INFLUENCE OF GEOGRAPHIC LOCATION, EMERGENCE SEASON AND DEGUTTING METHOD ON QUALITY OF *GONIMBRASIA BELINA*

By

Obert Nobert Madimutsa

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Department of Food Science and Technology
School of Agricultural Sciences and Technology
Chinhoyi University of Technology, Chinhoyi, Zimbabwe

Supervisor: Dr Faith Angeline Manditsera
Co Supervisors: Dr Juliet Mubaiwa and Dr Lesley Macheka

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DECLARATION

I hereby declare that the work reported in this thesis and submitted at the Department of Food Science and Technology, School of Agricultural Sciences and Technology at Chinhoyi University of Technology for a Master of Philosophy degree is my original work. I confirm that it has not been previously submitted for a degree at any Higher Education Learning Institution.

04/10/2023

Obert N. Madimutsa Date

Student

As the candidate's supervisors we agree to the submission of this thesis

Manditsera

04/10/2023

Dr. F. A. Manditsera Date

Mubaiwa

04/10/2023

Dr. J. Mubaiwa Date

LMacheka

04/10/2023

Dr. L. Macheka Date

DEDICATION

I dedicate this dissertation to my family. To my caring wife Paidamoyo, for all the love, patience, and support both financially and emotionally. To my lovely children Tavonga, Curtis and Chloe, thank you for your patience and teaching me to slow down and enjoy those precious moments in life.

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ABSTRACT

Edible insects such as *Gonimbrasia belina* (mopane worm) consumption contributes to the sustainable supply of protein and other nutrients (iron and zinc) for low socio-economic communities. Moreover, mopane worms are a potential source of bioactive compounds such as phenolic compounds, which have antioxidant properties. However, the nutritional composition of foods of animal and plant origin is affected by different factors, including processing method, emergence season and sourcing geographical area though the above factors are cited as affecting the nutritional composition of edible insects, and there is limited information about their specific effects on mopane worm for full utilization of the resource. Mopane worms are bivoltine, producing two generations per rainy season which is also postulated to affect their nutritional value. Effects of emergence season, geographic location, and pre-processing techniques on their nutritional composition are relevant for dependable usage of mopane worm. The acceptance of mopane worm by consumers and its contribution to food security is well studied. However, there is still a knowledge gap in terms of food safety and anti-oxidant properties associated with this edible insect. Finding an answer to these areas has many implications; from enhancing the productivity of mopane worm and to developing better strategies of utilization of this important natural resource. In this study, the influence of geolocation and emergence season on mopane worms' nutritional composition was investigated. Proximate, mineral composition and fatty acid profile were analysed on degutted mopane worm samples collected over two mopane worm emergence seasons (November to December 2020 and 2021, April to May 2021, from three locations in Gwanda district, Zimbabwe. A further analysis on the effect of degutting method on the mopane worm's proximate composition, antioxidant capabilities, chemical, and microbial safety was conducted. Natural degutting process is when mature mopane worm remove their gut contents before they burrowing in the soil. Manual degutting process is when actively feeding mopane worm's gut is pressed outside in order to remove the gut contents. Crude protein was determined by Kjeldahl method, crude fat by Soxhlet extraction, ash by incineration in a muffle furnace and crude fibre by chemical digestion followed by ashing. Mineral and heavy metal content was determined on an Atomic absorption spectrophotometer after wet ashing. Phosphorus was determined calorimetrically on a UV-visible spectrophotometer. Total phenolic content was determined by the folin-ciocalteau assay whilst the total flavonoid content was determined by the aluminium chloride assay. The crude protein content of mopane worms ranged between 52.5±0.21

and 59.9±0.18 % DM and differed significantly (Bonferroni-correction $\alpha < 0.002$) ($p<0.05$) between seasons, location and degutting method. Naturally degutted mopane worms had higher crude protein than manually degutted samples. Depending on the season, location, and degutting method, mopane worms' fat content varied considerably (Bonferroni-correction $\alpha < 0.002$) ($p<0.05$) between 12.1±0.06 and 19.0±0.05 % DM. Season, location, and degutting technique all had a significant (Bonferroni-correction $\alpha < 0.002$) ($p<0.05$) impact on the mineral content of mopane worms. Potassium (1195.3±0.4 – 1759.9±0.2), magnesium (104.6±0.3 – 225.5±0.3) and calcium (51.2±0.4 – 145.5±0.3) were the most abundant macro-elements (mg/100g DM), whilst iron (10.6±0.2 – 21.6±0.2) and zinc (12.7±0.1 – 17.9±0.4) were the most abundant micro-elements (mg/100g DM). Manually degutted samples had significantly higher ($p<0.05$) concentrations of zinc (16.1±0.3 mg/100g) and phosphorus (610.0±3.4 mg/100g). Mopane worm fat was found to contain high concentrations of oleic (ω-9) and linoleic (ω-6) and palmitoleic (ω-7) fatty acids using an Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR). The antioxidant activity against the DPPH radical (53.8±1.4 %), ABTS radical (97.8±0.18 %), Fe^{2+} metal chelating activity (56.6±0.01 %), PFRAP (0.25±0.01), total phenolic content (740±2.4 mg gallic acid eq/100 g) was high for naturally degutted samples, whilst total flavonoid content was high in manually degutted (16.8±0.02 mg QE/100g). However, samples that were naturally degutted exhibited a larger microbial load (TBC, coliforms, *E. coli*, as well as yeast and moulds) than samples that were manually degutted ($p<0.05$). *S. aureus*, *Salmonella spp* and heavy metals were not detected in naturally and manually degutted samples. The results showed that mopane worms can be used as an alternative protein source and a strong source of antioxidants. Harvesting mature mopane worm (naturally degutted) has an advantage of ensured high levels of macro and micro-nutrients and antioxidants but poses a great risk in terms of microbial safety. These results also suggest that mopane worm, could have a substantial role in reducing protein, zinc, and iron deficiencies in target communities.

PREFACE

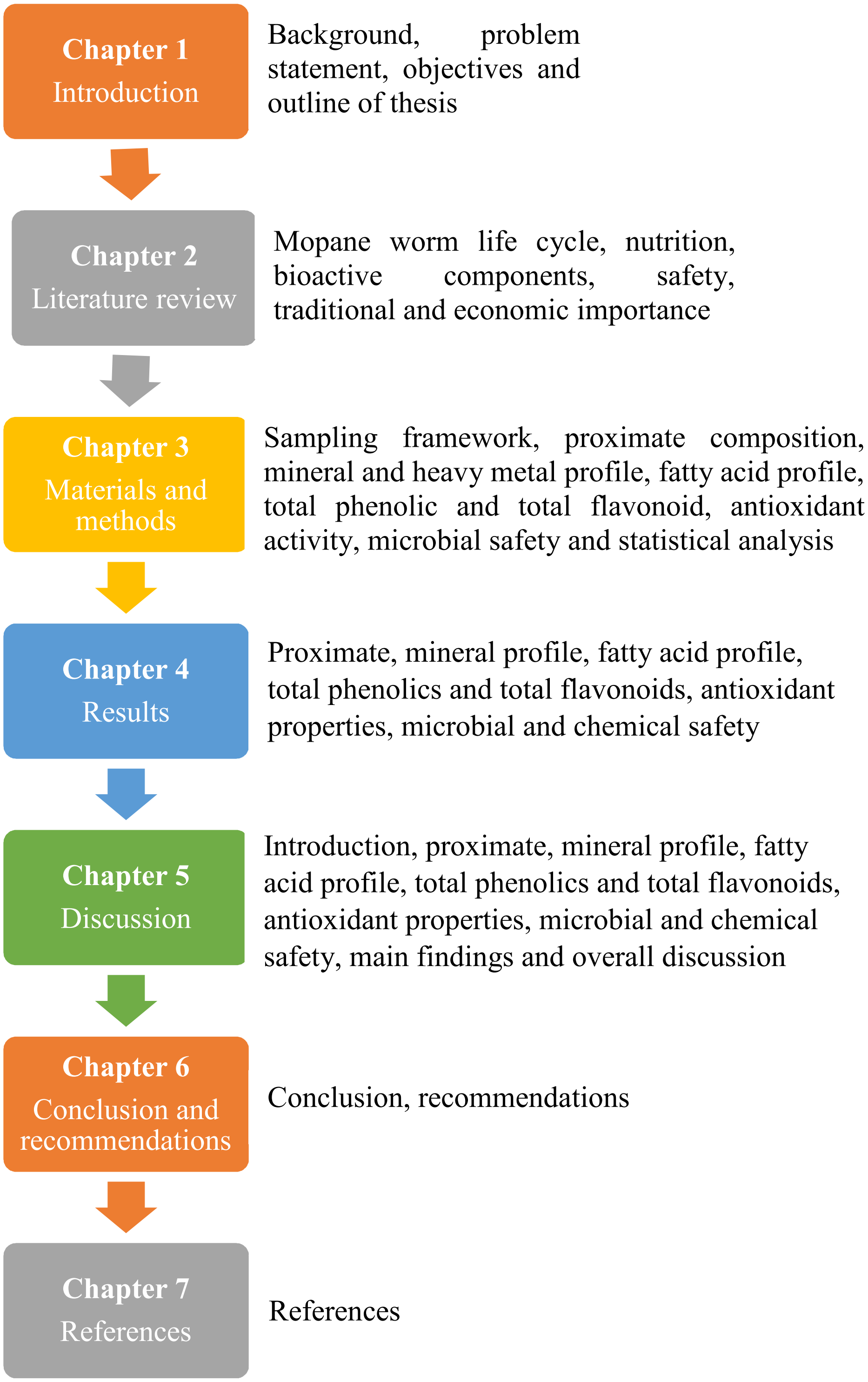

Figure 1: Outline of thesis

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Chapter 1: General Introduction

1.1 Background

Food security worldwide, particularly in developing countries like Zimbabwe, is a critical issue that must be addressed (Moreki *et al.*, 2012). It is widely assumed that the world's population would reach 9 billion people by 2050 (FAO/IFAD/UNICEF/WFP/WHO, 2018). To feed this growing population, existing food production will have to treble. Land is a finite resource, and increasing the amount of land dedicated to agriculture is rarely a practical option. There are currently 821 million undernourished people in the globe (WFP, 2019), with 178 million children under the age of five suffering from stunting due to malnutrition (UNICEF/WHO/WB, 2018), the majority of whom live in Sub-Saharan Africa. Food consumption and production methods must be re-evaluated for a better future. Inefficiencies must be addressed, and food waste must be reduced. For a sustainable future, new means of growing food are required. Edible insects present a significant and mostly untapped possibility to meet the world's nutritional needs while using significantly less water, feed, and land than other forms of livestock (Dossey, 2013; Shockley and Dossey, 2014; van Huis *et al.*, 2013). Over 2000 insect species are thought to be included in human diets and the nutrition provided by several of them matches or exceeds that found in conventional diets (Premalatha *et al.*, 2011). Edible insects are a good source of "complete" animal crude protein, fats (essential fatty acids like omega 3), minerals (Fe, Zn, and Ca), and vitamins (retinol, riboflavin, niacin, and thiamine) (Finke, 2013). Because edible insects are unquestionably high in nutrients, including them in one's regular diet could enhance nutrition in impoverished countries (Moreki *et al.*, 2012). Around 470 insect species are known to be edible in Africa, with mopane worms (*Gonimbrasia belina*) being among the most popular, followed by grasshoppers (*Ruspolia differens*), beetles (*Eulepida mashona*), and termites (*Isoptera*) (van Huis, 2020).

The mopane worm is one of Zimbabwe's most popular edible insect (Kwiri *et al.*, 2014). The crude protein content of the mopane worm, which is a significant nutrient, ranges from 48 to 61 percent. The insect is also high in fat (16–20%), with important fatty acids accounting for 40% of the fat (Glew *et al.*, 1999; Headings and Rahnema, 2002). According to Moreki *et al.*, (2012) and Simone *et al.*, (2013), mopane worm is a good source of minerals such as calcium (average of 1.6g/100g), zinc (average of 0.19g/100g), and iron (average of 1.27g/100g). Although the nutritional value of the mopane worm has been previously documented, further information on changes associated with diet, emergence season, and growth environment is still needed. The nutritional value of an insect is influenced by its diet, season, developmental stage, sex, species, and growth environment, according to van Huis and Oonincx (2017). Furthermore, mopane worms may have beneficial bioactive compounds and though this has yet to be shown; thus, a study to determine their presence is necessary. A study of mopane leaves from various habitats in the Venetia-Limpopo Nature Reserve indicated an average of 63.2g (dry weight basis) of total polyphenolic compounds and 0.59g (dry weight basis) of condensed tannin– protein ratio (Hrabar *et al.*, 2009). As a result, since an insect's nutritional composition is heavily influenced by its diet, it's quite likely that bioactive compounds are sequestered from the feed by the insect. Polyphenols, for example, are supplementary non-nutritional elements that are normally present in minute amounts in food and play an essential role as health-promoting substances, including antioxidants, antiallergic, and immunosuppressive qualities (Teodoro, 2019).

Mopane worm emergence are seasonal. The first generation of mopane worms emerges from pupation between November and December, and the second generation between April and May (bivoltine), whilst in more arid regions such as Namibia they are univoltine (producing one generation per rainy season) (Ditlhogo *et al*., 1996; Thomas, 2013). The difference in season of emergence is postulated to have an effect on the nutritional composition of edible insects (Ssepuuya *et al*., 2019). In addition, the nutritional composition of foods of animal and plant origin (Romotowska *et al*., 2016) and that of edible insects, such as *Ruspolia differens* (Ssepuuya *et al*., 2019), is also affected by the sourcing geographical area. Hlongwane *et al.* (2022) focused on an influence on proximate (excluding fibre), Fe and Zn for samples from different countries whilst Madibela *et al.* (2009) reported the influence on ash and crude crude protein for samples from Botswana. Though the above factors are cited as affecting the nutritional composition of edible insects, there is limited information about their specific effects. It is difficult to generalize these effects for all insects due to factors like different insect matrix, species, feed substrates, developmental stage and geographical origin or habitat (Mutungi *et al*., 2019). However, there is limited information on the influence of season of emergence for mopane worm collected from different locations in the same climatic region. This research was therefore aimed at determining the effect of season of emergence and geolocation on the nutritional composition of mopane worms.

The distribution of the mopane worm is closely linked to that of its primary host, the mopane tree (*Colophespermum mopane*) (Hrabar *et al.*, 2009). In most locations, mopane worms are bivoltine, meaning that two generations are produced each year, the first between November and January and the second between March and May (Stack *et al.*, 2003; Ghazoul, 2006). In view of the potential mopane worm nutritional benefits among the world's most vulnerable people, including those in Zimbabwe, the goal of this study is to investigate how the nutritional, bioactive components, microbial safety and heavy metal contamination of the mopane worm are affected by harvest location, emergence season and degutting method.

Safety of the edible insects is of concern. A comprehensive literature review by Ruzengwe *et al.* (2022) revealed that postharvest processing methods of the edible insects can influence safety and nutritional composition. Findings from a study by Murefu *et al.*, (2019) indicated that the safety of wild harvested edible insects is influenced by the processing methods. Evidence from literature show that many insects are collected from wild habitats or likely to be reared in environments that potentially originate microbiological hazards (Belleggia *et al.*, 2020; Mancini *et al.*, 2019;; Wynants *et al.*, 2019; Van der Fels-Klerx *et al.,* 2018). The rich nutritional profile of insects offers a suitable substrate for the growth of unwanted microorganisms, such as spoilage and pathogenic ones, when the conditions are suitable (Braide *et al*., 2011; Klunder *et al*., 2012). In Africa, freshly harvested or semi-processed insects find their way to rural open-air markets, with some favourite species reaching urban markets and restaurants (Mmari *et al.,* 2017; Baiyegunhi *et al.,* 2016). The processes leading to delivery of the edible insects to the end-consumer are highly variable, as the techniques and practices in collection or harvesting, aggregation, handling, preliminary processing, packaging, storage, and transportation vary widely. In order to fully benefit from mopane worm and optimise its nutrient retention and safety, it is important to understand the impact of postharvest processing methods, such as degutting. On these aspects, Ruzengwe *et al.* (2019) reiterated that the bioavailability of the nutrients in edible insects and their products can be influenced by the processing method, which needs to be considered when implementing food-based strategies. A food matrix is typically altered as a result of food processing, which may increase or reduce the bioavailability of nutrients (Fernandez-Garcıa *et al*., 2009a). Insect processing has the potential to enhance nutritional quality, safety, flavour, and shelf life, according to (Williams *et al*., 2016).

1.2 Problem statement

According to Romotowska *et al.* (2016), a number of variables, such as the processing technique, emergence season, and the geographic region of sourcing, have an impact on the nutritional content of foods with animal and plant origins. Despite the fact that the aforementioned parameters are cited as having an impact on the nutritional makeup of edible insects (Ssepuuya *et al.*, 2016), there is insufficient knowledge on their precise effects on mopane worm to fully utilize the resource. The acceptance of mopane worm by consumers and its contribution to food security is well studied. However, there is still a knowledge gap in terms of food safety and antioxidant properties associated with this edible insect. Finding an answer to these areas has many implications; from enhancing the productivity of mopane worm and to developing better strategies of utilization of this important natural resource.

1.3 Justification of study

Agenda 2063 which emphasis 'reduction of hunger especially amongst women and youth to 20% level by 2023' (AU, 2014). Nutritional variation due to emergence season and geolocation will provide information that is key in domestication of the mopane worm for realization of its optimum nutritional content. Sustainable Development Goal (SDG) 2 – 'end hunger, achieve food security and improved nutrition and promote sustainable agriculture' by 2030 (FAO, 2020). Optimization and sustainable harvesting of mopane worm can contribute significantly as an additional health benefit apart from macro-nutritional (e.g. protein) content in target communities. The lack of systematic work to ensure product quality is a major drawback in the edible insect sector. It is critical to appropriately assess the raw ingredients utilized in any formulation to avoid underperformance and economic waste (Moskowitz, 2008). In order to formulate nutritionally sufficient meals, an accurate assessment is essential.

1.4 Research design

1.4.1 Research objectives

The aim of the study is to investigate the influence of geographical location, season of emergence and degutting method on the nutritional and bioactive properties of mopane worms.

Specific objectives

a. To assess the effect of emergence season (bivoltine) on the nutritional composition of mopane worms.

b. To determine the effect of geographic location of harvest on the nutritional composition of mopane worms.
c. To assess the effect of degutting method on nutritional, bioactive and microbial and chemical safety of mopane worm.

1.4.2 Research questions

To develop effective interventions on the optimal harvesting stage of mopane worm for nutritional benefits, it is important to understand the influence of factors like season of emergence, the location of harvest and last but not least the degutting methods. Therefore, **Chapters 3, 4, 5 and 6** presents a study conducted in 2020 and 2021 to understand the influence of season of emergence and geolocation of harvest on nutritional composition of mopane worm, in an attempt to address the *first and second research questions*.

RQ1: Does season of emergence and geolocation of harvest have an influence on the nutritional composition of mopane worm?

RQ2: What is the influence of degutting method on nutritional, bioactive properties and chemical and microbial safety of mopane worm?

2 Chapter 2: Literature Review

2.1 Mopane worm life cycle

There are four possible stages in the insect life cycle: egg, larvae, pupa, and adult. Not all insects will go through all four stages. It depends on the type of metamorphosis their species follows. All insects start out as eggs.

The life cycle of the mopane worm is reported to have four phases (figure 2-1). The Emperor moth lays eggs (50–100 eggs) on the leaves of the mopane in the initial phase of the mopane worm's life cycle. In ten days, the eggs hatch and the second phase begins. The larvae feed on the leaves in the second phase and develop into a fully developed worm. The fully developed worm's size varies depending on the locality, variation, and nourishment. Some worms have been known to grow to be 9-10 cm long. Before attaining maximum size, the worm is said to moult four times (Mujuru *et al.*, 2014). The mature worms travel down the tree into the earth at Instar V (burrowing). Harvesting the worms at this time is perfect since the nutritional composition is at its peak and the worms have completed feeding on the leaves. Because there is less nourishment (leaves) in the intestine, worms taken at this stage contain the least amount of frass and are less bitter.

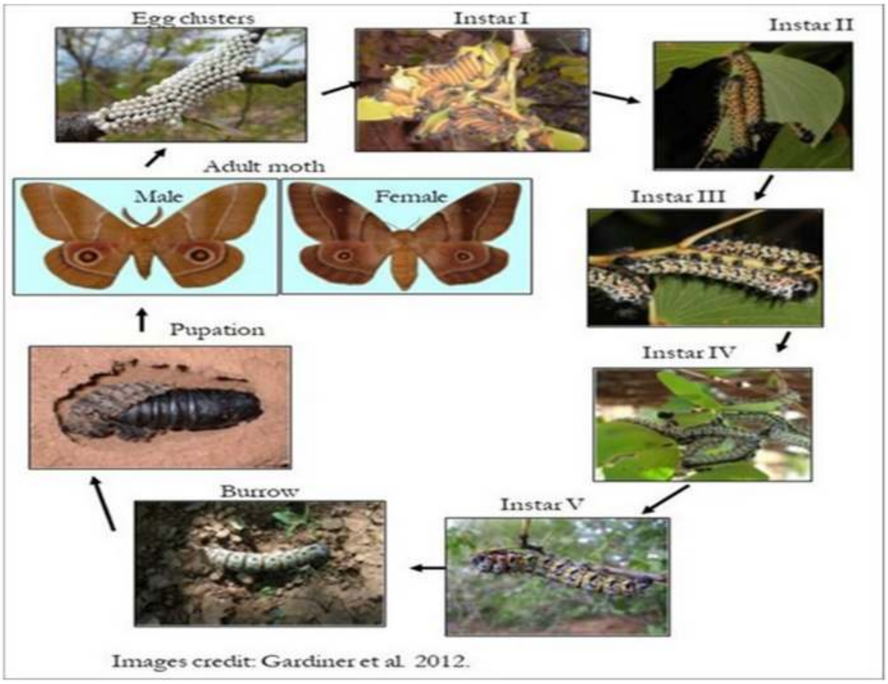


Figure 2: The life cycle of *G. belina*

To ensure the continuation of the life cycle and a future outbreak in later months, good harvesting practice mandates that a small percentage of the worms, ideally 10%, be left unharvested and allowed to reach the ground. The worm pupates after burrowing 15 cm into the ground. They build a rigid cocoon and remain dormant for months, sometimes up to seven months, depending on moisture, temperature, and environment conditions. The mothing stage occurs when the moth emerges at the start of summer. The Emperor moth's main goal is to mate and deposit eggs for 2–3 days without food, after which the moth dies (Stack *et al.*, 2003).

2.2 Harvesting and Processing

The mopane moth is *bivoltine* throughout most of its distribution, meaning there are usually two generations each year from December to January and April to May (Hrabar 2006; Akpalu *et al.* 2007). Mopane worms are harvested from both the ground and from trees, usually the fourth instar stage, and the last stage before pupation (fifth instar). According to Kozanayi and Frost (2002), harvesters prefer to gather mopane worms from shorter trees because they are more convenient to access. To find the mopane worm, harvesters search for droppings beneath big trees. Rural harvesters typically gather adult mopane worms as they begin to crawl down the trees, but during their emergence, it is not possible as there is not enough mature mopane worms and the tendency is to collect premature mopane worms. Mopane worms that are mature and ready to be harvested can be distinguished by their toughness and prominent spikes.

The most challenging and labour-intensive step in processing the mopane worm has been demonstrated to be removing its intestinal contents (van Huis, 2013). This is particularly true if the larvae are harvested before they are ready to pupate. It has been reported that when larvae are completely grown, their bodies are filled with a "yellow nutritive material" and their guts are relatively empty (naturally degutted). Traditionally, the larvae are squeezed by being placed between the thumb and forefinger in order to remove their gut contents. Depending on the size of the larvae and the presence of undigested material, the pressure during squeezing is regulated. Because the larvae's spines can pierce hands and cause bleeding, this procedure presents difficulties for harvesters and increases the risk of mopane worm contamination. Thus, use of gloves while degutting gave both injury protection and a decreased risk of contamination, according to Taylor (2003a). Following the degutting process, mopane worms are then washed with water and dried. According to research by Mujuru *et al.* (2014), drying can decrease the volume (size) of mopane worms due to moisture content loss. Hot ash drying, which requires less time to dry and results in fewer and smaller spikes on the worms, is the most popular drying technique among processors. In some cases, the worms could be boiled before being sun dried. However, due to the worms' noticeable spikes and smaller size, this method is not very popular (Mujuru *et al*. 2014). The multilayer water in the mopane worm matrices was removed in substantial quantities, which is what caused the reduced size. These modifications lower market value because buyers view them as immature as a result of the shrinking.

2.3 Nutritional composition

The variation in nutritional composition of edible insects from different researches is high, owing to real variation between insects taken from different settings or rearing circumstances (Finke and Oonincx, 2014), as well as method of analysis (Payne *et al.*, 2016). Each of the tens of thousands of insects has an own nutritional profile. The nutritional content of insects varies depending on their metamorphic stage and diet, even within species. Holometabolous insects, particularly their larval phases, appear to have more fat and less crude protein than hemimetabolous insects, which do not have larval or pupal stages. This is expected since holometabolous insects do not consume throughout the pupation transition from larva to adult, but they require enormous amounts of energy to complete their metabolic alterations that transform their bodies from larva to adult form (Simpson and Raubenheimer, 2001). Crude protein, fat, vital amino acids, and fatty acids, as well as vitamins and minerals, are abundant in many species (Bukkens, 1997; Rumpold and Schluter, 2013). Diet has been found to be a major driver in the chemical makeup of insects in previous research (Oonincx and van der Poel, 2010).

The most significant aspect affecting the insect's nutritional value is its feed and age. Older yellow mealworm larvae have more fat reserves, which they use as a source of energy during transformation (McClements *et al*., 2003). Age causes the lipid quality of superworm (*Z. morio*) larvae to decline (Kulma *et al*., 2020). Insect females are expected to be more nutritionally valuable than males due to reproductive activity. This was demonstrated for the *Zonocerus variegatus* grasshopper and house cricket, females had more crude protein, ash, fiber, carbohydrates, and vitamins A and B than did males (Ademolu *et al*., 2017; Kulma *et al*., 2019) The nutritional composition of edible insects is generally impossible to describe with any degree of accuracy. There are significant variances among species, as well as within species, depending on factors including geolocation, emergence season, feed, and whether the insects are wild harvested or reared (Rumpold and Schluter, 2013). The nutritional profile of insects is influenced by even their stage of development (Kulma *et al*., 2020). This research aimed at demystifying the influence of emergence season, geolocation and degutting method on the nutritional quality of mopane worm.

2.3.1 Crude protein content

The crude protein content on a dry-matter basis of insects range between 7 and 91 %; and many species contain approximately 60 % crude protein (Finke and Oonincx, 2014). The digestibility of crude protein from insects is highly variable, partly because a part of the amino acid in cuticular crude protein is bound to chitin, a polysaccharide and component of the exoskeleton of insects. According to Rumpold and Schlüter (2013), who compiled 236 nutrient compositions, edible insects in general meet the requirements of the WHO for amino acids with high values for phenylalanine and tyrosine and sometimes being rich in tryptophane, lysine and threonine. Most edible insects provide satisfactorily the required essential amino acids. Yi *et al.*, (2013) extracted and characterised crude protein fractions from three mealworm species and one cricket species. They concluded that crude protein content of the insect species was comparable with conventional meat products. Promising in terms of future food applications is that insect crude proteins can form gels using the soluble fractions obtained by a simple aqueous extraction procedure.

Crude proteins represent the main component of the nutrient composition of mopane worms with an average content of 58% on a dry basis in larvae (Glew *et al.*, 1999). However, the amount of crude proteins depends on the processing method used, as highlighted by Bukkens (1997), who noted that dried mopane worms had a relatively higher percentage than dry-roasted ones (57% and 48% respectively). The amino acid content of larvae compares quite well with soybean, with significant proportions of lysine, tryptophan, and methionine, which are limiting in maize and legumes respectively (DeFoliart, 1989) (Table 2-2). The crude protein score has been found to be at or above the WHO ideal standard in all essential amino acids (Table 2-1). Generally, insect crude protein has low digestibility (Bukkens, 1997). Depending on processing methods, crude protein values and digestibility vary (Dreyer and Wehmeyer, 1982). For example, when dried and traditionally prepared, crude protein digestibility (D) values of about 86%, assimilability (A) of 79% and net crude protein utilization (NPU) of 68% have been reported for mopane worms. These values compare quite well with conventional crude protein sources (Bergeron *et al*., 1988). The relatively low digestibility values could be attributable to the presence of chitin, a nitrogen containing carbohydrate.

Table 2-1: Essential amino acid content of *G. belina* compared with the WHO ideal crude protein

Amino acid	***% of total amino acid***	***(% amino acid/ideal) x 100%***	***WHO ideal***
Tryptophan	1.2	109	1.1
Threonine	5.7	168	3.4
Isoleucine	4.5	161	2.8
Leucine	6.5	98	6.6
Lysine	7.4	128	5.8
Methionine + cysteine	4.2	168	2.5
Phenylalanine + tyrosine	11.7	186	6.3
Valine	5.7	163	3.5
Histidine	3.1	111	2.8

Adopted from FAO/WHO/UNU, 2007

Table 2-2: Amino acid content of *G. belina*

Amino acid	***Amount (mg/g dry weight)***
Tryptophan	5.62
Threonine	27.4
Isoleucine	21.5
Leucine	31.2
Lysine	35.8
Methionine	10.0
Cysteine	10.4
Phenylalanine	25.5
Tyrosine	30.8
Valine	27.5
Arginine	28.5
Histidine	15.0

Alanine	25.2
Aspartame	53.0
Glutamate	60.8
Glycine	22.6
Proline	24.6
Serine	27.1
Total crude protein content	482.52

Adopted from Bukkens (1997); Glew *et al. (1999);* Kwiri *et al.* (2020)

2.3.2 Fat content

Fat represents the second largest portion of the nutrient composition of edible insects, ranging from 13 % for Orthoptera (grasshoppers, crickets, locusts) to 33 % for Coleoptera (beetles, grubs) (Rumpold and Schluter, 2013). The fat content of edible insects ranges from 7 to 77 g/100 g dry weight and the caloric value of insects varies between 293 and 762 kcal/100 g dry weight (Ramos-Elorduy, 1997). These values depend on insect diet and insect species. For instance, worms and termites are known to contain more fat (Bukkens, 2005) and, according to DeFoliart (1992), some insects contain more essential fatty acids, such as linoleic and/or linolenic acids, compared with meat. Lipids in edible insects have been shown to contain considerable amounts of polyunsaturated essential fatty acids such as linoleic and linolenic acids (Womeni *et al.*, 2009), which the human body cannot synthesize them and should be provided for in the diet (Michaelsen *et al.*, 2009). The larvae of the African palm weevil are considered a delicacy in Nigeria. The lipid content (on a dry weight basis) of this larva (67 %) is higher than the amount found in most conventional crude protein foods such as beef, chicken, egg, and milk (Ekpo and Onigbinde, 2005). In developing countries, this can be an advantage as malnutrition here is often more a problem of energy deficiency than crude protein deficiency (DeFoliart, 1992). The fatty acids of insects are generally comparable with those of poultry and fish in their degree of unsaturation but contain more polyunsaturated fatty acids (Finke and Oonincx, 2014; Rumpold and Schluter, 2013). In insects, the stage of development has been shown to contribute significantly to the energy value, for example, the larvae or pupae are usually richer in energy compared to adults (Bukkens, 1997).

Mopane worms contain about 15 % fat (Hobane, 1994), of which fatty acids constitute 75 % of the crude lipid fraction (Glew *et al.*, 1999). About 38 % of the fatty acids were saturated and 62 %

were unsaturated (Rumpold and Schluter, 2012). Mopane worm contain a significant proportion of palmitic acid at 32 %, oleic acid, a monounsaturated fatty at 34 % and linolenic acid, a polyunsaturated fatty acid at 19.6 % (table 2-3). As noted by Bukkens (2005), the fatty acid composition of edible insects is a function of the insects' diet during growth and development. The presence of unsaturated fatty acids makes insects-based food products prone to oxidation.

Table 2-3: Fatty acid composition of mopane worm

Fatty Acid	**% Composition**
Saturated Fatty Acids	
Myristic (C14:0)	1.15
Palmitic acid (C16:0)	31.9
Stearic acid (C18:0)	4.7
Monounsaturated Fatty Acids (MUFAs)	
Palmitoleic (C16:1)	1.8
Oleic acid (C18:1)	34.2
Polyunsaturated Fatty Acids (PUFAs)	
Linoleic (C18:2)	6.02
Linolenic (C18:3)	19.6
Arachidonic (C20:4)	0.5
Total unsaturated fatty acids	62.12
Total saturated fatty acids	37.75

Adopted from Bukkens (2005); Rumpold and Schluter, (2012); Womeni *et al*. (2009)

2.3.3 Micronutrients

It has been reported that the mopane worm contains a significant amount of important minerals. For instance, the level of iron, a mineral important in prevention of anaemia was present at an average of 300 µg/g dry matter (Table 2-4). This compares well with iron content in beef, which is around 60 µg/g (Bukkens, 2005). Stack *et al.* (2003) attributed the high iron content is attributable to the mopane worm diet, the leaves they feed. In addition, calcium a mineral important for bone integrity was present at 2730 µg/g dry matter. Notable was also the presence of appreciable amounts of zinc, a mineral important for the normal functioning of the immune system.

The amount of zinc in mopane worms was reported to be around 140 µg/g dry matter (Glew *et al.*, 1999). Mopane worm did not show significant proportions of sodium but were quite rich in potassium with levels of about 15 800 µg/g dry matter.

Table 2-4: Mineral content of *G. belina* (ug/g dry matter)

Mineral	**Composition (ug/g dry matter)**
Calcium	2730
Iron	304
Potassium	15 800
Magnesium	1 850
Sodium	18.8
Phosphorus	6 340
Zinc	142

Adopted from Glew *et al.* (1999); Headings and Rahnema 2002; Kwiri *et al.* (2020)

2.4 Bioactive components

Edible insects may provide organic compounds with bioactive effects on human health in addition to providing macronutrients and micronutrients to human diets (Roos and Van Huis, 2017). Additional research is necessary to substantiate claims, particularly those relating to bioactivity that is favourable to health, due to the wide variety of edible insects and their variance in nutrient levels at the larval, instar, and adult stages. New findings about the potential health benefits are constantly being discovered, and the rise of the highly diverse food groups represented by edible insects signifies a wide area of potential bioactive compounds that need scientific verification.

Bioactive compounds are present in small quantities in foods, mainly in fruits, vegetables, and whole grains, and provide health benefits beyond the basic nutritional value (Gökmen, 2016). Most of the bioactive compounds have antioxidant, anticarcinogenic, antiallergenic, anti-inflammatory, and antimicrobial properties.

Epidemiological studies indicate that high consumption of foods rich in bioactive compounds with antioxidant activity, including vitamins, phytochemicals, and mainly phenolic compounds, such as flavonoids and carotenoids, has a positive effect on human health and could diminish the risk of numerous diseases, such as cancer, heart disease, stroke, Alzheimer's, diabetes, cataracts, and age-related functional decadence (Hassimotto *et al.*, 2009; Siriwardhana *et al.*, 2013). Some examples of bioactive compounds are carotenoids, flavonoids, carnitine, choline, coenzyme Q, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine.

Phenolic compounds, including their subcategory flavonoids, are present in almost all plants and have been found extensively in cereals, legumes, nuts, olive oil, tea, red wine, vegetables, and fruits. They mostly have antioxidant properties and some studies demonstrated favorable effects on cardiovascular diseases risk factors.

Colophospermum mopane leaves are generally not favoured by vertebrate browsers and are used only in drought years, implying that they likely contain plant defence compounds such as phenolic and tannins possibly signifying the presence of some bioactive compounds in mopane worm (Gardiner, 2005). Similarly, a study on the mopane leaf chemistry in the Venetia-Limpopo Nature Reserve found an average of 63.2 g (dry weight basis) of total polyphenolic compounds and 0.59 g (dry weight basis) of condensed tannin-protein ratio. However, research on the bioactive substances found in mopane worms has not been done, thus it is essential to conduct a study to determine their concentrations.

2.4.1 Antioxidants

Chemical substances known as antioxidants prevent oxidation when cells are exposed to free radicals. To increase shelf life or lower health hazards, they can be added to food products or found naturally in food. The development of functional foods has increased dramatically as a result of antioxidant activity in food. Edible insects are a potential source of a variety of peptides due to their high protein content, which may lead to antioxidant activity. A number of peptides discovered in edible insect species, such as B. mori and A. domesticus (Di Mattia *et al*., 2019) and the edible wasp Vespa affinis (Dutta *et al*., 2016), demonstrated antioxidant capabilities in in vitro investigations. To learn more about the direct advantages of antioxidants present in edible insects for human health, more research is required.

Impact of diet on insect phenolic composition

The main source of phenolic compounds in insects is thought to be herbivore eating habits. *P. icarus* larvae, pupae, and adults raised on alfalfa and crown vetch displayed a phenolic profile that was identical to that of the particular host plant that included the flavonoid kaempferol-3-O-glucoside as a main component. In addition, kaempferol-3,7-di-O-glucoside, which is thought to be a biotransformation by product of kaempferol or kaempferol-3-O-glucoside in plants, was found in the larvae.

Phenolic chemicals have a variety of bio-activities that are linked to chronic diseases, including antioxidant, anti-inflammatory, and anticancer effects, among others. Additionally, due to the growing concern over microbial resistance to conventional antibiotic treatments and the interest in developing clean-label food preservatives that will prevent the use of synthetic compounds in the food industry, the antimicrobial bio-activity of plant phenolic compounds has been extensively studied against a variety of pathogenic and non-pathogenic microorganisms (Cushnie and Lamb, 2005; Daglia, 2012; Farhadi *et al*., 2019). To yet, only the antioxidant bioactivity of insect phenolics has been evaluated. Nonetheless, it is commonly accepted that the crude protein and peptide fractions in insects like tropical banded crickets (Gryllodes sigillatus), mealworm (Tenebrio molitor) and desert locusts (Schistocerca gregaria) are responsible for their overall bioactivity towards oxidation, inflammation, hypertension, and glycaemic inhibition (Zielińska *et al*., 2017, 2018; Hall and Liceaga, 2020; Hall *et al*., 2018). A few of the phenolic chemicals found in insects, such kaempferol and quercetin, have demonstrated bioactivity when isolated from plant sources, which raises the prospect for more bioactive activities.

2.5 Safety of edible insects as food

In spite of issues with food security in the majority of developing nations, consumer knowledge of food safety is rising globally. Despite related bacteria that may affect the safety of mopane worms as food, their consumption is on the rise. Both domesticated and wild harvested edible insects may harbor harmful microbes such as bacteria, viruses, fungus, and protozoa (Vega and Kaya 2012). Similar to meat products, mopane worms are nutrient- and moisture-rich, creating an environment that is conducive to microbial survival and growth (Klunder *et al*., 2012). Basically, in Africa, mopane worm harvesting, processing, packaging, and storage procedures are largely considered as inadequate and the major sources of their spoiling by bacteria or fungi include water of poor quality, insect vectors and soil (Kwiri *et al*. 2014). Mopane worms are usually maintained in unclean polypropylene woven bags, dirty plastic or metal buckets, and dirty clay pots that have previously been used to keep paint or fertilizer (Allotey *et al*. 1996; Nyakudya 2004). This may raise the risk of end product contamination and deterioration. In a study conducted in Botswana, the quality of sun-dried mopane worms decreased when the inside meat underwent colour changes as a result of mould growth. *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Phycomycetes* species, some of which generate mycotoxin, were the most common fungal isolates discovered. Following quantification, it was discovered that aflatoxins ranged from 0 to 50 g per kilogram of product, above the 20 g per kg FAO maximum acceptable level. This suggests that consuming contaminated foods over an extended period of time could be harmful to your health. However, conventional processing techniques like boiling, roasting, and frying are frequently used to enhance sensory qualities like flavour and palatability, and they also have the additional benefit of enhancing food safety.

Concerns concerning the handling of edible insects, food processing methods, hygiene, and general food safety have increased due to global consumer expectations for safer and healthier foods. FAO (2010b) only classified insects as "health foods" provided they were gathered from forested regions and were typically clean and chemical-free, as is the case with the majority of African caterpillars. Based on the exposed knowledge gap, the objective of this study was to determine the effect of degutting method on the proximate, antioxidant properties, chemical and microbial safety of mopane worm. Findings from this study will help in the adoption of sustainable harvesting methods for reducing pressure on mopane worm populations, including habitat management and small-scale controlled rearing and more importantly, on optimizing safety of the mopane worm.

2.5.1 Chemical hazards

Pesticide residues such as organophosphorus pesticides, and chlorinated pesticides such as benzene hexachloride (BHC), lindane and aldrin have been reported in edible insects. The source of the pesticides was from chemicals used to control the insects. Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), dioxins, organophosphate flame retardants (PFRs), pesticides (vinyl toluene), copper, and zinc are some of the chemical hazards previously reported in edible insects (Porma *et al*., 2017). Low quantities of arsenic have reportedly been found in wild-caught *Agrotis infusa* (*Bogong moths*) Green *et al*., (2001). Presence of heavy metals in wild harvested edible insects is mostly related to contamination from the feed and storage containers. In most instances the selected harvesting and storage containers include empty fertilizer bags and empty paint buckets (figure 2-2). Thus the risk of chemical contamination is high during these post-harvest practices.

Figure 3: Empty paint buckets used during mopane worm harvesting

Studies have identified heavy metals like mercury, cadmium, lead, and zinc as chemical dangers in wild harvested edible insects. Significant amounts of nickel, lead, and cadmium were found in Anapleptes trifasciata (a beetle), Rhynchophorus phoenicis larvae (Banjo *et al*., 2010). In a study on the safety of mopane worms in South Africa, significant bioaccumulation of cadmium and copper was found (Greenfield, Akala, and Van Der Bank, 2014). The same study also discovered manganese levels that were 20–67 times greater than FDA guidelines. The researchers found two probable sources of these metal contaminants: food consumed by worms and pollution that land on leaves.

2.5.2 Biological hazards

The main issues regarding the safety of edible insects in Africa were found to be primarily biological threats. The emperor moth (*Bunaea alcinoe*) caterpillar was the subject of a study by Braide *et al*. (2011), which revealed the presence of pathogenic microbes such *Staphylococcus aureus, Bacillus cereus, Proteus, and Escherichia coli*. Among the investigated edible insects' species, it was also possible to find mycotoxins accumulation as well as Aspergillus, Penicillium, and Fusarium proliferation. The presence of *E. coli* and fungi indicate improper hygiene practices by the insect vendors and inadequate processing which resulted in post-processing contamination (Braide *et al*., 2011). According to Klunder *et al*. (2012), the major source of contamination in wild harvested insects is soil. Soil can have faecal matter from animals present in the area resulting in contamination with pathogens such as *Escherichia coli.* More so, consumption of contaminated food by the insects can also increase the amount of microflora found in the gut of edible insects.

2.6 Mopane worm distribution

Mopane woods can be found from southern Angola, through northern Namibia, and into the eastern half of Botswana. Zimbabwe is arguably the country with the largest area, extending into agricultural regions IV and V. Additionally, the mopane woodlands extend into Northern South Africa, Eastern Mozambique, and Southern Zambia. Low rainfall and a propensity for drought are characteristics of these forests, making cultivation challenging and unprofitable (Kwiri *et al.* 2014). In these areas, the mopane worm develops into a substantial product for both money creation and dietary augmentation.

Colophospermum mopane (figure 2-4) are a common larval food plant of the mopane moth (*Imbrasia belina*). Fundamental to the life cycle of phytophagous insects is the location of a suitable plant for oviposition, especially for species with relatively immobile larvae, or monophagous species. Frequently, it is assumed that females select host plants that provide qualitatively and quantitatively the best food for their larvae, as natural selection should favour a positive relationship between adult oviposition preference and offspring performance. In mopane woodland, *Colophospermum mopane* Kirk ex J. Léonard, trees (commonly known as 'mopane') tend to dominate and generally comprise 90% of the total woody plant biomass (Guy, 1981). This deciduous, xeric savanna woodland species is well-known for its suite of chemical defences (Ferreira *et al.*, 2003), yet it is the main host species for the larvae of the mopane moth (*Imbrasia belina*) (Westwood, 1849; Alloy *et al.*, 1996) and, where dominant, it represents the single host species for mopane worms (Hrabar, 2005).

Figure 4: Mopane trees (Colophospermum mopane) (Orwa *et al*., 2009*)*

Although tree species diversity is low within mopane woodland, there is high variability in the growth form and density of mopane trees within a habitat. While mopane trees typically grow to approximately 10m in height, or else occur as low scrub of 1–2 m high, stands of trees up to 20 m high exist (on deep, nutrient-rich alluvial soil) and are termed 'cathedral mopane' (Van Wyk, 1993). Associated with these height differences is a difference in plant architecture, as shrubs are usually multi-stemmed, while taller trees tend to be single stemmed (Fraser *et al*, 1987). The principal cause of these differences in tree height has been identified as variation in the soil composition, particularly depth and pH (O'Connor, 1992) and moisture stress (Hempson *et al.*, 2007). Variable foliar chemical composition is therefore also expected between habitat types (Kraus *et al*, 2004). Furthermore, densities of mature mopane in woodland vary greatly, ranging from <10 trees/ha in arid north western Namibia (Viljoen, 1989) to 481 trees/ha in southeastern Zimbabwe (Kelly and Walker, 1976) and 2740 trees/ha in northern South Africa. This high degree of variability within mopane woodlands thus results in very different habitat types for mopane moth larvae, which may in turn influence host preference by ovipositing female mopane moths at the habitat level.

Host preference at the individual tree level might also be important for mopane moths, as the mobility of larvae affects the way in which adults perceive the vegetation. For species that move readily between plants while feeding, the vegetation could be perceived as a single continuous food source, with average or aggregate attributes. By contrast, species confined to one or a few trees might perceive the vegetation as an array of food sources, each with individual traits (Keshet and Rausher, 1989). The relative immobility of mopane worms (they tend to only move to a new host tree once leaves on the initial host are depleted) might, therefore, require host plant selection by adults at the individual tree scale. However, eruptive population dynamics of phytophagous insects could be due to a lack of selectivity by ovipositing females, as there is no within generation feedback between deteriorating food resources and natality (Price, 1994).

Mopane worms are abundant in Gwanda and sporadic in Tsholotsho though the two areas have similar climatic conditions. A study carried out by Nunu *et al.* (2019) revealed that the differences in crude crude protein levels in leaves, non-extractible tannins, extractible tannins, and natural detergent fibres in Tsholotsho and Gwanda districts were not statistically significant. Findings showed differences in tree size and leaf length whilst the differences of all other variables (non-extractible tannin, extractible tannin crude crude protein levels and natural detergent fibres) relating to leaf sample analysis were not statistically significant. Findings on soil sample analysis pointed out that Gwanda had higher pH, phosphorus, and potassium levels whilst nitrates were significantly higher in Tsholotsho. Differences in the tree sizes and leaf sizes of the samples from the two sites show that there could be host selection based on these variables (Nunu *et al.*, 2019). There is need to envisage mopane worm farms in areas where the worms occur naturally to ensure consistency in supply. Mopane Worms Enterprises (MWE), a four-year-old business venture located near the arid eastern diamond fields of Marange, breeds worms and teaches other farmers how to do so. It also grows seedlings of the mopane tree, the worms' favourite food.

2.7 Traditional use and economic importance of mopane worm

Entomophagy is heavily influenced by cultural and religious practices, and insects are commonly consumed as a food source in many regions of the world. In most Western countries, however, people view entomophagy with disgust and associate eating insects with primitive behavior (FAO/WUR, 2013). This attitude has resulted in the neglect of insects in agricultural research. Insects are not used as emergency food to ward off starvation but are included as a planned part of the diet throughout the year or when seasonally available (Moreki *et al.*, 2012). Insect gathering and rearing as mini livestock at the household level or industrial scale can offer important livelihood opportunities for people in both developing and developed countries. In developing countries, some of the poorest members of society, such as women and landless dwellers in urban and rural areas, can easily become involved in the gathering, cultivation, processing, and sale of insects (FAO/WUR, 2013). These activities can directly improve their own diets and provide cash income through the selling of excess production as street foods.

Moreki *et al.* (2012) reported that when mopane worms were in season, the sale of beef was seriously affected. In the early 1980s, annual sales of mopane entering commerce were estimated by the South African Bureau of Standards to be 1600t; this did not include those privately collected and consumed (Dreyer and Wehmeyer, 1982). According to Mujuru *et al.* (2014), hundreds of tons of mopane worm are exported annually from Botswana and South Africa to Zambia and Zimbabwe. A similar caterpillar trade, involving other species, exists further north in Africa. Insects are not only sold widely in the village markets of the developing world, but many of the favourites make their way to urban markets and restaurants. The income generated by the mopane worm harvest provides many families with funds to purchase household items such as clothing, school materials and basic utensils (N'Gasse, 2004: Stack *et al.*, 2003). Vast numbers of people partake in the mopane harvest: the nutritional and economic incentives are so high that many are willing to travel hundreds of kilometers across the mopane woodlands in search of the insects (Kozanayi and Frost, 2002).

In the Limpopo Province in South Africa, the trade and consumption of mopane worms contributed to rural household food security (Baiyegunhi *et al.*, 2016). However, overexploitation and commercialization threaten the long-term management of the mopane woodlands, and a balance need to be found between sustainable harvesting of mopane caterpillar and improving the livelihoods of the rural poor (Baiyegunhi *et al.*, 2016). Strategies proposed are delaying the supply of the stock to the market and practices to maintain enough fifth-instar mopane worms, as well as safeguard the host tree against exploitation and ways to preserve the pupae (Gondo *et al.*, 2010). Also, restrictive harvesting periods have been proposed but there are doubts about its effectiveness (Akpalu *et al*, 2007). As the occurrence of mopane caterpillar is erratic and periodically fails to produce worms of harvestable size, there is now an increased interest in developing domestic farming techniques of the worms at the household level. Cereals have low iron and zinc bioavailability, and it has been attempted to enrich cereals with mopane caterpillar in Zimbabwe

(Gabaza *et al.*, 2018). However, the bio-accessibility of iron and zinc was not improved, it only increased the iron and zinc content of the enriched fermented cereals. Also, the nutritional potential of the mopane worms has been studied in diets through its use in fortified blended foods formulations (Kwiri *et al.*, 2014). Allergic reactions to the consumption of mopane caterpillar are possible (Kung *et al.*, 2011; Okezie *et al.*, 2010).

3 Chapter 3: Materials and methods

3.1 Sampling and sample preparation

3.1.1 Influence of emergence season and geolocation

A total of 24 kg of manually degutted mopane worms were collected from each of the three seasons of November to December 2020 and 2021, and April to May 2021. The samples were collected from three locations in the Gwanda district: Ward 21 (Timber farm, 21° 3′ 59.24″ S; 28° 59′ 46.382″ E), Ward 22 (River block, 20° 56′ 58.55″S; 29° 20′ 8.758″E), and Ward 13 (Insidi, 20° 50′ 48.283″ S; 29° 1′ 31.598″ E) (Figure 3-1). For each geolocation as shown in Figure 3-4 and 3-6, three harvesting camps were identified, and four harvesters were randomly selected from each camp. From each harvester, 2 kg of manually degutted mopane worms (3rd and 4th instar) were collected by picking 0.5 kg of manually degutted mopane worm from four quarters of the area occupied by the degutted mopane worms to obtain an 8 kg sample. They were then placed in one container and mixed thoroughly to make one composite sample per harvesting geolocation (ward), giving a total of 24 kgs from each geolocation (ward).

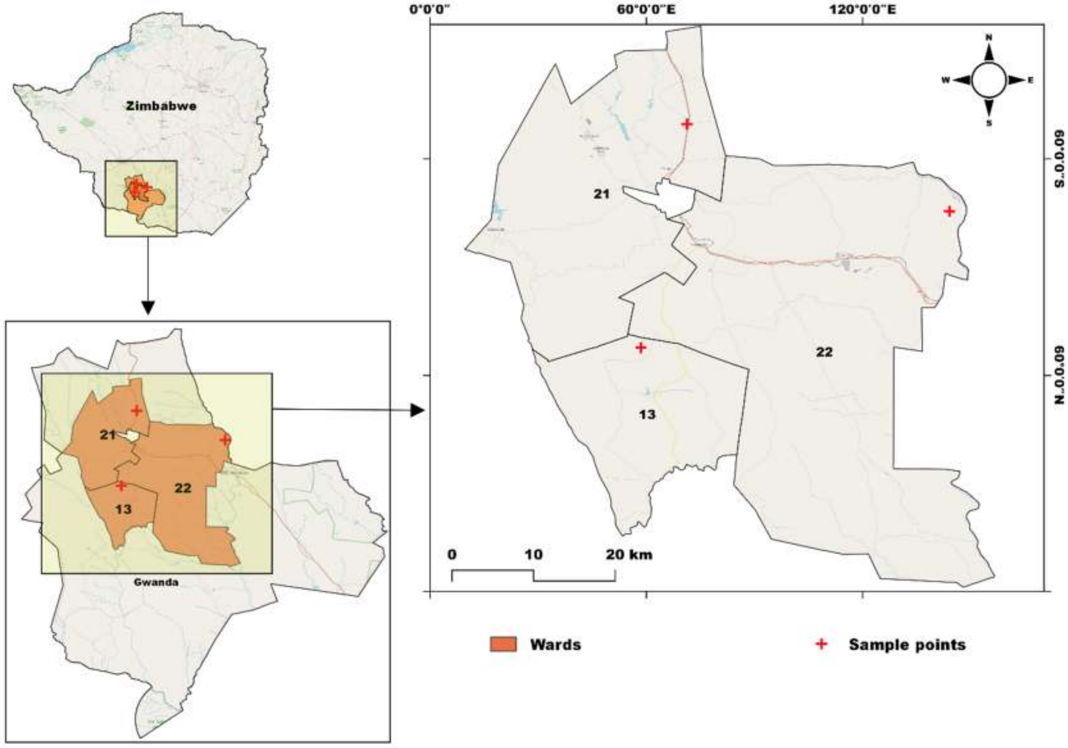


Figure 5: Map of Zimbabwe showing mopane worm sampling geolocations in Gwanda district. Ward 21 is Timber Farm, 22 is River block, 13 is Insidi.

The mopane worms were then rinsed with cold deionized water, placed in zip-lock polyethylene bags and transported to the laboratory at Chinhoyi University of Technology in a cool box with flaked ice. At the laboratory, the samples were kept at -18 °C until analysis were done. In preparation for analysis, each composite sample was subdivided into two (A and B) and each duplicate was independently analysed. Before analysis, 1 kg of each sample was allowed to defrost for at least 30 minutes, followed by crushing in an electrical blender (AE Electrical, Harare, Zimbabwe) at high speed for 10 seconds.

3.1.2 Influence of degutting method

Approximately 18 kg each of both manually and naturally degutted (figure 3-2) fresh mopane worm were obtained from two sampling locations in Gwanda district (Ward 21 - 21° 3′ 59.24″ S; 28° 59′ 46.382″ E) and Ward 22 - 20° 56′ 58.55″S; 29° 20′ 8.758″ E) (figure 3-3). Manually degutting is a process done by harvesters of removing the mopane worm gut contents by pressing its gut out followed by washing with water whilst naturally degutting is when the mature mopane worm empts its gut contents in preparation for pupation. The sampling points in Gwanda district were selected based on their importance in the mopane value chain according to Zimbabwe Vulnerability Assessment Committee (ZimVAC) 2020 survey (which indicated that more than 50% counts for mopane worm availability in the area).

Figure 6: A-Manual degutting process and B-naturally degutted mopane worm about to burrow

(B)

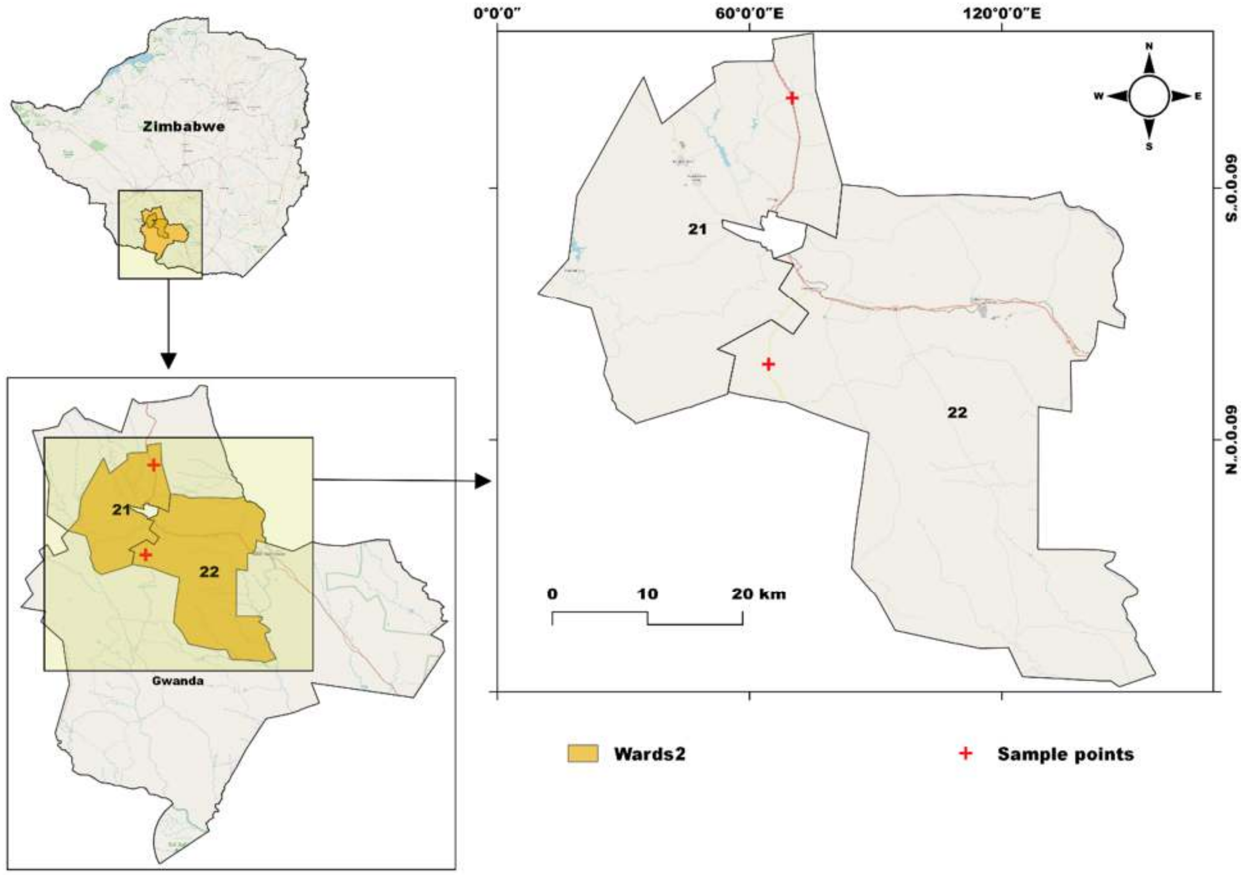


Figure 7: Map of Zimbabwe showing mopane worm sampling geolocations in Gwanda district. Ward 21 is Timber Farm and 22 is River block

For each ward three harvesting camps were randomly identified and three harvesters randomly selected per each harvesting camp (Figure 3-5 and 3-7). From each harvester 1 kg of fresh mopane worms (both manually and naturally degutted) were collected to obtain 9 kg sample per ward. Samples from each sampling point were mixed together to make a composite sample and cleaned with deionized water. The mopane worms were collected according to local harvesting practices (manually degutted - a combination of handpicking from the host trees and shaking the host trees followed by degutting and naturally degutted were collected on the ground when they were in the process of burrowing into the ground). Samples were packed in zipping polyethylene bags before freezing. The samples were transported to laboratory on flaked ice in cooler boxes and stored at −18 °C until further analyses.

Prior to analysis, approximately 100 g of frozen mopane worm samples were allowed to thaw. Samples for nutritional composition, heavy metals and bioactive properties analysis were split into two duplicate samples (labelled A and B). Samples for microbial analysis were packed separately into polyethylene bags under sterile conditions and frozen awaiting analysis. Before analysis, samples were allowed to defrost, followed by crushing in an electrical blender (AE Electrical, Zimbabwe) at high speed for 10 seconds.

The mopane worms were then rinsed with cold deionized water, placed in zip-lock polyethylene bags and transported to the laboratory at Chinhoyi University of Technology in a cool box with flaked ice. At the laboratory, the samples were kept at -18 °C until analysis were done. In preparation for analysis, each composite sample was subdivided into two (A and B) and each duplicate was independently analysed. Before analysis, 1 kg of each sample was allowed to defrost for at least 30 minutes, followed by crushing in an electrical blender (AE Electrical, Harare, Zimbabwe) at high speed for 10 seconds.

3.2 Sampling framework

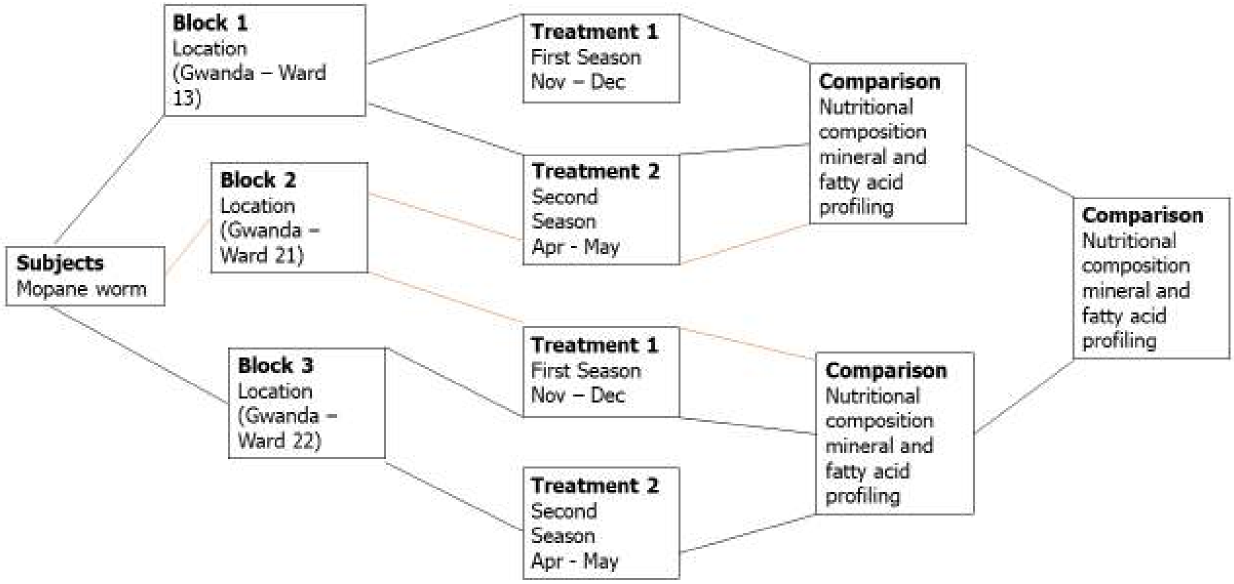


Figure 8: Sampling design for the influence of emergence season and geographic location

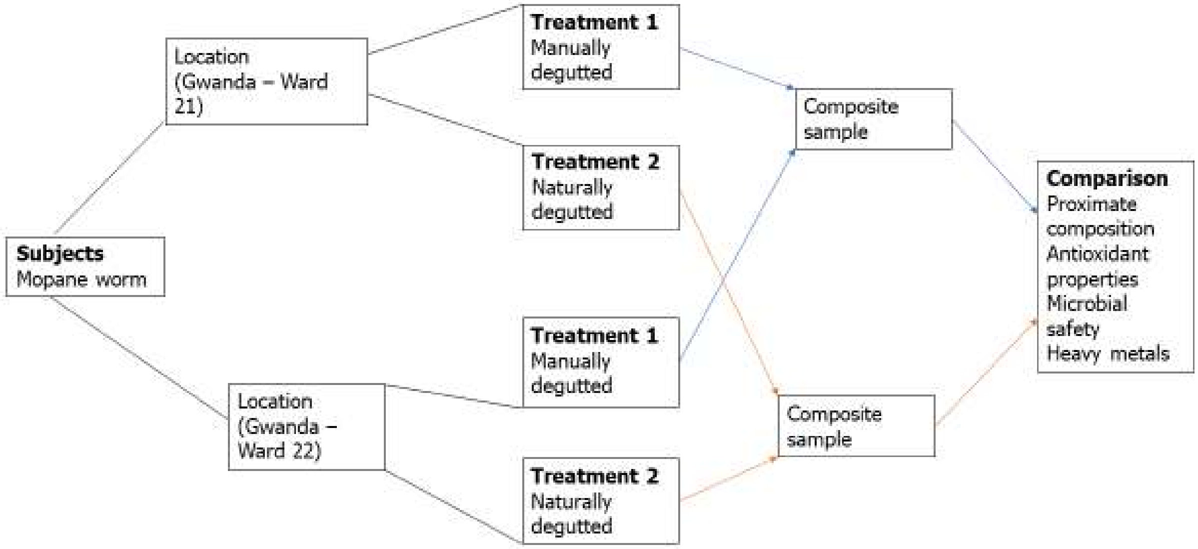


Figure 9: Sampling design for the influence of degutting method

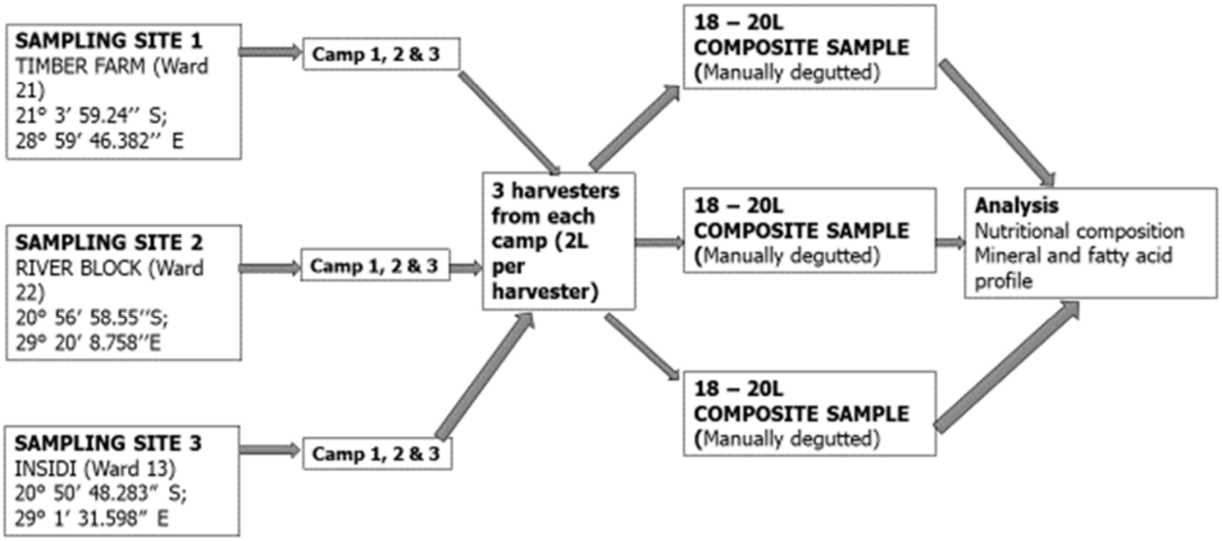


Figure 10: Sample collection framework for influence of emergence season and geographic location

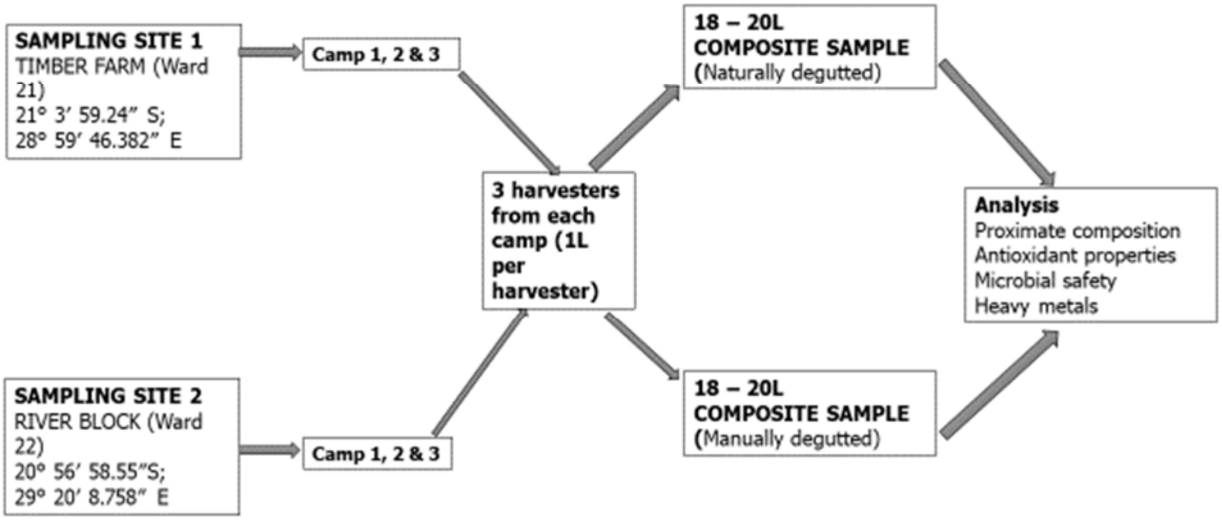


Figure 11: Sample collection framework for the influence of degutting method

3.3 Proximate composition

Moisture content was determined by drying in the forced air oven (Fischer Scientific, South Africa) at 105°C for 3 h as previously described by Nielsen (2010). Crude protein was determined by the Kjeldahl method following the method used by Mihaljev *et al.* (2015) with minor modifications. A nitrogen to crude protein conversion factor of 5.6 was used as suggested by Boulos *et al.* (2020).

Crude fat was gravimetrically determined using the Soxhlet extraction procedure according to Nielsen (2010). Briefly, the sample (5 g) was refluxed with technical grade petroleum ether (Merck (Pvt) Ltd, Johannesburg, South Africa) for 8 h and the extraction solvent evaporated under vacuum by a rotary evaporator (Büchi R-144, Büchi Labortechnik, Flawil, Switzerland) at 50 °C to obtain the fat. The fat obtained was then kept for use for fatty acid profiling.

Ash content was determined by heating the sample in porcelain crucibles in a muffle furnace (Scientific, Johannesburg, South Africa) at 600 °C for 6 hours according to the procedure in AOAC (2005).

Crude fibre was determined using the procedure previously described by Anaduaka *et al.* (2021) with minor adjustments. Each of the fresh (ground) mopane worm samples (2.0 g) (F1) was placed into a 300 ml conical flask, to which 200 ml of 0.128 M H_2SO_4 was added and boiled for 30 min.

Thereafter the material was filtered through Whatman filter paper 42 (Merck, Johannesburg, South Africa) followed by washing three times with hot water (80 °C) and the residue was placed back into the conical flask. Following the addition of 200 ml of 0.223 M NaOH, the sample was heated for 30 min before being filtered through Whatman filter paper 42 (Merck, Johannesburg, South Africa). The residue was then washed with 25 ml of 0.128 M H_2SO_4, three 50 ml portions of water and 25 ml of ethanol. Thereafter, the residue was transferred into a pre-weighed crucible (F2), dried at 130 °C for 2 h, cooled in a desiccator and weighed (F3). The samples were then ignited for 30 min at 600 °C, cooled in a desiccator and reweighed (F4). The percentage crude fibre was calculated as shown in equation 1:

$$\text{Crude fibre (\%)} = \frac{mi}{\text{mtot}} \text{ x } 100 \qquad \text{Equation (1)}$$

Where m_i – mass (g) of residue (F_3-F_2) minus mass of ash (F_4-F_2) and m_{tot} – is the total mass (g) of mopane worm sample (F_1)

3.4 Mineral and heavy metal profiling

Mineral profiling was according to the procedure previously described by Paul *et al.* (2014) with minor modifications. Wet ashing with 65 % nitric acid and 70 % perchloric acid (both from Sigma-Aldrich, St. Louis, MO, USA) was used to determine the mineral (Ca, Mg, K, Na, Fe, Zn, Mn, and Cu) and heavy metals (Cr, Cd, Co, Ni, Pb and Al). The limit of detection (LOD) and of quantification (LOQ) was 0.01 mg/g and 0.033 mg/g respectively. Briefly, 0.5 g of ground fresh mopane worm, 5 ml of 65 % HNO_3 and 10 ml of $HClO_4$ were added and heated until the solution was clear. After cooling, the solution was transferred to a 100 ml volumetric flask. The results were read on an atomic absorption spectrophotometer (AAS) (PinAAcle 900F, Perkin Elmer, Massachusetts, USA). Extraction, detection, identification, and quantification was in duplicate for each sample.

3.5 Phosphorus content

Phosphorus content was measured using colorimetric method according to Pulliainen and Wallin (1996) on an Ultraviolet-visible spectrophotometer (Lampda 365- UVSN410020, Perkin Elmer Massachusetts, USA). The samples were dry-ashed in the presence of zinc oxide, and total phosphorus content was measured calorimetrically as molybdenum blue at 430 nm. All determinations were in duplicate using a 2 x 2 randomized design.

3.6 Fatty acid profiling

Fatty acid profiling was done using a Spectrum Two Attenuated Total Reflection (ATR) – Fourier-transform Infrared (FTIR) (Perkin Elmer, Massachusetts, USA) interfaced with an ATR sampling accessory with a single bounce diamond crystal as previously described by Dong *et al*. (2020) with minor modifications. Spectra, in the absorbance mode, were measured from 4000 cm^{-1} to 600 cm^{-1}, by accumulation of 64 scans at a spectral resolution of 4 cm^{-1}. A reference background spectrum of air was scanned under the same instrument conditions before each sample measurement. Spectra were processed with spectrum 10 FTIR spectroscopy software (version 5.2.1, Perkin Elmer, Massachusetts, USA). A small drop of mopane worm fat obtained from Soxhlet extraction was placed on the surface of the diamond attenuated total reflection (ATR) crystal and the sample spectrum obtained. Identification was done by correlation with library fatty acid spectrums of standards.

3.7 Determination of total phenolic and flavonoids

3.7.1 Preparation of mopane worm extract

Extraction was done according to the method described by Vhangani and van-Wyk (2013) with minor alterations. Mopane worm (2 g) previously defatted by Soxhlet extraction with petroleum ether was mixed with 10 ml of 70 % methanol. The mixture was shaken for 48 h on a mechanical shaker followed by centrifuging (Thermo Electron Corporation Jouan MR1812, Waltham, MA, USA) for 15 min at 3500 rpm. The supernatant was collected and kept at -20°C until further use. The supernatant was used for the determination of the total phenolic, total flavonoid content, IC50 against 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) and 2.2'-azino-bis-(ethylbenzothiazoline-6-sulfonic) acid radical (ABTS), Fe2+ chelating and Potassium ferricyanide reducing antioxidant power (PFRAP).

3.7.2 Total phenolic content

The total phenolic content (TPC) was determined using the spectroscopic method as described by Ainsworth *et al*. (2007). Mopane worm extract (1 ml), and 1 ml of 10% Folin-Ciocalteu's reagent (Sigma-Aldrich Chemie, Steinheim, Germany) were mixed with 13 ml of distilled water and 5 ml of 7% Na2CO3 solution. The mixture was kept for 2 h at room temperature in a dark place. The blank solution was also prepared by replacing the sample with 1 ml methanol. Thereafter, absorbance was read at 760 nm on a UV-VIS spectrophotometer (UV-Vis 1900i, Shimadzu, Japan). Total phenolic content expressed as Gallic acid equivalent (mg GAE) per 100 g of the mopane worm sample was determined using a gallic acid standard curve (Equation 2).

$$y = 0.0011x + 0.2571 \qquad \text{Equation (2)}$$

Where y is the absorbance and x is the concentration of total phenolic content as GAE (µg/ml)

3.7.3 Total flavonoid content

Total flavonoids were determined using the method described by Jimoh *et al*. (2010) with minor adjustments. To each test tube 1.5 ml methanol, 0.1 ml aluminium chloride (Sigma-Aldrich, St Luis, USA) solution, 0.1 ml potassium acetate (Merck (Pvt) Ltd, Modderfontein, South Africa) solution and 2.8 ml distilled water were added and mixed well. The solutions were filtered through Whatman filter paper number 1 and the absorbance was read at 415 nm on a UV=VIS spectrophotometer. Total flavonoids were evaluated and expressed as mg quercetin equivalents per 100 g of the mopane worm sample.

3.8 Antioxidant activity determination

3.8.1 DPPH radical scavenging activity

The 1.1-diphenyl-2-picryl-hydrazyl radical scavenging (DPPH-RS) assay was performed according to the method of Vhangani and van-Wyk (2013) with minor modifications. In a nutshell, 3 ml of DPPH (0.12 mM) in 99% methanol was combined with 1 ml of mopane worm extract. This was incubated in the dark for 30 minutes. Absorbance was then read at 517 nm using a UV-Vis spectrophotometer. The control was prepared similarly using ascorbic acid (0.1%) as a positive control and water as the negative control by replacing the sample with 70 % methanol. The IC50 value was estimated using a fitted line (linear regression) calculated using the formula:

$$\text{IC50} = \frac{50 - c}{m} \qquad \text{Equation (3)}$$

Where: c is y intercept and m is gradient of the fitted line.

3.8.2 Determination of ABTS•+ radical scavenging activity

The analysis was according to the method of Chatsuwan *et al*. (2018). ABTS stock solution (7.4 mM) stock solution and 2.45 mM potassium persulphate were mixed at a ratio of 1:1 (v/v) and left in the dark for 14 h to produce the 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS•+) radical. After that, the ABTS•+ solution was diluted at the 1:50 (v/v) ratio using 95% ethanol so as to obtain an absorbance of 1.00 at 734 nm. ABTS•+ solution (2.85 ml) was then mixed with the mopane worm extract (0.15 ml) and the mixture incubated in a dark place at room temperature for 6 min. The absorbance was then read using a UV-VIS spectrophotometer at 734 nm. The negative and positive controls were prepared using distilled water and Trolox (0.1%) respectively. The IC50 value was estimated using a fitted line (linear regression) calculated using the formula in equation 3:

3.8.3 Determination of Fe^{2+} chelating activity

The Sudan *et al*. (2014) method was used to quantify the produced extracts' chelating impact on ferrous ions with a few minor adjustments. Mopane worm extract (1 ml) was mixed with 1.85 ml of distilled water and 0.05 ml of 2 mM $FeCl_2$. The mixture was left for 10 min at room temperature after the addition of 0.1 ml of 5 mM ferrozine which initiated the reaction. Absorbance was then read on a UV-Vis spectrophotometer at 562 nm. The percentage of chelating activity was calculated as follows:

$$\% \text{ chelating activity} = \frac{A0 - A1}{A0} \times 100 \qquad \text{Equation (4)}$$

Where: A0 is the absorbance of the negative control (water) control and A1 is the absorbance of the mopane worm extract.

3.8.4 Potassium ferricyanide reducing power (PFRAP)

Minor adjustments were made to the method used by Athukorala et al. (2006) to determine the reducing power. The phosphate buffer (0.2 M, pH 6.6) and the 1% potassium ferricyanide were mixed with the mopane worm extract (1.0 ml). This was then vortexed for 10 s and then incubated at 50°C in a water bath for 20 min. Thereafter, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture, and then vortexed for 10 s. From the solution 2.5 ml was then pipetted out and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. Absorbance was then measured at 700 nm using a UV-Vis spectrophotometer.

3.9 Microbial safety

Crushed fresh mopane worm were subjected to serial dilutions in peptone water (10^{-1} to 10^{-6}). Plate Count Agar (PCA) (Merck, South Africa) was used for Total Bacterial Count (TBC) and was incubated aerobically at 37°C for 48 h in conformity with Association of Official Analytical Chemists (AOAC) protocols (Maturin and Peeler, 2001). Enumeration of the total coliforms was done using Violet Red Bile Agar (VRBA) (HiMedia Laboratories Pvt. Ltd, India). Escherichia coli

(E. coli) colonies were enumerated on a selective chromogenic medium, Tryptone Bile X-Glucuronide Agar (TBX) (Oxoid Ltd, Basingstoke Hampshire, England) as blue/green colonies. S. aureus was enumerated on a Mannitol salt agar (Oxoid Ltd, Basingstoke Hampshire, England) which gives yellow colonies in their presence in conformity with Bacteriological Analytical Manual (2001) (Bennet and Lancette, 2001). Salmonella spp. enumeration was done using Xylose Lysine Deoxycholate agar (XLDA) (Oxoid Ltd, Basingstoke Hampshire, England) incubated at 35-37°C for 24 h. The selective medium gave Salmonella spp. as red colonies with black centers (Bennett and Lancette, 2001). Yeasts and moulds enumeration was done on Potato Dextrose Agar (PDA) (Merck, 1 Friesland drive, South Africa) and incubated in the dark at 25°C for 5 days.

3.10 Statistical analysis

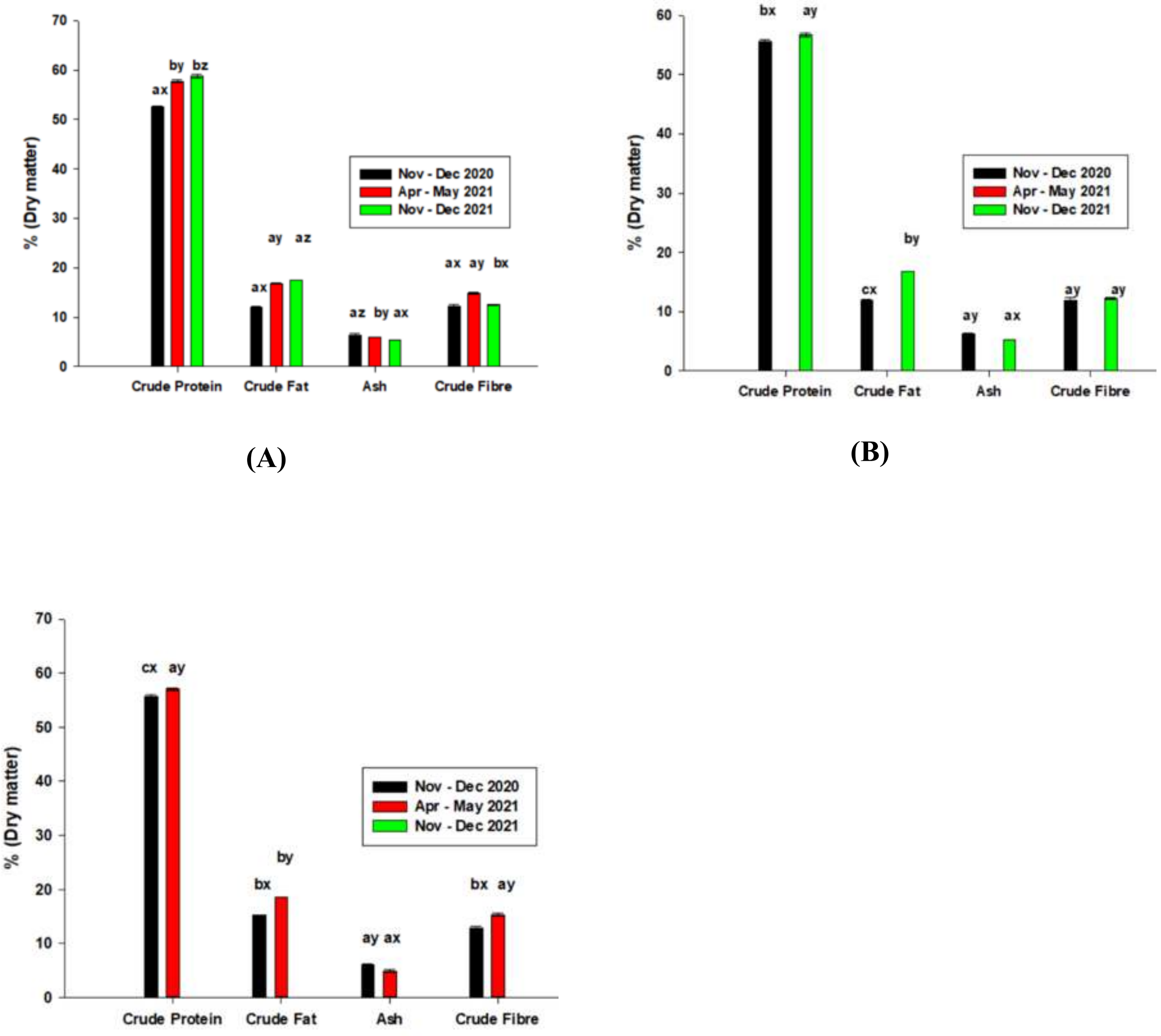
Statistical analysis was done using Sigma plot for windows (Version 12, IBM Corporation, Armonk, New York) software. Variation in nutritional composition of mopane worm between season and geolocation was assessed using One Way ANOVA at 5% level of significance. The Fisher LSD test was performed to separate means. A Bonferroni correction of p values was done, and a family-wise error rate calculated. Shapiro-Wilk test for normality was used. Variation in degutting method on nutritional composition, bioactive properties and microbial and chemical safety of mopane worm was assessed using a student's t-test.

4 Chapter 4: Results

4.1 RQ1: Does season of emergence and geolocation of harvest have an influence on the nutritional composition of mopane worm?

4.1.1 Proximate composition

Generally, proximate composition of mopane worm is significantly (Bonferroni-correction α < 0.002) affected by both season of emergence and geolocation. The proximate composition of mopane worms collected from different emergence seasons and geolocation are shown in Figure 4-1. The family-wise error rate for proximate analysis was 0.65 which then reduced to 0.41 after the calculation of the Bonferroni-correction α (0.002). Mopane worms harvested during the November to December season had the highest crude protein (58.8±0.35 % DM) and ash (6.6±0.2 % DM) whilst those harvested during the April to May season had the highest crude fat (18.6±0.04 % DM) and crude fibre (15.3±0.27% DM). The results also revealed that emergence season significantly (Bonferroni-correction α < 0.002) affected the proximate composition of mopane worm. For all geographic locations, samples from the three different emergence seasons had significantly different levels of crude protein, crude fat and ash. When compared to the November to December 2020 season, mopane worm from the April to May 2021 season had significantly (Bonferroni-correction α < 0.002) higher levels of crude protein, crude fat and crude fibre, as well as lower levels of ash. November to December 2020 season had the highest ash content.



(A)

(B)

(C)

Figure 12: (A – Timber farm, B – River block and C – Insidi): Proximate composition (dry matter) of mopane worm collected from three geolocations.

Values are mean ±SD (n=4). Different superscript letters x, y, z represent significant differences (Bonferroni-correction $\alpha < 0.002$) in the same geolocations showing the effect of season of emergence; whilst superscripts a, b, c represent significant differences (Bonferroni-correction $\alpha < 0.002$) showing the effect of geolocation. For River block and Insidi mopane worms did not emerge in the April to May 2021 and November to December 2021 seasons respectively.

4.1.2 Mineral profile

In general, both season of emergence and geolocation had a significant (Bonferroni-correction $\alpha < 0.002$) influence on mineral composition of mopane worm. Mineral composition of mopane worms from three different locations are shown in Table 4-1. The family-wise error rate for proximate analysis was 0.65 which then reduced to 0.41 after the calculation of the Bonferroni-correction α (0.002). The results show that mopane worm is rich in both micro- and macro elements. The Ca content ranged from 51±0.4-146±0.3 mg/100g DM) and higher values of

146±0.3 mg/100g were from the November to December season. Similarly, the Fe content obtained in this study ranged between 10.6±0.2 – 21.6±0.16 mg/100g and higher values of 21.6±0.16 mg/100g obtained in November to December season. Zinc content ranged from 13.8±0.5 – 17.9±0.4 mg/100g with the highest value of 17.9±0.4 mg/100g from the April to May season.

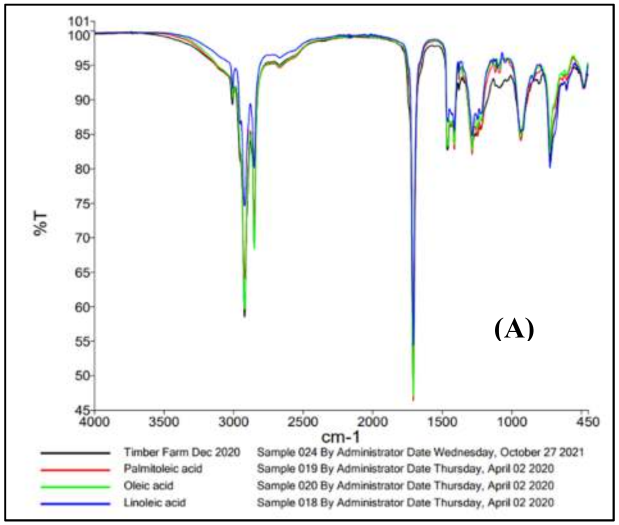
Table 4-1: Mineral (mg/100 g dry matter) composition of mopane worm

Mineral	**Emergence season and Geolocation**								
	Timber Farm			**River Block**			**Insidi**		
	Nov-Dec 2020	Apr-May 2021	Nov-Dec 2021	Nov-Dec 2020	Apr-May 2021	Nov-Dec 2021	Nov-Dec 2020	Apr-May 2021	Nov-Dec 2021
Macro-elements									
Ca	141.1 ± 0.4^{bC}	74.1 ± 0.5^{ay}	146.0 ± 0.3^{cY}	66.9 ± 0.4^{aA}	*	70.1 ± 0.2^{bX}	130.9 ± 0.4^{bB}	51.2 ± 0.4^{ax}	*
Mg	219.9 ± 0.4^{bC}	160.5 ± 0.4^{ax}	225.5 ± 0.3^{cY}	104.6 ± 0.3^{aA}	*	119.6 ± 0.3^{bX}	216.1 ± 0.3^{bB}	208.8 ± 0.9^{ay}	*
K	1684.7 ± 0.3^{bB}	1267 ± 0.2^{ay}	1702.1 ± 0.2^{cX}	1734.9 ± 0.3^{aC}	*	1759.9 ± 0.2^{bY}	1569.0 ± 0.3^{bA}	1195.3 ± 0.4^{ax}	*
Na	51.4 ± 0.2^{bB}	23.1 ± 0.3^{ay}	58.3 ± 0.3^{cX}	80.0 ± 0.2^{aC}	*	88.8 ± 0.2^{bY}	32.0 ± 0.3^{bA}	13.4 ± 0.3^{ax}	*
P	590.1 ± 3.4^{cC}	310.2 ± 3.2^{ax}	561.9 ± 3.6^{bY}	540.0 ± 3.7^{bA}	*	523.0 ± 2.7^{aX}	560.0 ± 3.1^{bB}	490.2 ± 1.0^{ay}	*
Micro-elements									
Fe	19.4 ± 0.5^{bC}	13.3 ± 0.6^{ay}	21.6 ± 0.16^{cY}	15.0 ± 0.5^{aA}	*	16.5 ± 0.2^{bX}	17.2 ± 0.5^{bB}	10.6 ± 0.2^{ax}	*
Zn	13.8 ± 0.5^{aA}	17.9 ± 0.4^{cy}	15.1 ± 0.30^{bX}	15.4 ± 0.4^{aC}	*	17.3 ± 0.2^{bY}	14.3 ± 0.4^{aB}	14.8 ± 0.4^{ax}	*
Mn	3.2 ± 0.3^{bB}	2.9 ± 0.5^{ax}	4.0 ± 0.18^{cX}	4.5 ± 0.3^{aC}	*	5.5 ± 0.3^{bY}	2.8 ± 0.4^{aA}	3.5 ± 0.5^{by}	*
Cu	$<0.01^{aA}$	$<0.01^{ax}$	$<0.01^{aX}$	0.5 ± 0.1^{bB}	*	0.3 ± 0.1^{aY}	$<0.01^{aA}$	0.4 ± 0.3^{by}	*

Values are mean ±SD (n=4). Different superscript letters a, b, c represent significant differences (Bonferroni-correction $\alpha < 0.002$) in the same geolocations showing the effect of season; whilst for the same season superscripts A, B, C; x, y and X, Y for Nov – Dec 2020, Apr – May 2021 and Nov – Dec 2021 seasons respectively represent significant differences (Bonferroni-correction $\alpha < 0.002$) showing the effect of geolocation, * No mopane worm emergence occurred thus no samples were collected.

4.1.3 Fatty acid profile

The results in Figure 4-2 show nine intense peaks at 3010.78, 2922.69, 2853.22, 1708.67, 1463.22, 1431.36, 1411, 1227 and 934 cm-1 in all the nine spectra. Only three fatty acids were present namely, oleic acid (C18:1, ω-9), palmitoleic acid (C16:1, ω-7) and linoleic acid (C18:2, ω-6). Ibarra *et al*. (2015) reported intense peaks for oleic acid on an FTIR spectrum at 2923, 2854, 1708, 1464, 1285 and 936 cm-1 whilst Rahman *et al*. (2014) observed intense peaks at 3010, 2923, 2854, 1708, 1464, 1431, 1412, 1227 and 935 cm-1 for both palmitoleic acid and linoleic acid. The results indicate that the fat in mopane worm is highly unsaturated. More so, the results show that the total number of fatty acids in mopane worm are not affected by either season or geolocation. The only influence could be in terms of their concentrations which was not quantified in this research. From the spectrums in Figure 2: H, it is clear that the peak heights differs significantly, indicating a difference in concentrations. The percentage transmittance at 3011 cm-1 (89.35%), 1431 cm-1 (86.7%) and 1227 cm-1 (85.97%) was high in the November to December 2020 season. Contrary, in the April to May 2021 season the percentage transmittance at 2917 cm-1 (62.99%), 2849 cm-1 (72.68%) and 1463 cm-1 (82.88%) was high. In November to December 2021 season the highest percentage transmittance was at 1706 cm-1, 1411cm-1 and 934cm-1 which was 55.99%, 85.08% and 85.67% respectively. The highest percentage transmittance was high in Timber farm in all the three seasons.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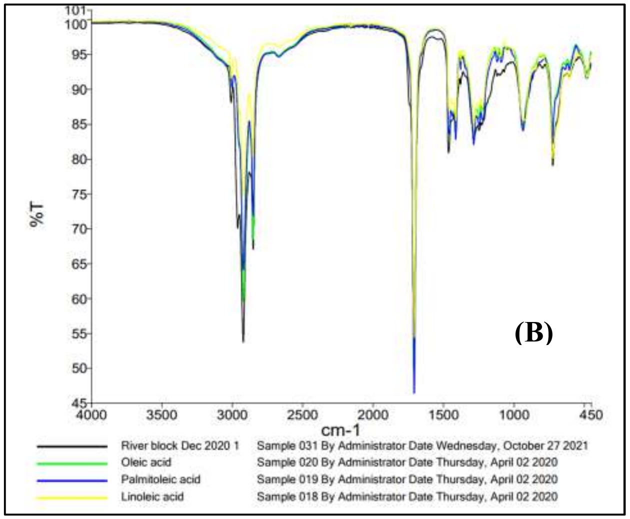
Timber Farm Dec 2020 Sample 024 By Administrator Date Wednesday, October 27 2021
Palmitoleic acid Sample 019 By Administrator Date Thursday, April 02 2020
Oleic acid Sample 020 By Administrator Date Thursday, April 02 2020
Linoleic acid Sample 018 By Administrator Date Thursday, April 02 2020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River block Dec 2020 1 Sample 031 By Administrator Date Wednesday, October 27 2021
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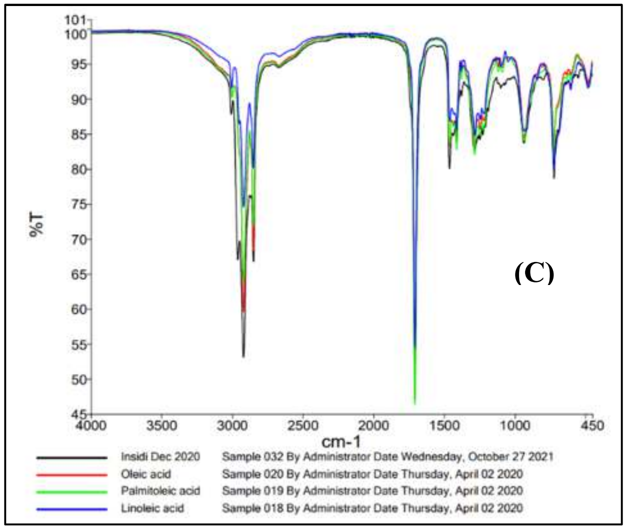
Palmitoleic acid Sample 019 By Administrator Date Thursday, April 02 2020
Linoleic acid Sample 018 By Administrator Date Thursday, April 02 2020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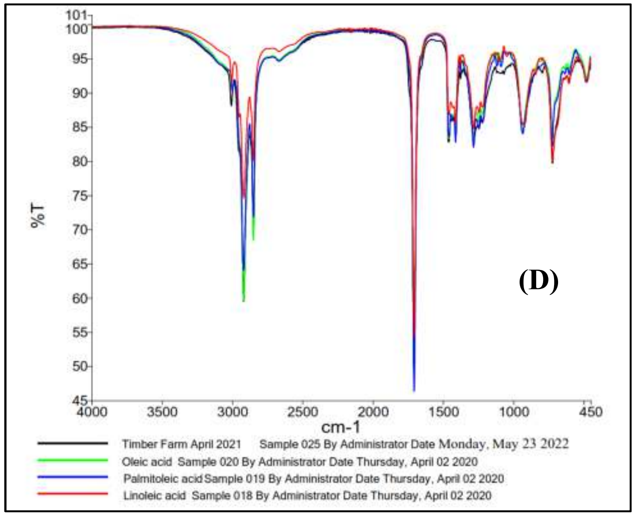
Insidi Dec 2020 Sample 032 By Administrator Date Wednesday, October 27 2021
Oleic acid Sample 020 By Administrator Date Thursday, April 02 2020
Palmitoleic acid Sample 019 By Administrator Date Thursday, April 02 2020
Linoleic acid Sample 018 By Administrator Date Thursday, April 02 2020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Timber Farm April 2021 Sample 025 By Administrator Date Monday, May 23 2022
Oleic acid Sample 020 By Administrator Date Thursday, April 02 2020
Palmitoleic acid Sample 019 By Administrator Date Thursday, April 02 2020
Linoleic acid Sample 018 By Administrator Date Thursday, April 02 2020

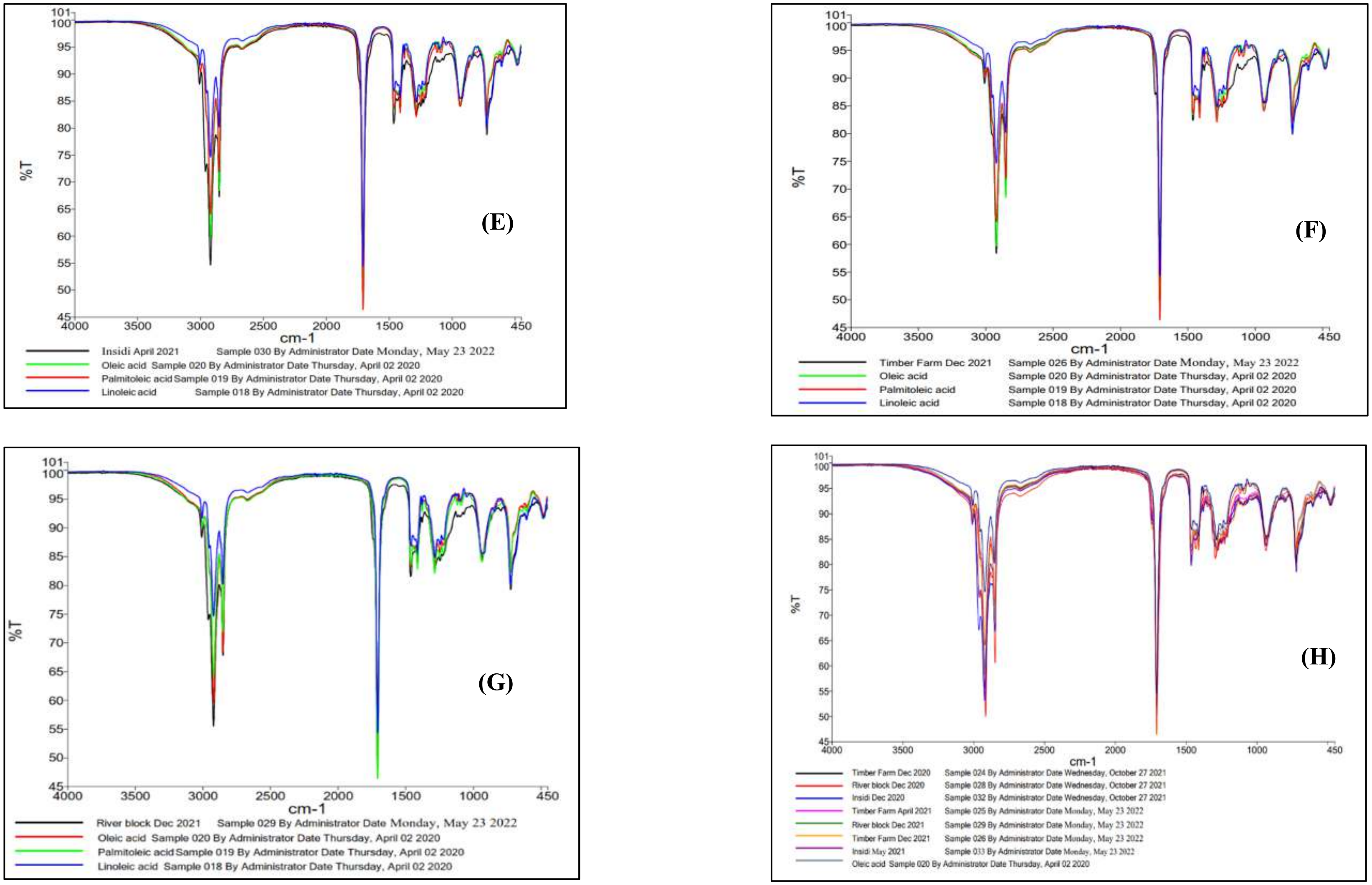

Figure 13: FTIR Spectrums (A – H) for oils from mopane worm obtained from different locations and seasons compared to reference spectrums from fatty acid standards.

4.2 RQ2: What is the influence of degutting method on nutritional, bioactive properties and chemical and microbial safety of mopane worm?

4.2.1 Proximate composition

The proximate composition of naturally and manually degutted mopane worm is depicted in Table 4-2. Crude protein (58.7±0.25 – 59.9±0.18 % DM) is the dominant nutrient in all two degutted mopane worms, followed by crude fat (17.6±0.03 – 19.0±0.05 % DM). Generally, naturally degutted mopane worm samples had the highest crude protein (59.9±0.18 % DM), crude fat (19.0±0.05 % DM) and ash (5.3±0.01 % DM) content whilst manually degutted samples had the highest crude fibre (12.7±0.11 % DM). The results also revealed that method of degutting significantly ($p < 0.05$) affected the proximate composition of mopane worm. Naturally degutted mopane worm samples had a crude protein content 1.2 % higher, a 1.4 % higher fat content and a 0.85% higher ash content than manually degutted samples. A greater difference (2.1%) was in the crude fibre of naturally and manually degutted mopane worms.

Table 4-2: Proximate composition of mopane worm

Degutting method	**% Dry basis**			
	Crude Protein	**Crude Fat**	**Crude Fibre**	**Ash**
Naturally	59.9 ± 0.18^b	19.0 ± 0.05^b	10.6 ± 0.08^a	5.3 ± 0.01^b
Manually	58.7 ± 0.25^a	17.6 ± 0.03^a	12.7 ± 0.11^b	4.5 ± 0.03^a

Values are mean ± SD (n=4). Different superscript letters represent significant differences ($p<0.05$) between the degutting methods.

4.2.2 Total phenolic content and total flavonoids

The total phenolic content of both naturally and manually degutted mopane worm samples are shown in table 4-3. Naturally degutted mopane worm samples had the highest total phenolic content (740.1±2.4 mg GAE/ 100g). Generally the degutting method has a significant ($p<0.05$) influence in the total phenolic content of mopane worm.

The total flavonoid content of both naturally and manually degutted mopane worm is shown in table 4-3. Manually degutted mopane worm had significantly ($p<0.05$) higher (16.8±0.02 mg QE/ 100g) total flavonoids than naturally degutted. This may be attributed to the fact that it is highly likely that some mopane tree leaves are left behind during manual degutting and influence the total flavonoid content of the mopane worm. Thus, the high antioxidant activity in naturally degutted mopane worm maybe attributed to the higher total phenolic content and the protein peptides that act as electron donors and can react with free radicals to transform them into stable compounds.

Table 4-3: Total phenolic content and total flavonoid content of mopane worm

Sample	**Total phenolic content (mg GAE/ 100g)**	**Total flavonoid content (mg QE/ 100g)**
Naturally degutted	$740.1^{b}\pm2.4$	$11.6^{a}\pm0.02$
Manually degutted	$620.0^{a}\pm3.6$	$16.8^{b}\pm0.02$

Values are mean ± standard deviation (n=6); means with different superscripts are significantly different ($p < 0.05$).

4.2.3 Antioxidant properties

DPPH-RS of naturally and manually degutted mopane worm

The IC50 value for manually and naturally degutted mopane worm samples is illustrated in figure 4-3. The IC50 value was lower for naturally degutted samples (49.2±1.39 mg/g). IC50 is the concentration of an antioxidant containing substance required to scavenge 50 % of the initial DPPH radicals. The lower the IC50 value, the more potent is the substance at scavenging DPPH and this implies a higher antioxidant activity. Degutting method significantly affected the antioxidant activity of mopane worm.

ABTS-RS of naturally and manually degutted mopane worm

The $ABTS^{+}$ radical scavenging activity of naturally and manually degutted mopane worm are depicted in figure 4-3. Naturally degutted mopane worm samples had a lower IC50 values (59.1±0.18 mg/g). There was a significant difference ($p < 0.05$) observed between the naturally and manually degutted samples.

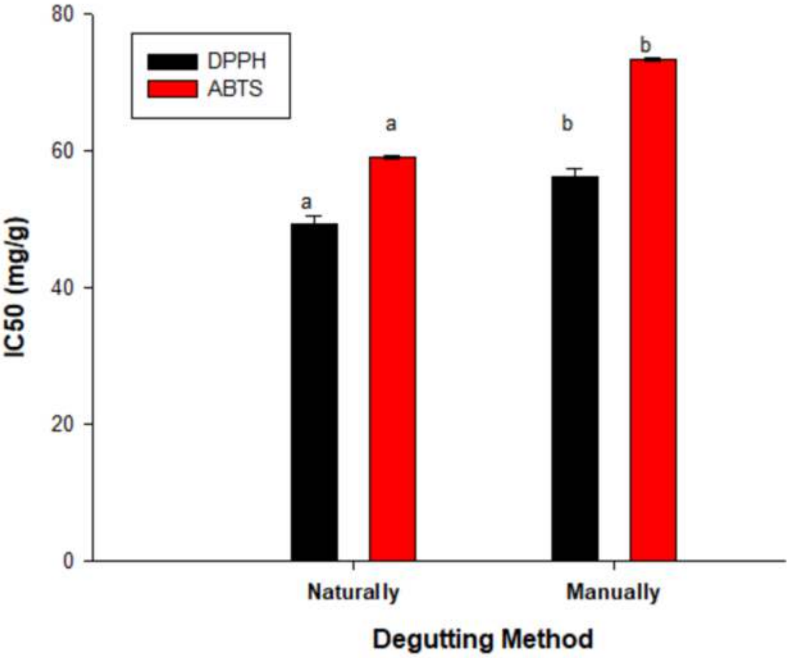

Figure 14: IC50 against DPPH and ABTS of naturally and manually degutted mopane worm.

Values are mean ± standard deviation (n=4); means with different superscripts are significantly different ($p < 0.05$).

Metal Chelation of naturally and manually degutted mopane worm

Results of chelation ability of Fe^{2+} by naturally and manually degutted mopane worm samples are shown in figure 4-4. In this study, the highest chelating ability was observed in naturally degutted mopane worm samples (47.3±0.01 %) than in manually degutted mopane worm samples (43.6±0.01 %).

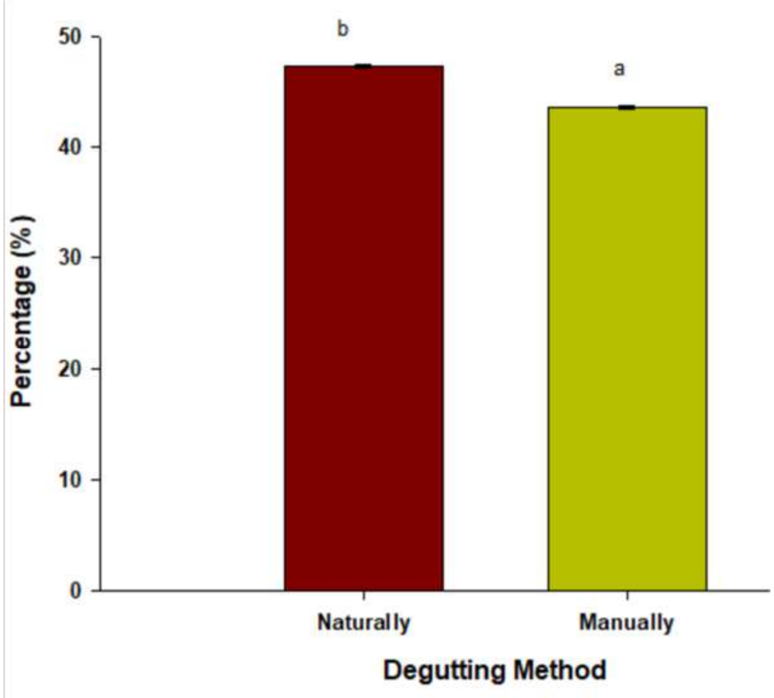

Figure 15: Iron (II) chelation activity of naturally and manually degutted mopane worm.

Values are mean ± standard deviation (n=4); means with different superscripts are significantly different ($p < 0.05$).

Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) of naturally and manually degutted mopane worm

In this study, the ability of naturally and manually degutted mopane worm samples to reduce Fe^{3+} to Fe^{2+} was investigated, and the results are depicted in figure 4-5. Naturally degutted had the highest reducing power (RP) (0.25), while manually degutted had the lowest reducing power (0.21). A significant difference ($p < 0.05$) was observed between naturally degutted and manually degutted mopane worm samples.

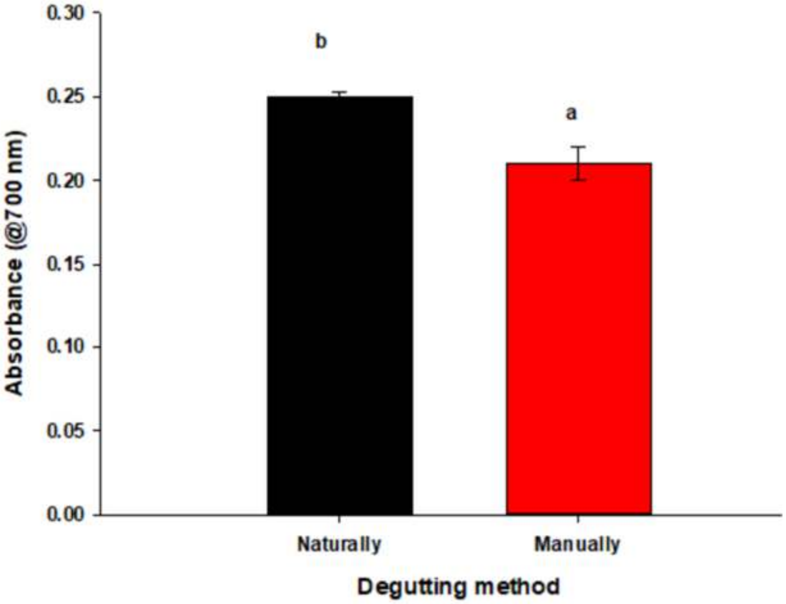

Figure 16: Reducing power activity of naturally and manually degutted mopane worm.

Values are mean ± standard deviation (n=4); means with different superscripts are significantly different ($p < 0.05$).

4.2.4 Microbial and chemical safety

Heavy metal profile

Heavy metal profile of manually and naturally degutted mopane worm are shown in table 4-4. The results show that no heavy metals were detected in both naturally and manually degutted mopane worm.

Table 4-4: Heavy metals (mg/100g dry matter) profile of mopane worm.

Heavy metal	**Degutting Method**	
	Natural	**Manual**
Cd	<0.01[a]	<0.01[a]
Co	<0.01[a]	<0.01[a]
Cr	<0.01[a]	<0.01[a]
Ni	<0.01[a]	<0.01[a]
Pb	<0.01[a]	<0.01[a]
Al	<0.01[a]	<0.01[a]

Values are mean ±SD (n=4). Different superscript letters represent significant differences ($p<0.05$) between the degutting methods. *Limit of detection (LOD) is 0.01mg/g and limit of quantification is 0.033 mg/g.*

The microbial load of both naturally and manually degutted mopane worm is shown in table 4. Degutting method has a significant ($p<0.05$) influence on the microbial load of mopane worms. Naturally degutted mopane worm samples had higher microbial content (TBC, coliforms, *E. coli* and yeasts and moulds) than manually degutted. *Salmonella spp.* and *S.aureus* were not detected in all the samples.

Microbial safety

The microbial load of both naturally and manually degutted mopane worm is shown in table 4-5. Degutting method has a significant ($p<0.05$) influence on the microbial load of mopane worms. Naturally degutted mopane worm samples had higher microbial content (TBC, coliforms, *E. coli* and yeasts and moulds) than manually degutted. *Salmonella spp.* and *S.aureus* were not detected in all the samples.

Table 4-5: Microbial composition of naturally and manually degutted mopane worm

Degutting	**Log (cfu/g)**				**Log (cfu/25g)**	
method	**TBC**	**Coliforms**	***E.coli***	**Yeast & moulds**	***Salmonella spp.***	***S.aureus***
Naturally	7.58 ± 0.04^{b}	4.14 ± 0.07^{b}	2.25 ± 0.04^{b}	6.98 ± 0.03^{b}	ND	ND
Manually	6.36 ± 0.10^{a}	3.31 ± 0.17^{a}	1.54 ± 0.07^{a}	6.15 ± 0.17^{a}	ND	ND

Values are mean ±SD (n=4). Different superscript letters represent significant differences ($p<0.05$) between the degutting methods

5 Chapter 5: Discussion

5.1 Introduction

Mopane worm is a natural resource rich in protein, fats, vitamins, amino acids, and minerals such as zinc and iron. Consumption of this insect is a traditional practice in Zimbabwe. It is consumed for its nutritional value and play an essential role in human nutrition. However, there is a lack of knowledge about the influence of season of emergence, geolocation of harvest and degutting method on the nutritional composition of mopane worm. This research aimed at getting insights into aforementioned factors.

This chapter summaries the major findings and discusses how these findings could contribute to the development of interventions for optimising the mopane worm harvesting for maximum nutrient harnessing accompanied by both chemically and microbiologically safe product. This chapter is organised as follows. First, sections 5.2 and 5.3 presents the major findings for the two research questions.

5.2 RQ1: Does season of emergence and geolocation of harvest have an influence on the nutritional composition of mopane worm?

5.2.1 Proximate composition

The crude protein content obtained from Timber farm in the April to May 2021 season was 5.2 % higher than that from November to December 2020 season whilst it was 1.3 % higher for the same seasons in Insidi Overall, the November to December 2021 season had the highest crude protein content (58.8±0.35 % DM) whilst the November to December 2020 season had the lowest crude protein content (52.5±0.21 % DM). The fat content was 4.7% higher in April to May 2021 season than in November to December 2020 season in Timber farm. Samples collected in the April to May 2021 season had a 3.3 % higher fat content as compared to those collected during the November to December 2020 season in Insidi. Ash content was higher in samples from the November to December 2020 season (6.4±0.2 % DM) as compared to that in samples from the April to May 2021 season (4.9±0.2 % DM). There was a 0.4% difference in the ash content for samples collected from Timber farm. A high difference in ash content (1.1%) was observed in samples collected from Insidi. In Timber farm the crude fibre obtained in April to May 2021 season was 2.6 % higher than that from the November to December 2020 season whilst its was 2.5 % higher in Insidi for the same period.

In general, the highest crude protein (58.8 % DM) was obtained in Timber farm whilst crude fat (18.6 % DM), ash (6.6 % DM) and crude fibre (15.3 % DM) was obtained in Insidi. Geolocation had a significant influence on proximate composition, with samples from River block having low ash (5.3 % DM), crude fat (12.0 % DM) and crude fibre (12.0 % DM) (Bonferroni-correction $\alpha < 0.002$). However, there were no significant differences in ash content of samples from the three geolocations (Bonferroni-correction $\alpha > 0.002$). The only exception was for samples from Timber farm and Insidi collected in the April to May 2021 season which had a significant difference (Bonferroni-correction $\alpha < 0.002$). There were also no significant differences in crude fibre for samples from Timber farm and River block for the November to December 2020 season and those from Timber farm and Insidi for the April to May 2021 season.

Our results are similar to those from previous studies on the effect of geographical location on nutrient content of edible insects (Hlongwane *et al.,* 2022; Madibela *et al.*, 2009; Ssepuuya *et al.*, 2019). Hlongwane *et al.* (2022) reported significant differences in the nutrient composition of *G. belina* collected across four countries namely Zimbabwe, Botswana, Zambia and South Africa. Madibela *et al.* (2009) reported the chemical composition of mopane worm sampled from three sites in Botswana showed significant differences ash content from the three different sites. However, there was no significant differences in crude protein, acid detergent fibre and neutral detergent fibre.

The variations in proximate composition of mopane worm from different harvesting seasons and geolocation may be attributed to the leaf quality of the different seasons and locations. According to Styles and Skinner (1997) mopane tree leaves differ in chemical composition due to differences in environmental factors like rainfall patterns and soil type prevalent in the different specific ecological locations.

5.2.2 Mineral composition

There is a significant (Bonferroni-correction $\alpha < 0.002$) effect of the emergence season on the mineral composition of mopane worm. The mopane worm harvested in the November to December 2021 season had a significantly higher Ca (146.0±0.3 mg/100g), Mg (225.5±0.3 mg/100g), K (1759.9±0.2 mg/100g), Na (88.8±0.2 mg/100g), Mn (5.5±0.3 mg/100g) and Fe (21.6±0.16 mg/100g) whilst P (590.1±3.4 mg/100g) and Cu (0.5±0.1 mg/100g) were higher in the

November to December 2020 season. Only Zn (17.9±0.4 mg/100g) was highest in the April to May 2021 season. Generally, mopane worm harvested in the November to December emergence season had higher mineral content than those from the April to May season except for Zinc. However, comparing the same season over two years (November to December 2020 and 2021) the differences between the mineral values were lower than those between November to December 2020 and April to May 2021.

The highest Ca (146.0±0.3 mg/100g), Fe (21.6±0.16 mg/100g) and Zn (17.9±0.4 mg/100g) content were all from Timber farm samples. The lowest values of Ca (51.2±0.4 mg/100g) and Fe (10.6±0.2 mg/100g) were from Insidi whilst Zn (13.8±0.5 mg/100g) was from Timber farm. Results show significant differences (Bonferroni-correction $\alpha < 0.002$) in the mineral composition of mopane worm harvested in the different geolocations. Mopane worm collected in Timber farm were also high in Mg (225.5±0.3 mg/100g) and P (590.1±3.4 mg/100g) whilst those from River block were high in Na (88.8±0.2 mg/100g), K (1759.9±0.2 mg/100g), Mn (5.5±0.3 mg/100g) and Cu (0.5±0.1 mg/100g). Mopane worms from Timber farm (from all seasons) had no Cu. The highest phosphorus content (590.1±3.4 mg/100g) of mopane worms from all the three geolocations was obtained in Timber farm. These results corroborate findings from previous studies (Glew *et al.*, 1999; Hlongwane *et al.*, 2022; Madibela *et al.*, 2007; Moreki *et al.*, 2012). Madibela *et al.* (2007) reported a Ca content in the range 36 – 46 mg/100g and a Zn content of 10.6 – 11.9 mg/100g in a study on the effect of degutting on chemical composition of mopane worm. Similarly, Hlongwane *et al.* (2022) studied the effect of processing on chemical composition of mopane worm and reported a Zn content of 10.8 mg/100g. Glew *et al.* (1999) and Madibela *et al.* (2007) reported a zinc content 14.2 mg/100g and 14.0 mg/100g respectively for *G. belina* samples from the same country. On the other hand, Fe content of 29.1 mg/100g was previously reported by Hlongwane *et al.* (2022).

The results in this study are in agreement with findings from a study by Hlongwane *et al.* (2022) in which the authors reported a significant effect of geolocation on both Fe and Zn. The mineral profile results obtained can be attributed to the soil type on mineral composition of the vegetation, thereby influencing the nutrition composition of mopane worm (Joy *et al.*, 2015; Lehtovaara *et al.*, 2017).

It has been demonstrated that bio-fortification with Zn fertilizers and enhanced soil fertility management by using mineral and organic nutrient resources, can raise Zn concentration of major grain crops, including maize (Manzeke-Kangara *et al*., 2021). Soil type influences the mineral composition of the vegetation (Joy *et al*., 2015), including the mopane tree leaves on which mopane worms feed, and feed influences the nutrient content of mopane worm (Lehtovaara *et al*., 2017). Its therefore possible that the soils and hence the vegetation in the three geographical areas on which the mopane worm feed differ in mineral composition which translates into differences in mopane worm's mineral composition based on harvesting area. The difference in soil mineral composition is caused by differences in either the type or magnitude of the factors that influence soil nutrient composition, such as parent (rock) material, climate, soil particle size, pH, humus content, aeration, temperature, water content, root surface area and mycorrhizal development among others (Jackson, 2008).

5.2.3 Fatty acid profile

In all the spectra three intense peaks at 3010.78 cm-1, 2922.69 cm-1 and 2853.22 cm-1 are observed that can be attributed to –CHn groups. This confirms the existence of long alkyl group. The 3010.78 cm-1 peak is attributed from the stretching vibration of =C-H (Rohman *et al*., 2014), originating from unsaturated fatty acids in the mopane worm fat. Strong band absorptions were observed in the region of 3000 – 2800 cm-1 caused by corresponding of C-H stretching vibrations (Ramírez-Hernández *et al*., 2019). According to Ramírez-Hernández *et al*. (2019) the stretching vibrations of methylene (-CH2-) and methyl (-CH3) groups can be seen at frequencies of 2922.69 and 2853.22 cm-1 respectively. These methylene (-CH2-) and methyl (-CH3) groups are also observed at 1461.22 and 1412.36 cm-1 due to their bending vibrations. The large peak around 1708.67 cm-1 is due to C=O double bond (COO-) stretching vibrations. Deformation and bending of C-H and stretching vibration of C-O result in peaks in the fingerprint region (1500-450 cm-1) (Ramírez-Hernández *et al*. (2019).

Our results corroborate findings reported in other studies reported in literature. According to Womeni *et al*. (2009), lipids in edible insects have been shown to contain considerable amounts of polyunsaturated essential fatty acids such as linoleic and linolenic acids, which the human body cannot synthesize and should be provided for in the diet. Rumpold and Schluter (2013) reported that about 38% of the fatty acids in mopane worm are saturated whilst 62% are unsaturated, similar to our findings

Overall, the season (April to May 2021) had mopane worm with high crude fat and crude fibre and low values for Ca and Fe content whilst the November to December 2021 season had high values of crude protein and minerals (Ca, Fe and Zn). Contrary, the November to December 2020 season had high values of ash content and low values of Zn. Mopane worm samples obtained from Timber farm had high values of crude protein, Ca, Fe and Zn whilst those from Insidi were high in crude fat, crude fibre and ash content. The highest percentage transmittance for the fatty acids was in Timber farm. Generally, the November to December season had high transmittances when compared to the April to May season. The variations in proximate and mineral content of mopane worm from different harvesting seasons or geolocations as revealed in this study could be due to the differences in feed and soil type. Thus, further research is recommended were both the nutritional content of the mopane leaves and the soil are analysed to evaluate if there is any correlation to the mopane worm nutritional content. More so, it is important to look into how other variables, such as insect age (maturity stage) and reproductive stage may have an impact on the mopane worm nutrient composition.

5.3 RQ2: What is the influence of degutting method on nutritional, bioactive properties and chemical and microbial safety of mopane worm?

5.3.1 Proximate composition

Our findings are greater than the protein content (55.41%) of mopane worm reported by Kwiri *et al*. (2014). In the current study degutting method had a significant effect on the proximate composition of mopane worm. Naturally degutted mopane worm sorely consisted of the 5th instar mature larva whilst the manually degutted counterpart was predominantly composed of the actively feeding 3rd and 4th instar larva. Thus, the differences in the crude protein, crude fat and ash maybe attributed to variation due to the mopane worm growth stages. As the mopane worm mature, some nutrients concentrate in preparation for the pupation stage of its life cycle. The nutritional composition is at its peak at the 5th instar stage. During the pupation stage of its life cycle, the pupae remain dormant for months, sometimes up to seven months, depending on moisture, temperature, and environment conditions. During this period they no longer feed, thus greatly needs a source of nutrients for survival. According to Kwiri *et al*. (2020), mopane worm contains significant amounts of fibre commonly chitin. The high crude fibre in manually degutted samples maybe be attributed to the degutting process. When manually degutting, the gut is forced outside the mopane worm and thus affects the skin to the flesh ratio. This then affects the overall percentage of fibre in the manually degutted samples as opposed to the naturally degutted samples which has their gut still intact. The results of this study is partly in agreement with Madibela *et al*. (2007; 2009) who reported a significant improvement on the concentration of crude protein and acid detergent fibre by degutting mopane worm. Madibela *et al*. (2007; 2009) also reported a low ash content and condensed tannins in degutted mopane worm samples. However, the comparison was with un-degutted mopane worm samples.

5.3.2 Total phenolic content and total flavonoids

Total Phenolic Content of naturally and manually degutted mopane worm

The presence of total phenolic and total flavonoids may be due to the fact that mopane worm feed on mopane tree leaves which contain high levels of these compounds. The high total phenolic content in naturally degutted mopane worm may be attributed to the synergistic interaction between phenols and protein. The high total phenolic content in naturally degutted mopane worm may also be attributed to fact that there are phenolic compounds found within the gut. Since the naturally degutted samples have their gut still intact, thus the phenolic compounds associated with it contributed to the high values obtained. To date the total phenolic content of mopane worm has not been researched. Del Hierro *et al*. (2020) quantified total phenolic compounds of house cricket (*Acheta domesticus*) and mealworm (*T. molitor*) extracts and reported 5000 mg GAE/ 100g and 3800 mg GAE/ 100g. The results obtained in this study ranged from 620±3.6 to 740±2.4 mg GAE/ 100g, thus mopane worm generally contains low quantities of total phenolic compounds. Thus, the antioxidant activity of mopane worm may be attributed to the high protein content since the TPC is low.

Total flavonoids content of manually and naturally degutted mopane worm

Manually degutted mopane worm had significantly ($p<0.05$) higher (16.8±0.02 mg QE/ 100g) total flavonoids than naturally degutted. This may be attributed to the fact that it is highly likely that some mopane tree leaves are left behind during manual degutting and influence the total flavonoid content of the mopane worm. Thus, the high antioxidant activity in naturally degutted mopane worm maybe attributed to the higher total phenolic content and the protein peptides that act as electron donors and can react with free radicals to transform them into stable compounds.

5.3.3 Antioxidant properties

DPPH-RS of naturally and manually degutted mopane worm

The lower IC50 values in naturally degutted mopane worm maybe attributed to the fact that the gut contains some antioxidants and since in manually degutting it is removed thus some antioxidants are lost with it. Moreover, according to Vanga *et al*. (2022) soluble proteins contain peptides that act as electron donors and can react with free radicals to transform them into stable compounds. Thus, since naturally degutted mopane worm has a significantly higher protein content than manually degutted mopane worm therefore a higher antioxidant activity is highly likely in the former than the later. It is therefore highly recommended to harvest mature (naturally degutted) mopane worm since they contain high antioxidant activity.

In a study reported by Vanga *et al*. (2022) on *G. belina* flour, the DPPH-RS was 37.44% and the values are lower than those obtained in this study. The results, therefore, suggest that mopane worm could be scavenging agents and imply that they have the ability to react with free radicals. Thus, it can be safely proposed that edible insects can be used as novel functional components in food compositions.

ABTS-RS of naturally and manually degutted mopane worm

The $ABTS^+$ radical scavenging activity of naturally and manually degutted mopane worm are depicted in figure 3.7.4A. Naturally degutted mopane worm samples had a lower IC50 values (59.1±0.18 mg/g). There was a significant difference ($p < 0.05$) observed between the naturally and manually degutted samples. This may be attributed to the fact that naturally degutted mopane worm had a higher total phenolic content. Most polyphenols work as antioxidants in the body. This may be due to the fact that most of the antioxidants are found within the gut and thus, since during manual degutting process the gut is removed from the larva. Results of this study are in agreement with Vanga *et al.* (2022) who reported an ABTS-RS of 96.6% for *G. belina* flour. It was also observed that both naturally and manually degutted samples showed lower IC50 values against DPPH as compared to ABTS. These observations could be attributed to the difference in scavenging patterns of ABTS- radical scavenging and DPPH-radical scavenging. According to Chalamaiah *et al*. (2012), ABTS is more accessible to hydrophilic peptides, while hydrophobic peptides can interact easily with peroxyl radicals, such as DPPH. Most importantly, to our knowledge, this is the first study to establish the antioxidant indices of these two differently degutted mopane worm samples. This study’s findings have implications for the utilization of mopane worm as functional components in food.

Metal Chelation of naturally and manually degutted mopane worm

The results obtained in this study are comparable to those previously reported by Vanga *et al.* (2022) for *G. belina* flour (44.0 %). Ferrous ion (Fe^{2+}) is the most potent pro-oxidant among metal ions. This ion can interact with hydrogen peroxide in a Fenton reaction to produce the reactive oxygen species and hydroxyl free radical (OH), leading to the initiation and/or acceleration of lipid oxidation in food (Khantaphant *et al*., 2011). Therefore, the ability of these two differently degutted mopane worm samples to chelate Fe^{2+} suggests they can reduce or avoid the free radical formation. The results of this study are vital since they indicate that mopane worm possess considerable metal chelating activity, which is critical in antioxidant activity since it reduces the concentration of transition metals that catalyse lipid oxidation.

Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) of naturally and manually degutted mopane worm

The results in this study are in agreement with what was previously reported by Vanga *et al*. (2022) who got an RP of 0.26 for mopane worm flour. As articulated by Zieli ´nska and Pankiewicz (2020), due to their high protein nature, edible insects are, therefore, potential sources of bioactive proteins that could also possess antioxidant activity. The polyphenolic compounds reported in this study suggest can also contribute to the antioxidant activities of the mopane worms.

5.3.4 Microbial and chemical safety

Heavy metal profile

This may be due to the fact that mopane worm feed on mopane leaves mainly and no study has yet reported on the presence of heavy metals in mopane tree leaves. Contamination of mopane worm with heavy metals commonly is during to post harvesting practices, like in the case of use of empty paint containers as harvesting containers.

Microbial safety

According to Garofalo *et al.* (2019), raw edible insects generally contain high numbers of mesophilic aerobes, bacterial endospores or spore-forming bacteria, Enterobacteriaceae, lactic acid bacteria, psychotropic aerobes, and fungi, and potentially harmful species may be present. The mould content of the mopane worm is worrisome for both naturally and manually degutted, with naturally degutted having higher concentrations. This may be due to the fact that naturally degutted mopane worms are generally collected from the soil when they are about to barrow which maybe the source of contamination. Some moulds are mycotoxigenic specifically those of the genera Aspergillus, Penicillium, and Fusarium (Gashe *et al.,* 1997; Vandeweyer *et al.,* 2018). According to Braide (2012) bacterial populations are generally higher in the gut as compared to the skin thus higher counts of microbes (TBC, coliforms and *E.coli*) are in naturally degutted mopane worms as compared to manually degutted. During degutting process (manually), the gut is pressed out of the mopane worm followed by washing thus removing a significant amount of microflora. Thus, two groups of microbiota should be of interest when assessing the microbial safety of insects that are intrinsically associated with insects and those that are introduced from the environment (rearing, handling). *Salmonella spp* and *S. aureus* were absent in both samples (manually and naturally degutted mopane worm). Pathogenic *E. coli* from the gut of edible insects is rarely likely according to Vandeweyer *et al*. (2021).

Vandeweyer *et al*. (2021) reported that the three main bacterial groups that may pose a risk in insect foods are *Clostridium spp*. (particularly *C. perfingens* and *C. botulinum*), *Bacillus cereus* (*B. cereus sensu lato* (s.l.)), and the non-spore forming species *Staphylococcus aureus*. However, other classical foodborne pathogens such as *Salmonella spp*., *Campylobacter spp*., *Listeria monocytogenes*, or pathogenic *Escherichia coli* are rarely detected, whereas particularly spore-forming bacteria with pathogenic potential such as species of the *Bacillus cereus* group or *Clostridium spp*. may pose a food safety risk. Therefore, the importance of food processing techniques must be emphasized to ensure that the vegetative cells of pathogens are safely inactivated without promoting the growth of spore formers. Preventive treatments (e.g., blanching, microwave drying, boiling, etc.) that reduce the load of this bacterial community are recommended when using insect powder as a food ingredient (Osimani *et al*., 2018).

This study examined the contamination dynamics of the two different degutting methods of mopane worm, and has shed some light on the potential variations in contamination when similar processes are applied for different insect species. However, there is need of an in-depth study regarding the pathogenicity of *E.coli spp*. found in the gut of mopane worm and its possible source, whether its naturally part of the gut microbiota or its contamination and also the presence of mycotoxin producing moulds and finally quantification of the mycotoxins present.

5.4 Main research findings

For the health diets to be possible, it's crucial to comprehend how the emergence season, harvest site, and pre-processing technique affect the nutritional, bioactive and safety aspects of mopane worm. A study to ascertain the degree to which season and geolocation affect the nutritional composition of mopane worm was conducted (**Research Question 1**). Overall, the results indicate that mopane worm is high in crude protein (52.5 – 58.8 %), fat (12.0 – 18.6%), iron (10.6 – 21.6 mg/100g), zinc (13.8 – 17.9 mg/100g) and calcium (51.0 – 146.0 mg/100g). The findings show that the nutritional composition of mopane worm was significantly impacted by the emergence season and the harvesting site. Given the close relationship between the mopane worm larvae, mopane trees, and rainfall, climate change and weather variability will probably have an impact on the nutritional quality of mopane worms. The leaf quality of the various harvesting seasons and geolocations may be responsible for the changes in the mopane worm's proximate composition. Due to variations in environmental elements like rainfall patterns and soil types present in the several distinct biological areas, mopane tree leaves probably have varying chemical compositions. Importantly, the study revealed that the concentration of fatty acids in the mopane worm fat is also significantly affected by both season and location of harvest. Wild harvested edible insects spontaneously occur without any control over the environment or their feed. The degree to which the nutrient content is comparable for the same insect species taken from different geographic regions and seasons should therefore be established before making broad recommendations to employ wild harvested insects.

A desire to assess how the degutting approach affects the bioactive and safety (chemical and microbiological) features of mopane worms was sparked by revelations from the sampling process (pre-processing techniques) in this study (**Research question 2)**. Generally, edible insects undergo a pre-processing stage before they are processed for consumption. However, studies on the effect of pre-processing methods on nutritional, bioactive and safety aspects of edible insects are limited. The study revealed that naturally degutted mopane worm had high crude protein (59.9±0.18 %) and crude fat (19.0±0.05%) and no heavy metals were detected for all the mopane worm samples. The total phenolic content (740±2.4 mg GAE/100g) was also high in naturally degutted mopane worm whilst the total flavonoids (16.8±0.02 QE/100g) was high in manually degutted samples. The high total flavonoids in manually degutted samples could be attributed to the mopane leaf frass that is left during the process. Interestingly, the edible insect exhibited a strong antioxidant activity. The IC50 values against DPPH (49.2±1.39 mg/g) and ABTS (59.1±0.18 mg/g) radicals were low for naturally degutted mopane worm suggesting a higher antioxidant activity. The antioxidant activity against Fe^{2+} metal chelating (56.6±0.01%) and PFRAP (0.25±0.01) was high in naturally degutted mopane worm samples. Edible insects appear to be a promising source of food bioactives such as minerals, polyunsaturated fatty acids, fibre and phenolic compounds. Thus, they may be able to provide a wide range of food supplements and functional food ingredients for specific purposes. In general, the results showed that naturally degutted mopane worms are a better source of nutrients and bioactive compounds than manually degutted. As a matter of concern, the microbial safety of naturally degutted mopane worm was high for total bacterial count (7.58±0.04), coliforms (4.14±0.07), *E.coli* (2.25±0.04) and yeasts and moulds (6.98±0.03 log cfu/g). Most importantly *salmonella* and *S.aureus spp* were not detected. However, despite pre-processing having a detrimental impact on the nutritional composition of the edible insect, based on the required daily limits and the antioxidant activity level, it still remains a useful source of nutrients, suggesting its great potential to contribute towards nutrition security.

Table 5-1: Summary of research key findings

Chapter	**Objective**	**Main findings**
2	To assess the effect of emergence season (bivoltine) on the nutritional composition of mopane worms. To determine the effect of geographic location of harvest on the nutritional composition of mopane worms.	• Mopane worm is high in crude protein (52.5±0.21 – 58.8±0.35 %), fat (12.0±0.06 – 18.6±0.04 %), iron (10.6±0.2 – 21.6±0.16 mg/100g), zinc (13.8±0.5 – 17.9±0.4 mg/100g) and calcium (51.0±0.4 – 146.0±0.3 mg/100g) • Nutritional composition of mopane worm is significantly influenced by the emergence season and the harvesting site.
3	To assess the effect of degutting method on nutritional, bioactive and microbial and chemical aspects of mopane worm.	• Naturally degutted mopane worm had high crude protein (59.9±0.18 %) and fat (19.0±0.05 %).

- No heavy metals were detected for all the mopane worm samples
- The total phenolic content (740±2.4 mg GAE/100g) was also high in naturally degutted mopane worm whilst the total flavonoids (16.8±0.02 QE/100g) was high in manually degutted samples.
- The edible insect exhibited a strong antioxidant activity with naturally degutted samples having higher values

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5.5 Overall discussion

The challenges in eradicating hunger, food insecurity, and all forms of malnutrition continue to increase. In light of the severity of malnutrition and micronutrient deficiencies, combating undernutrition is essential for human wellbeing and development. Nutritious foods like edible insects can help diversify diets and boost nutrition. However, substantial and sustained consumption of nutrient rich foods is needed to attain nutrition security. The actual contribution of edible insects to nutrition largely depends on the availability of technical information about their nutritional composition and factors that influence them. Therefore, this thesis considered the factors that influence nutritional variation, which ranges from season of emergence, geographical location of harvest and pre-processing technique. This research focused on the influence of rainfall patterns, soil type, feed, and climate (all encompassed in a season or a particular location). Knowledge on these key aspects could be used for transforming the major global future food (edible insects) for a healthy and sustainable diets. Although uncommon in developed countries, malnutrition in children remains a scourge in many developing countries yet it is in these countries that edible insects are found in abundance. Thus, this thesis is important for food sovereignty in African countries since they have locally based solutions to malnutrition.

5.6 Methodological considerations

This thesis is based on a study conducted in Gwanda district, Zimbabwe, and generalisations are made on the use of edible insects for nutrition security in developing countries. However, it is important to note that each country has its own species of wild harvested edible insects, and also different climatic conditions in different regions of the same country. Although, there do exist some similarities in weather patterns and insect species.

The use of a generalized 6.25 factor has been previously reported to overestimate the crude protein content in insects. In this thesis, 5.6 nitrogen to crude protein conversion factor was used to calculate the crude protein content (Boulos *et al.,* 2020).

6 Chapter 6: Conclusion and Recommendations

RQ1: Does season of emergence and geolocation of harvest have an influence on the nutritional composition of mopane worm?

The results from the study revealed that the nutritional composition of mopane worm is affected by the season of emergence and geolocation, with the April to May season having the highest fat, and crude fibre content whilst the November to December season has the highest crude protein and ash content. More so, our study showed that the mineral profile of mopane worm is significantly affected by geolocation and emergence season with the November to December season having the highest values of Ca, Mg, K, Na, Zn, Fe and P. The April to May season had higher values of Zn, Mn and Cu. Mopane worm fat was highly unsaturated with oleic (ω-9), linoleic (ω-6) and palmitoleic (ω-7) the only fatty acids present. Emergence season had no significant effect on the number and type of fatty acid present. The value of the findings is that the new information obtained in this study can be applied to research on rearing of mopane worm since it would be beneficial to recommend the harvesting of mopane worm in certain seasons of emergence to leverage their different advantages in terms of concentration of specific nutrients. Alternatively, the study further demonstrated that the nutritional composition of mopane worm can vary with geographical location, which suggests the need to identify and map geographic areas that are superior sources of highly nutritive mopane worms.

RQ2: What is the influence of degutting method on nutritional, bioactive properties and chemical and microbial safety of mopane worm?

Natural degutting improves the nutrient content and antioxidant activity of mopane worm. Manual degutting on the other hand results in nutrient losses. Microbial load of mopane worm reduces when manually degutted. The research showed that mopane worms can be employed as a novel food additive, an alternative protein source, and a strong source of antioxidants. The advantage of harvesting mature mopane worms (naturally degutted) is that high amounts of minerals and antioxidants are guaranteed, but there is a danger in as far as microbial safety is concerned.

6.1 General conclusion

Mopane worms just like all other herbivores are affected by the quality of feed they consume. The value of the findings is that the new information obtained in this study can be applied to research on rearing of mopane worm since it would be beneficial to recommend the harvesting of mopane worm in certain seasons of emergence to leverage their different advantages in terms of concentration of specific nutrients. Alternatively, the study further demonstrated that the nutritional composition of mopane worm can vary with geographical location, which suggests the need to identify and map geographic areas that are superior sources of highly nutritive mopane worms. Furthermore, the research showed that mopane worms can be employed as a novel food additive, an alternative protein source, and a strong source of antioxidants. The advantage of harvesting mature mopane worms (naturally degutted) is that high amounts of minerals and antioxidants are guaranteed, but there is a significant danger to the safety of the microbes. Thus, large scale production of insect-based foods may help solve the looming global food insecurity problem and contribute to accomplishing the sustainable development goals set by United Nations.

6.2 Recommendations

The following are recommendations for further research based on the outcomes of the MPhil research.

a) In this study, we were able to show that season of emergence and geolocation of harvest influence the nutritional composition of mopane worm. This thesis did not investigate the possible influence of insect's maturity stage, soil type, and feed on the nutritional composition of mopane worm.

b) In order to preserve wild edible insect populations and avoid overexploitation, with the number of collected insects exceeding the capacity for regeneration capacity, the use of edible insects as a sustainable food source requires large-scale insect farming.

c) This study focused on fatty acid profiling of the fat present in mopane worm and amino acid profiling was not done. Therefore, further investigations are needed to elaborate in more detail the fatty acid and amino acid composition as influenced by season and location.

d) This thesis explored the total phenolic content of mopane worm. Further research is needed to gain insight on the different types of polyphenols by profiling using HPLCs or GC-MS or LC-MS.

e) This study also pinned on the following microbes: coliforms*, E.coli*, *salmonella*, *S.aureus*, yeasts and moulds and total bacterial count. The yeast and molds counts in mopane worm were worrisome, therefore it is highly likely that mycotoxin producing fingi (moulds) (Fusarium, Aspergillus and Penicillium) might be present. Thus, to further ascertain the microbial safety of edible insects, research is needed to gain insight in the pathogenicity of *E.coli* present and identification of the moulds and quantification of mycotoxins.

7 Chapter 7: References

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LIST OF PUBLICATIONS

1. **Madimutsa, O. N.**, Manditsera, F. A, Ruzengwe Matiza, F., Mubaiwa, J and Macheka, L. (2023). Effects of seasonal variation and geographical location on the nutritional composition of mopane worm (*Gonimbrasia belina*). (Accepted in Journal of Insects as Food and Feed). JIFF-2022-0157R1
2. Ruzengwe, F. M., Manditsera, F. A., **Madimutsa, O. N**., Macheka, L., Kembo, G., Fiore, A.,Ledbetter, M and Mubaiwa, J. (2023). Optimising mopane worm (Gonimbrasia belina)

processing for improved nutritional and microbial quality. *Journal of Insects as Food and Feed*, 1-12. https://doi.org/10.3920/JIFF2022.0046

3. **Madimutsa, O. N**., Mubaiwa, J., Ruzengwe Matiza, F., Manditsera, F. A and Macheka, L. (2023). Effect of degutting method on the antioxidant properties, chemical and microbial safety of *Gonimbrasia belina*. (To be submitted to Frontiers)
4. Bangira, C., **Madimutsa, O. N**., Manditsera, F. A., Bara, B., Sithole, R., Mubaiwa, J and Macheka, L. Influence of Soil Properties on Pre-pupal Burrowing, Pupation and Nutritional Quality of *Gonimbrasia belina* Westwood. (Submitted to International Journal of Tropical Insect Science)