemann, A., van Gaasbeek, T., Dagevos, H., & Jongen, W. (2002). Variables influencing food perception reviewed for consumer-oriented product development. *Critical Review in Food Science and Nutrition, 42*(6), 565-581.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy, 56*, 365-379.

Smith, D. M. (2010). Protein Separation and Characterization Procedures. 261-281.
Srivastava, S. K., Babu, N., & Pandey, H. (2009). Traditional insect bioprospecting-A Human food and medicine. *Indian Journal of Traditional Knowledge, 8*(4), 485-494.
St-Hilaire, S., & Cranfill, K. (2007). Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. *Journal of The World Aquaculture Society, 38*(2), 309-313.
Stack, J., Dorward, A., Gondo, T., Frost, P., F., T., & Kurebgaseka, N. (2003). *Mopane worm utilisation and rural livelihoods in Southern Africa.* . Paper presented at the International Conference on Rural Livelihoods, Forests and Biodiversity, Bonn.
Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B., & Van Campenhout, L. (2016). Microbial community assessment of mealworm larvae (Tenebrio molitor) and grasshoppers (Locusta migratoria migratorioides) sold for human consumption. *Food Microbiology, 53*, 122-127.
Sun, Q., A., S., Chinachoti, P., & Faustman, C. (2002). Effect of Water Activity on Lipid Oxidation and Protein Solubility in Freeze-dried Beef during Storage. *Journal of Food Science, 67*(7), 2512-2516.
Tabassum, A., Tasneem, A., & Abbasi, S. A. (2016). Reducing the global environmental impact of livestock production: the minilivestock option. *Journal of Cleaner Production, 112*, 1754-1766.
Tawodzera, G. (2011). Vulnerability in crisis: urban household food insecurity in Epworth, Harare, Zimbabwe. *Food Security, 3*(4), 503-520.
Tazi, S., Plantevin, F., Falco, C. D., Puigserver, A., & Ajandouz, E. H. (2009). Effects of light, temperature and water activity on the kinetics of lipoxidation in almond-based products. *Food Chemistry, 115*(3), 958-964.
Tiencheu, B., Womeni, H. M., Linder, M., Mbiapo, F. T., Villeneuve, P., Fanni, J., & Parmentier, M. (2013). Changes of lipids in insect (Rhynchophorus phoenicis) during cooking and storage. *European Journal of Lipid Science and Technology, 115*(2), 186-195.
Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. S., & Lakemond, C. M. M. (2014). Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Research International, 62*, 1087-1094.
van Huis, A. (2003). Insects as food in Sub-Saharan Africa. *Insect Science and its Application, 23*(3), 163-185.
van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review in Entomology, 58*, 563-583. doi: 10.1146/annurev-ento-120811-153704
van Huis, A. (2015). Edible insects contributing to food security? *Agriculture & Food Security, 4*(1), 1-9.
van Huis, A. (2016). Edible insects are the future? *Proceedings Nutrition Society, 75*(3), 294-305.
van Huis, A. (2017). Edible insects and research needs. *Journal of Insects as Food and Feed, 3*(1), 3-5.
van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., G., M., & Vantomme, P. (2013). Edible insects: Future prospects for food and feed security (Vol. 117). Rome: FAO Forestry Paper.
Vantomme, P. (2015). Way forward to bring insects in the human food chain. *Journal of Insects as Food and Feed, 1*(2), 121-129.
Vantomme, P., Mertens, E., van Huis, A., Klunder, H., & NWFP. (2012). Assessing the Potential of Insects as Food and Feed in assuring Food Security (pp. 38). Rome: Food And Agriculture Organization of The United Nations.
Verkerk, M. C., Tramper, J., van Trijp, J. C., & Martens, D. E. (2007). Insect cells for human food. *Biotechnology Advances, 25*(2), 198-202.
Vinceti, B., Termote, C., Ickowitz, A., Powell, B., Kehlenbeck, K., & Hunter, D. (2013). The Contribution of Forests and Trees to Sustainable Diets. *Sustainability, 5*(11), 4797-4824.
Wang, D., Bai, Y., Li, J., & Zhang, C. (2004). Nutritionalvalue of the field crickets Gryllus Testaceus walker. *Entomoligica Sinica, 11*(4), 9.
WHO/FAO/UNU. (2007). Protein and amino acids requirements in human nutrition *WHO Technical Reports Series* (pp. 284). Geneva: World Health Organisation.

Wienk, K. J. H., Marx, J. J. M., & Beynen, A. C. (1999). The concept of iron bioavailability and its assessment. *European Journal of Nutrition, 38*(2), 51-75.

Williams, J. P., Williams, J. R., Kirabo, A., Chester, D., & Peterson, M. (2016). Nutrient content and Health benefit of Insects. In A. T. Dossey, J. A. Morales -Ramos, & M. Guadalupe Rojas (Eds.), *Insects as sustainable food: Production, Processing and Food appplication* (pp. 61-84). Amsterdam: Elsevier.

Williams, P. G. (2007). Nutritional composition of red meat. *Nutr. Diet. ,, 64*((Suppl. 4)), S113–S119.

Yang, L., Siriamornpun, S., & Li, D. (2006). Polyunsaturated fatty acid content of edible insects in Thailand. *Journal of Food Lipids, 13*, 9.

Yen, A. L. (2009). Edible insects: Traditional knowledge or western phobia? *Entomological Research, 39*(5), 289-298.

Yen, A. L. (2015). Insects as food and feed in the Asia Pacific region: Current perspectives and future directions. *Journal of Insects as Food and Feed, 1*(1), 33-55.

Yeo, H., Youn, K., Kim, M., Yun, E. Y., Hwang, J. S., Jeong, W. S., & Jun, M. (2013). Fatty Acid Composition and Volatile Constituents of Protaetia brevitarsis Larvae. *Prev Nutr Food Sci, 18*(2), 150-156.

Yhoung-Aree, J., Puwastien, P., & Attig, G. A. (1997). Edible insects in Thailand: An unconventional protein source? *Ecology of Food and Nutrition, 36*(2-4), 133-149.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., Huis, A., & Boekel, M. A. J. S. (2013). Extraction and characterization of protein fractions from five insect species. *Food Chemistry, 141*.

Zhang, Y., Xinhui, W., Wei, W., & Jiaming, Z. (2014). Effect of boiling and frying on nutritional value and in vitro digestibility of rabbit meat. *African Journal of Food Science, 8*(2), 92-103.

Zhou, J., & Han, D. (2006). Proximate, amino acid and mineral composition of pupae of the silkworm Antheraea pernyi in China. *Journal of Food Composition and Analysis, 19*(8), 850-853.

Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., & Jakubczyk, A. (2015). Selected species of edible insects as a source of nutrient composition. *Food Research International, 77*, 460-466.

ZIMVAC. (2014). Zimbabwe Vulnerability Assessment Committee (ZimVAC) 2014 Rural livelihoods assessment.

SUMMARY

Entomophagy (practise of eating insects) is a traditional practice in many countries and has the potential to contribute to food and nutrition security. Edible insects are identified as a valuable source of proteins and micronutrients with the potential to contribute in alleviating malnutrition especially in developing countries where the practice is traditional. In these countries, the communities depend mostly on collection of the insects from the natural environments and the edible insect value chain is mainly traditional and limited to household level. However, knowledge on the actual contribution of wild harvested insects to nutrition security is still limited as there are limited studies to this effect. Thus, the aim of this study was to investigate how wild harvested edible insects can contribute to nutrition security in developing countries. In this study, we used two wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) consumed in Zimbabwe as case study.

Chapter 2, aimed to obtain insight into insect consumption patterns amongst rural and urban populations in Zimbabwe and the factors that may influence these patterns. Survey data of 200 urban and 175 rural respondents showed that insect consumption was significantly higher in rural (89.7%) than in urban (80.0%) areas. Rural respondents (63.9%) consumed insects more than three times a week on average as compared to urban (14.5%) respondents. Taste was the main motive of consumption for respondents in both the rural (89.2%) and urban areas (74.4%). Respondents in urban areas more often reported nutritional value (74.4%) and medicinal properties (28.1%) as important motives for consumption compared to rural respondents (51.0% and 15.3%, respectively). Availability of edible insects influences both urban (64.0%) and rural (83.0%) respondents' consumption of insects.

Despite the potential contribution of wild harvested edible insects to nutrition security, their nutritional composition is often unknown. Therefore, in **Chapter 3,** we investigated the nutritional composition and variation in the nutritional composition with respect to geographical place of harvest in Zimbabwe of two commonly consumed wild harvested insects i.e., *Eulepida mashona* (beetle) and *Henicus whellani* (cricket). The results showed that the protein content ranged between 52-56% (*Eulepida mashona*) and 59-70% (*Henicus whellani*), which is comparable to that of animal-based protein sources. Essential amino acids of both insect species did meet the WHO/FAO (277 mg/g protein) requirement for adult humans. In addition, the high tryptophan concentration (8.68mg/g protein) found in *Eulepida mashona* offers possibilities for using this insect in complementing this limiting amino acid in maize. The fat content of both species was low (<10%), but differed significantly between sampling districts. In addition, the PUFA/SFA and omega 6/3 ratios of both species are recommendable for a healthy diet. The iron (24.2- 52.9mg/100g) and zinc content (10.0-20.9mg/100g) are high for both species, making them a useful mineral-containing ingredient for food enrichment.

However, nutritional content is only beneficial when proteins are digestible and minerals bioaccessible. More so, processing can affect the digestibility and bioaccessibility of these nutrients. In **Chapter 4**, we therefore determined the effects of domestic processing on protein digestibility and mineral bioaccessibility of the two insect species. Boiling of both insects resulted in loss of protein as it leached into the boiling water. The raw insects had a higher protein *in vitro* digestibility than the boiled and roasted insects, and the maximal decrease in protein digestibility was around 25% for twice boiling of the beetles and for boiled and roasted crickets. For both insect species, boiling resulted in a non-significant loss of iron and zinc. Iron was the least bioaccessible mineral in both insects. Beetles had a much higher iron bioaccessibility (30.7%) as compared to crickets (8.11%). Boiling resulted in about 50% decrease in iron and zinc bioaccessibility in both species while roasting did not. Despite the decrease in protein digestibility and mineral bioaccessibility of the two species, consumers can still benefit from the high protein and mineral content.

Seasonality and geographical availability can limit the potential contribution of wild harvested insects to food and nutrition security. Traditionally, insects are sun dried. Therefore, it is necessary to extend shelf life of harvested insects by drying to improve availability. However, lipid oxidation can affect shelf life of dried insects. As such, in **Chapter 5** we investigated the influence of grinding, drying, and storage conditions on lipid oxidation, particularly the formation of secondary lipid oxidations compounds, in *Henicus whellani* and *Eulepida mashona.* Insects were dried at 40^{o}C (mimicking sun drying) and 100^{o}C (possible high temperatures that could be used to dry insects), and subsequently stored at 4^{o}C and 50^{o}C. Drying of *Henicus whellani* and *Eulepida mashona* at 40^{o}C resulted in a water activity of 0.35 and 0.43 respectively, while drying at 100^{o}C resulted in a water activity of 0.14 and 0.16, respectively.

Results showed a higher concentration of malondialdehyde for insects dried at 100 ^{o}C compared to those dried at 40 ^{o}C, indicating an increase in lipid oxidation for both insect. Monitoring the concentration of the volatile lipid oxidation products (VLOPs) revealed that lipid oxidation occurred during storage at 4^{o}C and 50^{o}C for both insect species. However, the peak concentration of VLOPs was at 4weeks of storage for insects dried at 40 ^{o}C, and at 2 weeks of storage for insects dried at 100 ^{o}C. The concentrations of VLOPs (hexanal, heptanal, nonanal, hexanoic acid, octatonic acid, and nonanoic acid) provided accurate information on the influence of drying and storage conditions. However, further sensory analysis is required to link the volatiles to perceived smell.

Chapter 6 discusses the integrated findings of the present study and presents a broader outlook on how wild harvested edible insects can contribute to the nutrition security of people who are currently consuming insects. The high protein and mineral content of wild harvested insects justifies the current traditional insect value chain. Suggestions on how consumers can get best of the nutritional value of

wild harvested insects are given. In addition, due to the shortfalls of the traditional insect value chain, possible pathways to transform the traditional insect value chain to a commercial insect chain are discussed.

ACKNOWLEDGEMENTS

As my PhD journey ends, I look back and I am grateful of the support I got from lot of people. First, I would like to thank my promotor, Prof. Vincenzo Fogliano and co-promotors Dr Catriona Lakemond and Dr Pieternel Luning for continuous support throughout my PhD. I thank you for guidance and support throughout my studies. Vincenzo, thank you for the insightful and fruitful discussions. You helped me especially to look at the bigger picture of my research work. Catriona, thank you for replying to the first email I sent with my draft concept note. Together with Pieternel, you agreed to become my supervisors and ever since then I received unwavering support from both of you. Pieternel and Catriona, I appreciate your expertise, constructive criticism and constant encouragement during this PhD journey. I am also grateful to Prof Cuthbert Zvidzai for valuable contributions and suggestions during my studies.

I wish to extend my gratitude to the administrative and technical staff of the Food Quality and Design Group. Lysanne Hoksbergen, Kimberley Boss and Corine van Heunen, thanks for always organising my trips and stay in the Netherlands. To Charlotte van Twisk, Geert Meijer, Frans Lettink, Xandra Bakker-de Haan, Erik Meulenbroeks, thanks for always being there to offer help whenever needed in the laboratory. Ruud Verkerk and Gert-Jan Hofstede, I appreciate the many talks we had and encouragement I got from you. Brigitte Verkerk and Laura, thanks for the cherished experiences we had in Zimbabwe in 2016 when the team of supervisors visited. Thanks to fellow PhD colleagues at FQD for the moral support.

I extend my appreciation to the management of Chinhoyi University of Technology (Professor David Simbi and your team) for your support especially granting me the study leave to undertake my PhD studies. Prof. Makuza, Dr A. Mpofu and my colleagues in the Food Science and Technology department, thank you for your support. Prof Robert Musundire ("Doc") thank you for introducing me to the edible insects' research. I appreciate the valuable discussions we always had about edible insects.

Dear Bug 1 (Liya), Bug 2 (Grace) and Bug 3 (Renske), I am thankful that you warmly welcomed me to the bug family as Bug 4. I cherish the bug discussions we had so as the outings and dinners we had as a family every time I was in the Netherlands.

To the present and past Zimbabwean PhD candidates of WUR: Lesley, Ruth, Juliet, Shingai, Sheila, Prosper, Plaxedis, Augustine and Oniward, thank you for the moral support. You never knew, how the talks we shared uplifted me. LECTA, thank you for the great experiences and moments we shared and especially for propelling my wings so that I fly high.

To the students, I supervised and worked with (Eva Wintjies, Madelief van Rooyen, Riccardo Bottiroli and Susan Doornbos) thank you for your valued contributions.

Renske and James thank you for being my paranymphs.

I would like to thank my parents (Tserayi and Francisca Manditsera) for your love, care, support and encouragement which not only started in my PhD journey but dated many years back. Thank you so much for the moral support, prayers and above all always believing that I can do it. You probably made many prayers for my PhD than I did for myself. Lesley, I cannot thank you enough. Thanks for your love and unending encouragement. To my Manyimo brothers, sisters, nieces and nephews, thank you for moral support and prayers. My siblings, Robie, Kuda, Jesy, Holie and your families, thank you for your prayers, encouragement and cheering me up. Reverend and Baba Macheka thank you your prayers and encouragement. Zviko and Simba thanks for cheering me up.

Lastly, if you have not been acknowledged in this thesis, I apologize for that, please rest assured that my gratitude is the same to you as to those mentioned above!

Faith A. Manditsera

ABOUT THE AUTHOR

Faith Angeline Manditsera was born on 25th of February in Mutoko, Zimbabwe. After completing her Advanced Level studies at Hartzell High School, Zimbabwe, Faith enrolled at the University of Zimbabwe for a Bachelor of Science degree in Food Science and Technology. She graduated in 2004 and thereafter was employed by Chinhoyi University of Technology as a teaching assistant in the Department of Food Science and Technology. In 2007, VLIR-UOS awarded Faith, a scholarship to pursue an MSc in Food Technology at Ghent University/KU Leuven. Upon completion of her MSc degree, Faith returned to Zimbabwe, back to Chinhoyi University of Technology but now as a lecturer, a position she still holds to date. In 2014, Faith was granted a NUFFIC fellowship to pursue sandwich PhD studies in the Netherlands. In September 2014, Faith started her PhD studies with the Food Quality Design, Wageningen University in collaboration with Chinhoyi University of Technology. The results of her PhD research are presented in this thesis.

LIST OF PUBLICATIONS

1. **Manditsera, F. A**., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. Food Research International, 121, 404-411. doi: https://doi.org/10.1016/j.foodres.2019.03.052
2. Murefu, T. R., Macheka, L., Musundire, R., & **Manditsera, F. A.** (2019). Safety of wild harvested and reared edible insects: A review. Food Control, 101, 209-224. doi: 10.1016/j.foodcont.2019.03.003
3. **Faith A. Manditsera**, Pieternel A. Luning, Vincenzo Fogliano, Catriona M.M. Lakemond (2019). The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. *Journal of Food Composition and Analysis*, 75, 17-25.
4. **Faith A. Manditsera**, Catriona M. M. Lakemond, Vincenzo Fogliano, Cuthbert J. Zvidzai and Pieternel A. Luning. (2018). Consumption patterns of edible insects in rural and urban areas of Zimbabwe: taste, nutritional value and availability are key elements for keeping the insect eating habit. *Food Security*, 10, 561–570.
5. Musundire, R., Zvidzai, C., Chidewe, C., Ngadze, R., Macheka, L., **Manditsera, F.,** Mubaiwa, J., & Masheka, A. (2016). Nutritional and bioactive compounds composition of *Eulepida mashona*, an edible beetle in Zimbabwe. Journal of Insects as Food and Feed, 2(3), 179-187. doi: 10.3920/jiff2015.00505
6. Musundire, R., Zvidzai, C. J., Chidewe, C., Samende, B. K., & **Manditsera, F. A**. (2014). Nutrient and anti-nutrient composition of Henicus whellani (Orthoptera: Stenopelmatidae), an edible ground cricket, in South-eastern Zimbabwe. International Journal of Tropical Insect Science, 34(4), 223-231. doi: 10.1017/s1742758414000484
7. Musundire, R., Mazodze, F., Macheka,L., Ngadze, R.T, Mubaiwa, J. and **Manditsera, F.A**. (2015) Eucalyptus grandis and Tagetes minuta leaf powders effectively protect stored maize against Sitophilis zeamais without affect grain organoleptic properties. *African Journal of Agriculture Research*, 10(2), 49-57.
8. Lesley Macheka, Ruth Tambudzai Ngadze, **Faith Angeline Manditsera**, Juliet Mubaiwa and Robert Musundire. (2013). Identifying causes of mechanical defects and critical control points in fruit supply chains: an overview of a banana supply chain. International. *Journal of Postharvest Technology and Innovation*, Vol. 3, No. 2, 2013. 109-122.
9. Lesley Macheka, **Faith Angeline Manditsera**, Ruth Tambudzai Ngadze, Juliet Mubaiwa, Loveness Kuziwa Nyanga. (2013) Barriers, benefits and motivation factors for the implementation of food safety management system in the food sector in Harare Province, Zimbabwe. *Food Control* 34. 126-131
10. Stephen T. Musasa, Brighton M. Mvumi, **Faith A. Manditsera**, Jonathan Chinhanga, Shepherd Musiyandaka and Claire Chigwedere (2013). Postharvest orange losses and small-scale farmers' perceptions on the loss causes in the fruit value chain: a case study of Rusitu Valley, Zimbabwe. *Food Science and Quality Management*. Volume 18.1-9

Overview of Completed Training Activities

Discipline specific activities

	Organizing institute (s)	Year
Courses		
Multivariate Analysis for Food Data And Scientists	VLAG	2014
Advanced Food Analysis	VLAG	2015
Sensory Perceptions and Food Preferences	VLAG	2015
Healthy Food Design	VLAG	2018
Sustainable and Healthy Diets: Synergies and Trade-offs	VLAG	2017
Conferences, workshops and symposia		
Tropentag (Oral)	Bonn University	2017
Global Food Security Conference (Poster)	Elsevier	2017
The Second International Conference Insects to Feed the World (Oral)	Wuhan University	2018
Edible Insect: The Value Chains Symposium (Oral)	VLAG	2018
First African Conference on Edible insects (Oral)	Chinhoyi University of Technology	2019

General courses and workshops

Information Literacy for PhD including Endnote Introduction	WGS	2014
The Essentials of Scientific Writing and Presenting	WGS	2014
Philosophy and Ethics of Food Science and Technology	VLAG	2015
Techniques for Writing and Presenting a Scientific Paper	WGS	2016
Project and Time Management	WGS	2016
Effective Behaviour in your Professional Surroundings	WGS	2016
Reviewing a Scientific Paper	WGS	2016
PhD Carousel	WGS	2017

Optional courses

Preparation of research proposal	Food Quality and Design	2014
PhD study tour to Italy	Food Quality and Design	2016
Weekly group meetings	Food Quality and Design	
PhD Symposium (Diversity in Science)	WUR PhD Council	2016
Scaling Up Nutrition -Academic platform	Food and Nutrition Council Zimbabwe	2018
Value chains	Chinhoyi University of Technology	2018
AgriFOSe2030 First stakeholder workshop	Chinhoyi University of Technology	2018
FAO ENACT TOT workshop	Michael Okpara University	2019
Organising committee member-First African Conference on Edible insects	Chinhoyi University of Technology	2019

Funding

The research described in this thesis was financially supported by the Netherlands Organisation for International Cooperation in higher Education (NUFFIC), Grant award number CF9421/2014.

Cover design by Lesley Macheka and Faith Manditsera
Printed by Digiforce (www.wwur.proefschriftmaken.nl)