IMPROVING ATTRACTIVENESS OF AN INSECT PEST THROUGH VALUE-ADDITION: A POSSIBLE INSECT MANAGEMENT STRATEGY

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MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE IN AGRICULTURE (PLANT PROTECTION), IN THE DEPARTMENT OF SOIL SCIENCE, PLANT PRODUCTION AND AGRICULTURAL ENGINEERING, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA.

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DEDICATION

This study is dedicated to Jesus Glad Tidings International Church.

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First and foremost, I like to thank the Almighty God for the courage, strength, wisdom and guidance throughout my years at the University of Limpopo and through completing this arduous task. May His Name be praised, now and forever. Amen.

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Plant Protection), has not previously been submitted by me or anybody for a degree at this or any other University; that it is my work in design and in execution and that all materials contained herein had been duly acknowledged.

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ABSTRACT

Attractiveness of insect pest for use as sources of food may be improved by providing information on preservation and relevant nutritional value. Nutritional composition in edible insects may depend on drying method and/or vegetation (location). Influence of drying method and location on nutritional composition of the African metallic wood boring beetle (*Sternocera orissa),* widely consumed in certain rural communities of Limpopo Province, South Africa, was investigated. Randomised complete block design in a 3 x 3 factorial arrangement was used with three drying methods (oven-drying, freeze drying, cooking method) and three locations (Khureng, Magatle, Ga-Masemola), with three replicates. Nutritional composition data were subjected to a two-way analysis of variance (ANOVA) and means were separated using Turkey Honestly Significant Differences (HSD) at 5 % level of significance. Relative to freeze-drying, oven-drying and cooking methods increased protein, carbohydrates, fat, energy, ash and dry matter content with the exception of cooking method, which decreased the moisture content. Compared to other locations, Ga-Masemola significantly increased fat and energy of the test beetle. Relative to the freeze-drying method, oven-drying and cooking increased ($P \leq 0.05$) essential and non-essential amino acids. Location did not have significant effect on the essential and non-essential amino acids of *S. orissa* across all the villages. Similarly, oven-drying and cooking increased K, P, Fe, Zn and Mg. Compared to locations, Ga-Masemola increased ($P \leq 0.05$) Fe of the test beetle. Results of the study suggested that oven-drying and cooking methods improved the nutritional composition of *S. orissa*, which has the potential of enhancing nutrition in marginal rural communities of Limpopo Province. Providing results of this study to rural communities through extension services has the potential of improving the attractiveness of this beetle to marginal communities, and thus, increasing harvesting and therefore, reduce insects population densities.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

The advent of synthetic pesticides resulted in most indigenous pest management tactics disappearing due to neglect or simply because they were viewed as being barbaric. However, due to the environment-unfriendliness of synthetic pesticides (Mashela, 2002), most indigenous pest management tactics are being revisited. Some of the tactics include collection of pest for consumption, which resulted in the pest population decreasing to below the damage threshold densities.

Entomophagy, the collection and consumption of insects had long been known, especially in regions where environmental conditions were adverse to have a dramatic effect in lowering the population densities of insects (Bodenheimer, 1951; Conconi and Pino, 1979; Hoffman, 1947; Lapp and Rohmer, 1937; Ruddle, 1973; Skinner, 1910; Thompson, 1954). In some parts of the world, insects which have pest status on crops are a major food source and are collected during their feeding stages, dried and stored in large quantities for consumption when food is scarce. Ordinarily, 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 (DeFoliart, 1992).

Worldwide, more than 1000 species of insects have been reported as traditional foods by human that are either at any stage of their life cycle and have been an important part of nutritional intake and also serve as an economic value in both pest management and household economies (Illgner and Nel, 2000). Raksakantong *et al*. (2009) reported a number of edible insect species in Africa, Asia, America and Australia, with South Africa, Southeast Asia and North America having the highest registers.

In South Africa, the collection and eating of insects is more prominent in the warmer provinces such as Mpumalanga, North West, Limpopo and KwaZulu Natal. Insects provide a good source of proteins, minerals, vitamins and energy, but also cost less than animal protein in marginal communities, where their consumption have averted many cases of malnutrition (Chavunduka, 1975). The most consumed insects are grasshoppers, termites, mopani worms, stink-bugs and African metallic wood-boring (*Sternocera orissa*) Buquet (Toms and Nonaka, 2005). These insects are consumed either at larval or adult stage. Grasshoppers, termites, beetles and crickets are collected and consumed in the adult stage, whereas the lepidopterans and other orders are mostly collected and consumed at the larval stages (Onore, 1997).

The buprestid (*S. orissa)* adult beetle belongs to the Order Coleoptera, Family Buprestidae and is known as the African wood-boring beetle because of its metallic or bronzed appearance (Skaife and Ledger, 1979). The buprestid (*S. orissa)* is about 3.5 - 4.0 cm long and weighs about 2.6 g. In South Africa, this beetle is a browser of economic and uneconomic trees and is preferred by the Pedi - and Tsonga - speaking people in Limpopo Province. Among the Tsonga speaking people, *S. orissa* is known as as *Shitambela*, whereas the Pedis call it *lebitsi-kgoma.* Indigenous host plants include pendoring (*Gymnosporia senegalensis*) and witdoring (*Acacia campylacantha*) in South Africa, which are widely used by browsers. Adults are harvested using hands in the early morning, when beetles are still lethargic and are roasted or fried after first removing the elytra (Junod, 1913) and are said to be more delicious than the mopani worms.

Buprestid beetles are heat-loving (thermophilous) and can tolerate very high temperatures. Thus, they are active in hot and sunny conditions, where they are very alert, making it difficult to catch them. Adults feed on pollen, foliage and nectar and can cause enormous damage on crops. Females lay eggs in bark crevices and larvae tunnel into wood and plant stems. Adults are relatively short-lived, whereas the immature stages can take as long as 35 years to complete their development (Chunram, 1974). Also, it has been reported that *S.*

orissa is manly in Namibia, Botswana, South Africa, Zambia and Mozambique. Quin (1959) reported that there is one generation per year. Adult females are bigger than their male counterparts and are harvested carrying packets of eggs inside their abdomen during mid December to late January.

1.2 Problem statement

Improving attractiveness of edible insect pests may improve food security and lower the insect population levels in marginal communities of Limpopo Province. The research proposes to investigate ways and means of improving the attractiveness of one economic insect pest, *S. orissa,* for collection and consumption among the local communities.

1.3 Motivation

The buprestid, *S. orissa*, is an important edible insect in Limpopo Province where it is mainly harvested for consumption over a short period (< than a month). The preservation of this univoltine, delicious insect has an opportunity to contribute towards increased harvesting of the insects and therefore reduce control cost and malnutrition experienced in marginal communities of Limpopo Province.

1.4 Aim and objectives

1.4.1 Aim

The aim of the study was to make *S. orissa* insect pest more attractive for harvesting and consumption among the marginal communities in Limpopo Province in order to reduce its population pest densities to non-pest status.

1.4.2 Objectives

Objective 1: To determine whether different drying methods and location will have an effect on proximate chemical analysis of *S. orissa.*

Objective 2: To determine whether different drying methods and location will have an effect on amino acids and essential mineral element composition of *S. orissa.*

1.5 Hypotheses

Hypothesis 1: Drying methods and location do not influence proximate chemical analysis of *S. orissa.*

Hypothesis 2: Drying methods and location do not influence amino acids and essential mineral element composition of *S. orissa*.

1.6 Format of mini-dissertation

Following this General Introduction, a Chapter on Literature Review ensues; with each of the subsequent chapters addressing each of the above hypotheses in sequence (Chapters 3 – 4). Findings in all chapters are then summarised and integrated through providing the significance of the findings, recommendations with respect to future research and the conclusion.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Due to global warming, high population densities of insect pests are being expected, whereas strategies and tactics to manage them are limited due to the withdrawal of many pesticides. Insect population densities were traditionally reduced through harvesting and consumption. However, these tactics are no longer attractive since there is scant information on the nutritional value of these insects and how best they can be preserved. The ensuing literature review focuses on the work done, work not done and existing gaps on the nutrition of insects.

2.2 Socio-economic aspect of insects

2.2.1 Insects as human food

A number of insects or their products have been used as food in some parts of East Africa (Ene, 1963), and are good sources of protein, minerals and vitamins (Defoliart, 1992). Most edible insects have a higher energy value than soybeans, maize, beef, fish, lentils, or other beans (İncekara and Türkez, 2009). In Central Africa, the people of the Democratic Republic of Congo eat caterpillars belonging to a few dozen species which have high calorific value with the protein content ranging from 45 to 80 %, and also are rich source of iron.

In Southern Africa, there are two edible insects that have caught researchers' attention, namely mopani worm and the edible stink-bug (Teffo *et al.*, 2007). Mopani worm is an important food for many people and is also used as a cash insect among the rural poor (Madibela *et al*., 2008). The first harvest of the first generation is done around mid December, whereas the last harvest takes place mid-April. Mopani worms are widely consumed in South Africa, Botswana, Zimbabwe and Zambia as they are preferred for their high

protein content. The worms are exported and imported from other parts of Southern Africa such as Botswana, Zimbabwe and Zambia (DeFoliart, 1995; Dreyer and Wehmeyer, 1982; Greyling and Potgieter, 2004; Moruakgomo, 1996). According to Ruddle (1973), the Pedi people of South Africa call mopani worms Masonja and prefer it to beef. It has been reported that when these worms are available, they seriously affect the sale of beef in the northern parts of South Africa.

The stink-bug is highly consumed in Venda areas where it is known as *Thongolifha* and is also used to cure hangovers. The insect is also imported from Zimbabwe and Mozambique to supply the Thoyandou market in South Africa (Dzerefos, 2005). In the Philippines, June beetles (*Melolonthine scarabs*), weaver ants (*Oecophylla smaragdina*), mole cricket and locusts are eaten in some regions. Locusts form an important dietary supplement during breaks, which apparently have become less common since the wide spread of insecticides (Gullan and Cranston, 1986).

In Australia, the aborigines use a widerange of insect foods, especially moth larvae. The larvae of wood or ghost moth are regarded as a delicacy and they contain 7 – 9 % protein, 14 – 38 % fat and 7 – 16 % sugars as well as being good sources of iron and calcium. Adults of the bong moth, *Agrotis infusa*, once formed another famous Aboriginal food, being collected in their millions from aestivating sites in narrow caves and crevices on mountain summits in south-eastern Australia (Gullan and Cranston, 1986).

2.2.2 Insects as animal food

The nutritive value of insects as feed for fish, poultry, pigs and farm-grown mink certainly is recognized in China, where feeding trials have shown insect-derived diets can be cost-effective alternatives to more conventional fish meal diets (Gullan and Cranston, 1986). The insects involved are primarily the larvae and pupae of house flies (*Musa domestica*), the pupae of silkworms (*Bombyx mori*) and the larvae of

mealworms (*Tenebrio molitor*) (Loomis *et al*., 1980). In India, poultry are fed the meal that remains after the oil has been extracted from the pupae of these insects (Gullan and Cranston, 1986). The house fly larvae fed to chickens can also recycle animal manure (Amano, 1985). Teotia and Miller (1973) reported that there is no adverse effect of the birds fed with pupal diet on carcass quality and taste. The studies conducted on nutritional potential of acridids indicated that acridids tissue contains more good quality protein than the commercially used animal protein sources in poultry feed.

Insects have been used as food for zoo animals. Pennino *et al*. (1991) reported mealworms both small (*Tenebrio molitor*) and large (*Xophobas morio*), waxworms (*Galleris mellonella*) and crickets (*Acheta domesticus*) as the most insects commonly fed to zoo animals. Dierenfeld (1993) points out that insects are a substantial part of, or the entire diet fed to numerous species of amphibians, reptiles, birds and mammals in zoological parks. Dierenfeld *et al.* (1995) reported that retinol and carotenoid analysis of 10 invertebrates commonly fed in zoos demonstrated low concentrations of vitamin A activity. A variety of carotenoids found particularly in free-ranging invertebrates consuming natural diets implies that insectivores may rely on carotenoid pigments as vitamin A precursors.

2.2.3 Insects used in medicines

Entemotherapy, which is the use of insects derived product as medicinal system and throughout the world insects have been used directly or indirectly as medicine (Costa-Neto, 2002; Teffo *et al*., 2007). According to Costa-Neto (2002), insects are being used live, cooked, ground, in infusions, in plasters and as ointments both to cure and prevent ailments and as well as in religious rituals. Ayieko and Oriaro (2008) were of the opinion that certain insects are held in high esteem sorely for witchcraft and medicinal values.

Insects have been reported to possess medicinal properties that help in the prevention and cure of different ailments; managing physical conditions such as pregnancy including improving libido (Van Huis, 2002). There are species which are particularly rich in calcium (e.g. *Tangoropsis flavinata*), protein (e.g. *Imbrasia epimethea*, *Imbrasia dione*, *Antheua insignata*) or iron (e.g. *Cinabra hyperbius*) which are given to people suffering from anaemia, or given to pregnant and breastfeeding women (Vantomme *et al*., 2004).

At least 42 insects in north-eastern Brazil of Bahia State have been reported as used in folk medicine (Costa-Neto, 2002) and in India, the use of folk medicine is also common (Hitchcock, 1962). Again insects have proven to be promising sources of drugs for modern medicine since they are found to possess immunological, analgesic, antibacterial, diuretic and antirheumatic properties (Yamakawa, 1998). A lot of folk remedies are administered in the form of teas made using the powder produced by grinding the toased or scraped part of the body of the insects. Herbalists from Feira de Santana, for example, recommended the use of tea that was made from the toasted exoskeleton of a grasshopper (*Tropidacris* sp.) to cure skin diseases and, most importantly, people suffering from stroke (Costa-Neto, 2002).

Costa-Neto (2002) found that in Feira de Santana, the ant is added to sugar and it is used in their daily life to sweeten their coffee or juices in order to have good sight, and the American cockroach is cooked and its tea is drunk to treat heartburn and, in addition, people from Matinha dos Pretos put the exoskeleton of a cochroach over wounds to promote their scarring. Sting-bug (*Tritoma* sp.) is also used to treat toothache and earache (Costa-Neto, 1994). Anticancer and anti-HIV properties were found in the ethanolic extracts of propolis of *Apis mellifera* collected from different parts of Brazil (Park *et al*., 2000).

2.2.4 Chemical composition of insects

Teffo *et al*. (2007) provided the preliminary data on the nutritional composition of the edible sting-bug that is largely consumed by the Venda people in Limpopo Province of South Africa. Their article reported the first nutritional information on this insect. Dried bugs consist of 35 % protein, 51 % fat, with an energy content of 2600 kJ/100 g. Amino-acid concentrations varied from 0.82 mg/100 g (for threonine) to 1.32 mg/100 g (valine). Mineral content was 1.2 g/100 g. Stink-bugs are, therefore, a good source of proteins, fats, amino acids, minerals and vitamins.

Omotoso and Adedire (2007) analysed nutrient composition, mineral content and the solubility of the protein of adult and larva stages of palm weevil (*Rhynchophorus phoenicis*). The early larva stage contained the highest moisture content of 11.94 %, while the adult had the least value of 4.79 %. The late larva stage had the highest protein content of 10.51 %, while adult contained 8.43 %. Ash content was high in early larva with a value of 2.37 % and lowest in adult with a value of 1.43 %. Early and adult stage contained, respectively, the highest (22.14 %) and lowest (17.22 %) fibre contents. The values of potassium, magnesium and iron in early larva stage were (455.00 ± 21.21), (60.69 ± 2.57) and (6.50 ± 3.40) mg/kg, respectively, while late larva stage recorded (457.50 ± 10.61), (43.52 ± 1.37) and (6.00 ± 1.10) mg/kg, respectively, and adult stage recorded (372.50 ± 24.75), (53.31 ± 1.88) and (22.90 ± 3.70) mg/kg. Chromium, phosphorus, nickel, calcium, lead, manganese and zinc were also detected. Copper was not detected in any of the samples.

Protein deficiency is a state of malnutrition in which insufficient amounts of protein is taken in for the body to utilize in order to produce energy. To alleviate protein deficiency and maintain a healthy society, attention has been directed over the past several decades to the development of new protein sources such as fish protein concentrate, single cell protein and soybean protein (Bhatia and Greer, 2008; Ferrianti and Fiechter,

1983; Mendez *et al*., 2002; Pariser *et al*., 1978; Sikka *et al*., 1979; Tannenbaum and Wang, 1975). However, there is still an estimated one billion people suffering from protein deficiency (WHO/FAO, 2007). Alternative protein resources are continuously being evaluated. Three insect species have received detailed nutritional evaluation in the SADC: (i) the termite (*Macrotermes falciger)* in Zimbabwe (Phelps *et al*., 1975), (ii) the mopane worm (*Imbrasia belina)* and (iii) stink-bugs, (*Encosternum* d*elegorguei*) in South Africa (Dreyer and Wehmeyer, 1982; Teffo *et al*., 2007). Despite the reports on edible insects in the SADC, very little information is available on nutritional qualities of insects like African metallic boring beetle (*Sternocera orissa)*, which are eaten in large quantity in certain communities.

The study by Malaisse (1997), on 24 caterpillar species reported proteins, fat, and energy value average of 63.5 ± 9.0 % , 15.7 ± 6.3 % and 457 ± 32 kcal per 100 g (numbers based on dry matter), respectively. That study further demonstrated that caterpillars compared have higher protein and fat contents and provide more energy per unit compared with meat or fish. Insect fatty acids are similar to those of poultry and fish in their degree of unsaturation (DeFoliart, 1992). Lepidopterous larvae contain appreciable amounts of polyunsaturated fatty acids (Fasts, 1970). Fresh ants are also rich in formic acid content, which is used in making salad dressing in the place of acetic acid found in vinegar (Chen *et al*., 1998).

2.2.5 Economic importance of insects

The use of edible insects may have massive economic impact considering that they form the largest volume of animal protein consumed by all carnivores in the terrestrial ecosystem (Chen *et al*., 1998). In Thohoyandou and other markets, selling of insects is still highly esteemed and valuable income had been made for the harvesters (Teffo *et al*., 2007). The mopani worm is one of the best known and economically important forestry resources products of the mopani woodland in southern Zimbabwe, Botswana and northern Limpopo Province (Bradley and Dewes, 1993; Timberlake, 1996) and are being exported and

imported from other parts of Southern Africa such as Botswana, Zimbabwe and Zambia (DeFoliart, 1995; Dreyer and Wehmeyer, 1982; Greyling and Potgieter, 2004; Moruakgomo, 1996). According to Madibela *et al*. (2008), mopani worm is an important natural resource for many people in Southern Africa and it is a source of protein and cash, especially among the poor, hunger-averse rural dwellers. Women and children were usually involved in the collection of insects but now through income earnings generated even youth and men are involved. Dried mopani worms are normally sold locally for between R 5 and R 10 per 100 g, R 20 per 200 g and R 1200 and R 1500 for an 80 kg bag of worms. Thohoyandou market vendors in Limpopo Province estimated a turnover of about R 20 000 (Makhado *et al*., 2009).

Chavunduka (1975) notes that in some areas of Zimbabwe, families depend and make a good living from selling caterpillars. Normally insects are not only sold widely in the village markets of the developing countries, but many of the favourites make their way to urban markets and restaurants. In Mexico two insects are found in urban restaurants which were formerly exploited to the United States and Europe. These are the white maguey (*Aegiale hesperiaris*) and Mexican caviar (*ahuahutle*) (DeFoliart, 1992). In South Africa, Crafford (1991) and van der Waal (1996) reported that edible locusts are widely consumed in most rural areas in the northeastern part of South Africa and make an important contribution to the diet of local people. Locusts such as *Crytacanthacris septemfasciata* provide good nutritional source by containing high protein and fats at a value of 46.1 % and 9.6 %, respectively (Adler, 1934).

2.2.6 Insect potential hazards

A lot of insects secrete toxins and also produce toxic metabolites or sequester toxic chemicals from food plants (Blum, 1978; Duffey, 1980; Wirtz, 1984). Other insects secrete chemical compounds as alkaloids, for example, fire ants (*Solenopsis*) venom contains 2, 6– diakylpiperidimes (MacConnell *et al.,* 1971); toluene and 0-cresol in longhorn beetles (Moore and Brown, 1971); anabolic steroids from species in the family

Dytiscidae (Schildknecht, 1970) as well as cyanogenic glycosides from the larva of the moth *Zyaena trifolii* (Jones *et al.,* 1962).

2.3 Improving insect attractiveness as food

Successful preservation of food improves its attractiveness to consumers. A good example is the Mopani worm. The drying technique is probably the oldest and most important method of food preservation practiced by humans today. Moisture removal prevents the growth and reproduction of microorganisms which cause decay and minimises moisture-mediated deterioration reactions. During drying most changes take place which include: structural and physio-chemical modifications that affect the final product quality, and the quality aspects involved in dry conservation in relation to quality of fresh products and applied drying techniques (Baysal *et al*., 2003). Nutritional changes that occur during drying include the following: caloric content did not change, but was concentrated into a smaller mass as moisture is removed, fibre structure did not change, vitamin A was fairly well retained under controlled heat methods, whereas vitamin C was mostly destroyed during blanching and drying. Thiamin, riboflavin and niacin were lost during blanching, but thre was a fairly good retention if the water used to dehydrate was consumed. Minerals may be lost during dehydration if soaking water is not used and Fe is not destroyed by drying.

2.3.1 Oven drying

Oven drying is the simplest way to dry food and it is also faster than sun drying or other methods. The oven is set on the lowest possible temperature for the specimen involved. According to Opstvedt *et al*. (2003), exposure of food samples to denaturing temperature may increase digestibility of native protein by unfolding the polypeptide chain and rendering the protein more susceptible to digestive enzymes. On the other hand, when proteins are exposed to some heat treatment, digestibility may be reduced to formation of disulphide bonds in protein (Oria *et al*., 1995; Stanley *et al*., 1998).

2.3.2 Sun-drying

Sun-drying is still a method used to preserve grains, vegetables, fruits and other agricultural products (Szulmayer, 1971). In most of the developing countries, the use of modernist machinery for drying has not being practice due to unaffordable costs to the majority of farmers (Okoro and madueme, 2004). However, sun-drying has not always been suitable to large farming scale, due to lack of ability to control to temperature fluctuations, high labour cost, large area requirement, insect and fungal growth, encroachment of insects, birds and rodents, etc.

2.3.3 Freeze-drying

Freeze-drying is a process whereby water is removed by dehydration, through sublimation of ice in the materials. It is also known for shelf-life longevity by preventing microbial growth and retarding lipid oxidation (Marques, *et al*., 2009). Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. During freeze-drying there may be changes of decline in antioxidant content due to degradation of certain compounds. Its operational cost is very high (Marques, *et al*., 2006). Freeze-drying was first developed for the preservation of blood plasma and later penicillin during World War II (Flosdorf, 1949).

2.4 Addressing the identified gaps

Sternocera orissa is a beetle mostly consumed by rural dwellers in Limpopo Province. Junod (1913) reported that *S. orissa* was eaten by the shepherds by first removing the elytra. Different preparation methods are employed by different communities and methods of preservation has not yet being studied. In the Pedi vicinity they are collected either early morning or late afternoon and are mostly preferred when prepared immediately. Very little is found in literature about insects preservation by employing the drying

methods as the principal action to reduce moisture and facilitate nutrients availability. Several factors can affect the nutritional content of insects and this is dependent on the processing methods used. Losses of nutrients will depend on the sensitivity of that nutrient when processing goes on. Heating can be beneficial and detrimental to nutrient content in food (Morris *et al.*, 2004). Drying method can improve the digestibility of foods by making some nutrients more available. This problem of nutritional composition in insects can be managed by employing decreased temperature and shorten the drying time. Nutritional losses during drying depend on the following (Morris *et al.*, 2004): (a) Drying temperature, (b) Drying time and (c) Storage condition.

2.5 Summary of the gaps to be investigated

The aim of the study was to investigate the effect of drying methods and location on nutritional composition of *S. orissa,* in order to improve its attractiveness to local people. Eventually, this would increase the harvesting of the insect, which would serve as a control strategy.

CHAPTER 3
INFLUENCE OF DRYING METHOD AND LOCATION ON PROXIMATE CHEMICAL COMPOSITION OF *STERNOCERA ORISSA*

3.1 Introduction

One of the principal responsibilities of agro-processing is to preserve nutrients through all phases of food acquisition, processing, storage and preparation (Niir Board, 2001). Food preservation is the process of treating and handling food to stop or greatly slow down spoilage accelerated by micro-organisms. Most food preservation methods include pasteurisation and/or drying. Pasteurisation requires food to be sealed after treatment to prevent recontamination, whereas drying methods allow food to be stored for long periods without any special containment. Common drying methods include heat and freeze drying.

Freeze drying is a technique whereby continuously frozen specimen are dehydrated by sublimation and vacuum, without significant loss of physical form or colour (Harbach and Harrison, 1983). Due to the absence of liquid water and low temperature employed in this process, most microbiological reactions cease with the final product having excellent quality (Ratti, 2001). The method does not change product flavour, smells and nutritional content but guarantees long product shelf life (Adam, 2004). Although oven drying is the most commonly used, the challenge is that it may not enhance nutrient stability and bioavailability (Niir Board, 2001; Potter and Hotchkiss, 1995).

Preservation of *S. orissa* is a challenge among most marginal communities in Limpopo Province. Taste and quality of the beetle are said to deteriorate rapidly through micro-organism spoilage, resulting in fewer beetles being harvested. Ideal preservation technology may improve the attractiveness of this beetle as food, resulting in large number being harvested, thus reducing the population densities of the beetle. The

objective of the study was to determine the effects of drying method and location on proximate chemical composition of S. *orissa*.

3.2 Materials and methods

3.2.1 Study location/area and harvesting

Beetles were harvested in December 2009 from acacia trees (*Acacia campylacantha*) in three locations: Khureng (24°33'53''S, 29°23'4''E), Magatle (24°27'19''S, 29°23'39''E) and Ga-Masemola (24°33'46''S, 29°38'57''E) in Limpopo Province, republic of South Africa. The three locations have the semi-arid climate, with rainfall of less than 400 mm per annum. Soil type in Khureng and Ga-Masemola is predominantly clay, whereas Magatle has sandy soil. Vegetation in Khureng and Magatle are bushveld types, whereas at Ga-Masemola there are mixed bushveld types. Harvesting was done using the beating method in the early morning, when beetles were still inactive on tree branches (Holm, 1984). The dislodged beetles were picked from the ground, put in ventilated plastic bags and transported in cooler boxes to the Limpopo Agro-Food Technology Station and stored for three days in the refrigerator at – 5 ºC prior to processing (Finke *et al*., 1989).

3.2.2 Experimental design and procedures

Randomised complete block design in a 3 x 3 factorial arrangement, where the three drying methods (freeze, heat and cooking) and three locations (Khureng, Magatle, Ga-Masemola) were each replicated three times. In each treatment, 30 beetles with elytras and wings removed were used. Oven-drying treatment achieved at 66 ºC, ran for 24 hours, whereas freeze drying at 085 mtorr (pressure) and – 55 ºC for 24 hours. Thirty beetles per treatment were cooked in 130-cm-diameter frying pan with 50 ml tapwater until all free water had evaporated and then fried without adding cooking oil. Beetles from all treatments

were individually ground using a coffee grinder and sealed in plastic bags using the impulse sealant (KS-300 POWER 400 w, Source: 220 v 50 Hz, Hongzhan).

3.2.3 Data Collection

Ash and moisture content were determined as described by Harris (1970). Organic matter was determined by heating samples at 550 ºC for 8 hours. An Allihan Condenser Soxhlet extraction apparatus was used to determine fat content with ether as an extractant, which was evaporated at 90 ºC and the fat left inside the beaker. Weight gained was used to calculate the fat content. Nitrogen was determined using the Kjeldahl method (Kjeltec, Tecator AB, Hoganas, Sweden) and the quantity of protein calculated as 6.25 x N (AOAC, 1984)

3.2.4 Data analysis

Analysis of variance was performed on treatments using Statistix 8.1 software (Statistix, Analytic Software, Statistix; Tallahassee, FL, USA, 1985-2003). Tukey Honestly Significant Differences (HSD) all – pairwise comparison test at 0.05 probability level was used to determine treatment differences among the means.

3.3 Results

Relative to freeze-drying, oven-drying increased protein, carbohydrates, fat, ash, dry matter and energy by 179 %, 151 %, 104 %, 177 %, 163 % and 150 %, respectively (Table 3.1). Oven drying increased the six variables more than hundred fold, except for moisture content which decreased by 92 %. Similarly, cooking increased protein, carbohydrates, fat, moisture, ash, dry matter and energy by 38 %, 30 %, 2 %, 22 %, 42 %, 32 % and 27 %, respectively (Table 3.1). Relative to freeze-drying, cooking and oven-drying methods increased the proximate chemical composition measured, except for oven-drying which decreased moisture content. Fat and energy content of *S. orissa* were significantly high at Ga-Masemola (Table 3.2),

whereas protein, moisture, dry matter, carbohydrates and ash were not significantly affected by location (Table 3.2).

Table 3.1 Proximate chemical composition mean values of *Sternocera 18rissa* under three drying methods viz; freeze drying, traditionally cooking and oven drying method

Proximate chemical composition	Drying method[y]			Relative to freeze method (%)[z]	
	Freeze	Cooking	Oven	Cooking	Oven
Ash	1.01c	1.44b	2.80a	42	177
CHO[b]	8.21b	10.69b	20.62a	30	151
DM[c]	36.55b	48.25b	96.26a	32	163
Energy	700.7b	894.3b	1785.3a	27	150
Fat	4.81b	4.92b	9.83a	2	104
Moisture	51.75a	63.45a	3.74b	22	− 92
Protein	22.51b	31.19b	63.00a	38	179

[y]Row means followed by the same letter were not different according to Tukey Honest Significant Difference Test at the probability level of 5 %.

Impact[z] = (treatment/freeze drying – 1) X 100

CHO[b] = Carbohydrate

DM[c] = Dry matter

Table 3.2 Influence of three locations on proximate chemical composition of *Sternocera orissa*

Proximate chemical composition	Location			P ≤
	Khureng	Magatle	Ga-Masemola	
Ash	1.82	1.76	1.66	Ns
CHO[b]	12.83	14.01	12.66	ns
DM[c]	57.93	58.74	64.38	ns
Energy	1028.3	1071.0	1281.0	**
Fat	3.72	5.12	10.72	**
Moisture	42.06	41.25	35.61	ns
Protein	39.54	37.83	39.33	ns

Difference Test at the probability level of 5 %.

ns = not significant at 10%, ** = significant at 5% level of probability

CHO[b] = Carbohydrate

DM[c] = Dry matter

3.4 Discussion

Relative to freeze-drying, the other two methods increased protein, which was in agreement with observations on fish (Opstvedt *et al*., 2003), Atlantic spider (Margues *et al*., 2010). Increases in protein under drying temperature of 66 °C may improve digestibility of proteins by unfolding polypeptide chains and rendering protein susceptible to digestive enzymes (Opstvedt *et al*., 2003; Potter and Hotchkiss, 1995). The protein content of *S. orissa* under all drying methods was higher than protein content of most conventional food (fish = 19.6 %; eggs = 12.9 %; milk = 4.0 %; pork = 19.0 %; beef = 18.4 %; chicken = 22.0 %; lamp =

16.1 %) as reported by Ghaly (2009). Consequently, S. *orissa* adult beetles may be a cheap and abundant source of good quality animal protein for marginal communities in Limpopo Province.

Relative to freeze-drying, other methods tested increased carbohydrate, which agreed with findings of Raguse and Smith (1965), who reported that heat treatment caused a progressive increase in soluble sugars. Generally, most enzymes are not inactivated by freeze drying (Smith, 1969). However, simple sugars undergo changes when exposed to drying temperature and they react with other constituents (Goering and Van Soest, 1967; Van Soest, 1962). Hudson *et al*. (1941) and Thompson and Wolfrom (1958) demonstrated that complex carbohydrates degradate under the influence of temperature through cleavage of basic matrix structures with probable shift of bonds, resulting in formation of water soluble sugars.

Relative to freeze-drying and cooking, oven drying method increased fat content. Fat is essential in diets as it increase the palatability of foods by absorbing and retaining their flavour (Aiyesanmi and Oguntokun, 1996) and it is also the main form in which energy is stored in insect larvae (Chapman, 1980; Gilmour, 1961; Wigglesworth, 1976). According to Martin *et al*. (1981), fats are vital in the structuring and biological functioning of cells and they help in transportation of nutritional essential fat-soluble vitamins.

Relative to freeze-drying, oven drying methods decreased the moisture content of *S. orissa,* which was in agreement with findings in plants such as *Vernonia amygdalina*, fermented cassava product and tomato (Aliero and Abdullahi, 2009; Faramade and Faramade, 2005; Kolawole *et al*., 2010). Morris *et al*. (2004) demonstrated that moisture removal by heat improved food digestibility, increased concentration of nutrients and made some nutrient more available. In cooking method, moisture content was relatively higher which could increase chances of deterioration by micro-organisms (Hassan *et al*., 2007).

Generally, the ash content of a sample is the reflection of the level of minerals the test sample contains (Omotoso and Adedire, 2007). Since cooking and oven-drying increased the ash content, the two treatments probably increased the level of nutrients elements.

CHAPTER 4
INFLUENCE OF DRYING METHOD AND LOCATION ON AMINO ACIDS AND ESSENTIAL NUTRIENT ELEMENTS OF *STERNOCERA ORISSA*

4.1 Introduction

Drying as a preservation method is a very important aspect of food processing (Hassan *et al*., 2007). Common drying method includes sun-drying, food dryers, air-drying, vacuum thermal-drying, oven-drying and freeze-drying. Oven-drying (Kaehler and Kennish, 1996; Robledo and Pelegrin, 1997) and freeze-drying (Norziah and Ching, 2000; Suzuki *et al*., 1996) are the two most widely used methods to preserve indigenous foods. The basic mechanism of oven-drying method is heating by hot-air convection (Wong and Chueng, 2001) at 65 ºC temperature to avoid adverse thermal reactions (Anderson, 1996). Freeze-drying was developed to overcome the problem of the loss of volatile compounds in food during conventional drying operations. Freeze drying is an effective method to extend the average lifespan of food given that it prevents the deterioration due to microbial growth or oxidation (Barbosa-Canovas and Vega-Mercado, 2000).

Drying could be an important factor affecting the nutritional value of insects either through chemical modifications or direct loss of essential nutrients elements (Wong and Chueng, 2001). Amino acids are the building units of protein (Erasmus, 2001; Ihokoronye and Ngoddy, 1985) and serve as body builders (Bhavan *et al*., 2010). The biological value of food protein is dependent upon its amino acid composition (Finar, 1975; Oyenuga *et al*., 1974; Pant and Tulsiram, 1969; Williams, 1960).

The nature and chemical composition of essential amino acids give a particular food its protein status. Generally, high amino acid food is in high demand in marginal communities, and sources of food with high amino acids are more attractive. The essential amino acid and nutrient element status of *S. orissa* is not

documented. The objective of the study was to determine the effects of drying methods and locality on amino acid and essential mineral elements composition of *S. orissa*.

4.2. Materials and methods

4.2.1 Study location/area and harvesting

Beetles were harvested in summer November 2009 – January 2010 from acacia trees (*Acacia campylacantha*) at three locations: Khureng (24°33'53''S, 29°23'4''E), Magatle (24°27'19''S, 29°23'39''E) and Ga-Masemola (24°33'46''S, 29°38'57''E) in Limpopo Province, South Africa. Climatic conditions, soil type and vegetation types were as described previously (Chapter 3). Harvesting was done in the early morning when beetles were inactive on tree branches using the beating method (Holm, 1984), which dislodges beetles from branches. The beetles were picked from the ground and put in ventilated plastic bags and transported to the Limpopo Agro-Food Technology Station in cooler boxes and stored for three days in the refrigerator at –5 ºC prior to processing (Finke *et al*., 1989).

4.2.2 Experimental design and procedures

Randomised complete block design arranged in a 3 x 3 factorial arrangement was used with three drying methods (freeze drying, heat drying, frying) and three locations (Khureng, Magatle, Ga-Masemola) were used as treatments with three replications. Thirty beetles from each location were defrosted at room temperature, with the elytra and wings removed and weighed the body. Oven-drying was done at 66 ºC temperature for 24 hours, whereas freeze drying was done at pressure of 085 mtorr at – 55 ºC for 24 hours. Thirty beetles per treatment were cooked in 130-cm-diameter frying pan with 50 ml tapwater until all free water had evaporated and then fried without adding cooking oil. Beetles from all treatments were individually ground using a coffee grinder and sealed in plastic bags using the impulse sealant (KS-300 POWER 400 w, Source: 220 v 50 Hz, Hongzhan).

4.2.3 Data collection

The analysis of amino acids after acid hydrolysis extraction, pre-column derivatisation, separation by HPLC and detection by fluorescence was employed to determine the composition of proteins (De Vries *et al*., 1980; Einarsson *et al*., 1983). Approximately 0.5 g of edible *S. orrisa* was digested with 7 ml concentrated HNO_3 and 3 ml $HCLO_4$ at 200º C (Zasoski and Barau, 1977). For Ca, Mg, P, K, Na and Fe an aliquot of the digested solution was subjected to Inductively Coupled Plasma-Optical Emission Spectrometric (ICP-OES), whereas selenium was determined by an Inductively Coupled Plasma-Mass Spectrometry (Chao-Yong and Schulte, 1985).

4.2.4 Data analysis

Analysis of variance was performed on treatments using Statistix 8.1 software (Statistix, Analytic Software, Statistix; Tallahassee, FL, USA, 1985-2003). Tukey Honestly Significant Differences (HSD) all – pairwise comparison test at 0.05 probability level was used to determine treatment differences among the means. When treatments were significant at the probability level of 0.05, the degrees of freedom and their associated sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the treatment means (Little and Hills, 1981).

4.3 Results

In essential amino acids of *S. orissa*, the partitioning of the degrees of freedom and their associated sum of squares demonstrated that the drying method treatment contributed 98 %, 98 %, 97 %, 97 %, 97 %, 97 %, 79 %, 69 % and 98 % to the TTV on isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine, respectively (Appendices 4.1 – 4.9). However, in most instances, location had no effect on essential amino acids (Appendices 4.1 – 4.9). Relative to freeze-drying method, the cooking and oven-drying method increased the essential amino acids of *S. orissa* (Table 4.1). Similarly, in

non-essential amino acids of *S. orissa*, the partitioning of the degrees of freedom and their associated sum of squares demonstrated that the drying method treatment contributed 94 %, 98 %, 55 %, 94 %, 68 %, 98 %, 97 % and 98 % to the TTV on alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, proline and serine, respectively (Appendices 4.10 – 4.17). However, in most instances, location had no effect on non-essential amino acids (Appendices 4.10 – 4.17). Relatively to freeze-drying method, the cooking and oven-drying methods increased the non-essential amino acids of *S. orissa* (Table 4. 2*)*. Location had no effect on both essential and non-essential amino acids (Table 4.3 and 4.4).

Table 4.1 Composition (g/100 g) of essential amino acids in *Sternocera orissa* as affected by freeze, cooking and oven drying methods

Essential amino acid	Drying method[y]			Relative to freeze method (%)[z]	
	Freeze	Cooking	Oven	Cooking	Oven
Isoleucine	0.73b	1.07b	2.39a	47	227
Leucine	1.14b	1.72b	3.82a	51	235
Lysine	0.94b	1.56b	3.08a	66	227
Methionine	0.25b	0.28b	0.62a	12	148
Phenylalanine	0.51b	0.67b	1.62a	31	218
Threonine	0.59b	0.97b	1.94a	64	229
Tryptophan	0.17b	0.31ab	0.57a	82	235
Tyrosine	0.46a	0.79a	1.51a	72	228
Valine	0.61b	0.83b	2.59a	36	325

[y] = Row means followed by the same letter were not different according to Tukey Honest Significant Difference Test at the probability level of 5 %.

Impact[z] = Relative to freeze method = [(treatment/Freeze – 1) × 100]

Table 4.2 Composition (g/100 g) of non-essential amino acids in *Sternocera orissa* as affected by freeze, cooking and oven drying methods

Amino acid	Drying method[y]			Relative to freeze method (%)[z]	
	Freeze	Cooking	Oven	Cooking	Oven
Alanine	1.09b	1.56b	3.36a	43	208
Arginine	0.93b	1.23b	3.00a	32	222
Aspartic acid	0.95a	1.69a	2.71a	78	185
Glycine	0.94b	1.40b	3.19a	49	239
Glutamic acid	1.49b	2.60b	4.45a	74	198
Histidine	0.46b	0.63b	1.48a	37	135
Proline	1.03b	1.38b	3.46a	33	236
Serine	0.62b	0.86b	1.96a	38	216

[y] = Row means followed by the same letter were not different according to Tukey Honest Significant Difference Test at the probability level of 5 %.

Impact[z] = Relative to freeze method = [(treatment/Freeze – 1) × 100]

Table 4.3 Composition (g/100 g) of essential amino acids in *Sternocera orissa* as affected by Khureng, Magatle and Ga-Masemola locations

Amino acid	Location			LSD 0.05
	Khureng	Magatle	Ga-Masemola	
Isoleucine	1.45	1.40	1.34	< 0.05
Leucine	2.31	1.14	1.72	< 0.05
Lysine	1.87	1.88	1.82	< 0.05
Methionine	0.38	0.38	0.39	< 0.05
Phenylalanine	0.90	0.98	0.92	< 0.05
Threonine	1.15	1.16	1.18	< 0.05
Tryptophan	0.37	0.41	0.27	< 0.05
Tyrosine	1.03	0.74	0.98	< 0.05
Valine	1.32	1.32	1.39	< 0.05

Table 4.4 Composition (g/100 g) of non-essential amino acids in *Sternocera orissa* as affected by Khureng, Magatle and Ga-Masemola locations

Amino acid	Location[y]			LSD 0.05
	Khureng	Magatle	Ga-Masemola	
Alanine	2.22	1.87	1.91	< 0.05
Arginine	1.72	1.79	1.65	< 0.05
Aspartic acid	2.09	1.36	1.90	< 0.05
Glycine	1.96	1.91	1.67	< 0.05
Glutamic acid	3.21	2.32	2.60	< 0.05
Histidine	0.89	0.86	0.82	< 0.05
Proline	1.91	2.08	1.87	< 0.05
Serine	1.12	1.19	1.12	< 0.05

In essential nutrient elements of *S. orissa*, the partitioning of the degrees of freedom and their associated sum of squares demonstrated that the drying method treatment contributed 69 %, 74 %, 25 %, 75 %, 92 %, 84 %, 93 %, 92 %, 92 %, 88 %, 97 % and 87 % to the TTV on calcium, chlorine, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and zinc, respectively (Appendices 4.18 – 4.29). However, in most instances, location had no effect on essential nutrient elements except for iron (Table 4.6). Relative to freeze-drying method, the cooking and oven-drying method increased the essential nutrient elements of *S. orissa*, except for potassium that was reduced by cooking (Table 4.5).

Table 4.5 Essential nutrient element composition (mg/100 g) of *Sternocera orissa* as affected by three drying methods, viz; freeze-drying, cooking and oven-drying methods

Nutrient element	Drying method[y]			Relative to freeze method (%)[z]	
	Freeze	Cooking	Oven	Cooking	Oven
Calcium	24.76b	42.43ab	64.00a	71	158
Chlorine	42.08b	61.44ab	97.50a	46	132
Cobalt	0.20c	99.47a	0.59b	496	195
Copper	28.03b	42.10ab	79.13a	50	182
Iron	48.72c	84.81b	120.40a	74	147
Magnesium	39.01b	51.42b	103.33a	32	165
Manganese	22.56b	27.67b	61.16a	22	171
Phosphorus	249.37b	339.55b	639.00b	36	156
Potassium	453.63b	367.68b	912.67a	−18	101
Selenium	0.46b	0.63b	1.12a	37	143
Sodium	34.58b	64.03ab	96.80a	85	180
Zinc	60.87b	110.40ab	114.73a	81	88

[y] = Row means followed by the same letter were not different according to Tukey Honest Significant Difference Test at the probability level of 5 %.

Impact[z] = Relative to freeze method = [(treatment/Freeze – 1) × 100]

Table 4.6 Essential nutrient element composition (mg/100 g) of *Sternocera orissa* as affected by three locations, viz; Khureng, Magatle and Ga-Masemola locations

Nutrient elements	Locations[y]			P ≤
	Khureng	Magatle	Ga-Masemola	
Calcium	34.76	56.24	40.18	ns
Chlorine	78.67	62.01	60.34	ns
Cobalt	99.42	0.57	0.27	ns
Copper	58.13	51.56	39.56	ns
Iron	80.36	78.02	94.95	**
Magnesium	62.23	75.86	55.65	ns
Manganese	32.30	37.85	41.24	ns
Phosphorus	401.55	444.02	382.35	ns
Potassium	556.91	626.16	550.91	ns
Selenium	0.70	0.73	0.78	ns
Sodium	65.45	67.20	62.77	ns
Zinc	108.86	102.36	104.78	ns

Difference Test at the probability level of 5 %.

ns = significant at 10 % level of probability, ** = significant at 5% level of probability

4.4 Discussion

Relative to freeze-drying, oven-drying and cooking methods increased aspartic and glutamic acid, which is in agreement with finding of seaweed by Mabeau *et al*. (1992) and Wong and Chueng (2001). Wilson and Walker (2000) reported that the hydrolysis procedure destroys or chemically modifies the asparagines, glutamine and tryptophan residues in protein. While asparagines and glutamine are converted to the corresponding acids (aspartic and glutamic acids) and are quantified with them and tryptophan is destroyed. That could have informed the lowest amounts of tryptophan in all drying methods and locations.

Relative to freeze-drying, oven drying method increased lysine and leucine, which agreed with findings of (Ekpe *et al*., 2007) on bush mango seeds (*Irvingia gabonensis*). The increase in amino acid content after cooking and oven-drying gjobserved might be due to the effect of antienzyme on trypsin inhibition as explained by Ekpo (2006). Findings by Hackler *et al*. (1965) and Stilings and Hackler (1965) reported that heat-processing temperature and time may alter the nutritional quality of protein and its amino acids composition.

Relative to freeze-drying, oven drying increased proline content. This is in agreement with observations on blue-green algae (*Spirulina platensis*) by Divakaran and Duerr (1987). Histidine content was the lowest in all drying methods. Histidine is a very oxidation sensitive amino acid and that may be the reason for its reduction. Histidine is involved in many metabolic functions including production of histamines, which takes part in allergic and inflammatory reactions. It also plays role in osmoregulation process (Abe and Ohmama, 1987).

Drying methods significantly influenced the mineral concentrations. Relative to freeze-drying, oven-drying increased majority of minerals concentration except for zinc (Table 4.5). The concentrations of minerals

ranged from 0.20 mg/100 g (Cobalt) to 912 mg/100 g (Potassium). The high concentration of potassium confirms the findings of Hassan *et al*., (2007) on *Leptadenia hastata* (Asclipiadaceae).

Furthermore, Na/K ratios across the drying methods were very low (Figure 1), which is interesting from nutrition point of view, since the intake of sodium chloride and diets with high Na/K ratio have been related to the incidence of hypertension (Zhou and Han, 2006).

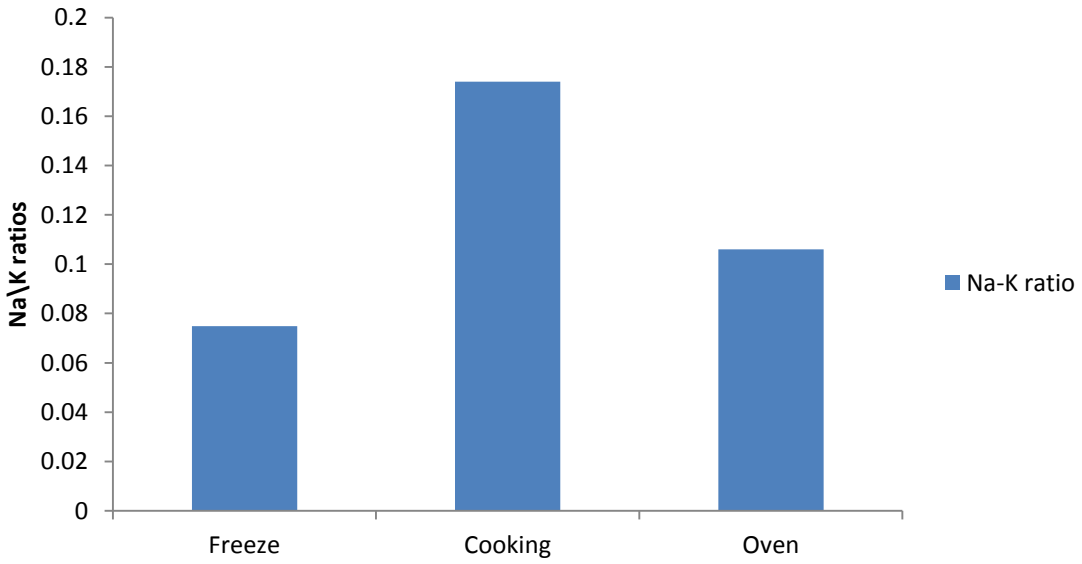

Figure 1. Na\K ratio of adult *Sternocera orissa.*

Oven-drying increased the potassium and lowered sodium. However, higher potassium with low sodium is a protective effect against excessive sodium intake (Hassan *et al*., 2007). The high potassium level in the heat treated samples may be an added advantage over the freeze-dried samples for use as therapy and are vital for bone development (Dzomeku *et al*., 1993). Most of the trace elements (Fe, Cu, Zn and Se) present in adult *S. orissa* are for physiological functions (Liu, 1996).

Relative to freeze-drying, oven-drying increased trace elements (Zn, Cu, Fe, Mn). Copper was found to be lower while Zn was found to be the highest. This result tallies with the work of Teeny *et al*. (1984) and Akinneye *et al*. (2010) on different fish species. Zinc was relatively high upon oven-drying, which might be attributed to contamination during drying process. Zinc is nutritionally important for its roles in immune system (Bhaskaram, 2002), for insulin secretion (Chausmer, 1998), in the release of vitamin from the liver (Hwang *et al*., 2002) and as an enzyme (Boron *et al*., 1988).

CHAPTER 5
SUMMARY, CONCLUSION AND RECOMMENDATIONS

A large number of insect pest are being regarded as food in some parts of the world. If insects become more widely accepted as a reasonable source of nutrients, especially protein, in societies, this will lower the insect pest reaching damage threshold. Insects can, therefore, be collected on readily available substrate to provide a sustainable and nutritional supply of protein for human consumption in rural areas of Limpopo Province.

The nutritional value of edible insects have the potential to provide income and jobs to rural dwellers in Limpopo Province of South Africa through people who capture, rear, process, transport and market the insects. Consumption of edible insects could be greatly enhanced if they can be brought into the human diet using modern and improved preservation and processing methods.

The relatively high nutritional composition of *S. orissa* when prepared using readily available technologies such as drying and cooking, renders this beetle a probable candidate for use in amelioration of malnutrition in marginal communities of Limpopo Province. However, there is need to determine nutritional composition of *S. orissa* under varying times and temperature regimes in order to establish the level where the tested variables could be optimized.

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APPENDICES

Appendix 3.1 Analyses of variance for ash under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	5.238	98	263.69	≤ 0.01
Location	2	0.042	1	2.14	≥ 0.10
Error	4	0.039	1		
Total	8	5.320	100		

Appendix 3.2 Analyses of variance for carbohydrates under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	258.763	96	66.40	≤ 0.01
Location	2	3.244	1	0.83	≥ 0.10
Error	4	7.794	3		
Total	8	269.801	100		

Appendix 3.3 Analyses of variance for dry matter under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	6007.13	98	159.69	≤ 0.01
Location	2	74.07	1	1.97	≥ 0.10
Error	4	75.24	1		
Total	8	6156.44	100		

Appendix 3.4 Analyses of variance for energy under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2007890	93	145.11	≤ 0.01
Location	2	109761	5	7.93	≤ 0.05
Error	4	27673	1		
Total	8	2145324	100		

Appendix 3.5 Analyses of Variance for fat under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	49.321	35	12.99	≤ 0.01
Location	2	82.320	59	21.69	≤ 0.01
Error	4	7.592	6		
Total	8	139.233	100		

Appendix 3.6 Analyses of variance for moisture under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	6007.13	98	159.69	≤ 0.01
Location	2	74.07	1	1.97	≥ 0.10
Error	4	75.23	1		
Total	8	6156.44	100		

Appendix 3.7 Analyses of variance for protein under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2726.66	97	75.86	≤ 0.01
Location	2	5.19	0	0.14	≥ 0.10
Error	4	71.89	3		
Total	8	2803.74	100		

Appendix 4.1 Analyses of variance for isoleucine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	4.646	98	110.99	≤ 0.01
Location	2	0.018	0	0.43	≥ 0.10
Error	4	0.083	2		
Total	8	4.748	100		

Appendix 4.2 Analyses of variance for leucine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	11.918	98	101.33	≤ 0.01
Location	2	0.052	0	0.45	≥ 0.10
Error	4	0.235	2		
Total	8	12.206	100		

Appendix 4.3 Analyses of variance for lysine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	7.250	97	56.87	≤ 0.01
Location	2	0.006	0	0.05	≥ 0.10
Error	4	0.254	3		
Total	8	7.510	100		

Appendix 4.4 Analyses of variance for methionine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	0.262	97	65.92	≤ 0.01
Location	2	0.001	0	0.02	≥ 0.10
Error	4	0.008	3		
Total	8	0.271	100		

Appendix 4.5 Analyses of variance for phenylalanine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2.160	97	76.15	≤ 0.01
Location	2	0.008	0	0.31	≥ 0.10
Error	4	0.056	3		
Total	8	2.225	100		

Appendix 4.6 Analyses of variance for threonine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2.923	97	85.42	≤ 0.01
Location	2	0.001	0	0.02	≥ 0.10
Error	4	0.068	3		
Total	8	2.992	100		

Appendix 4.7 Analyses of variance for tryptophan under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	0.240	79	14.92	≤ 0.01
Location	2	0.033	11	2.09	≥ 0.10
Error	4	0.031	10		
Total	8	0.305	100		

Appendix 4.8 Analyses of variance for tyrosine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	1.744	69	5.35	≥ 0.10
Location	2	0.144	6	0.44	≥ 0.10
Error	4	0.652	25		
Total	8	2.540	100		

Appendix 4.9 Analyses of variance for valine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	7.066	98	88.70	≤ 0.01
Location	2	0.011	0	0.14	≥ 0.10
Error	4	0.159	2		
Total	8	7.237	100		

Appendix 410 Analyses of variance for alanine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	8.604	94	48.28	≤ 0.01
Location	2	0.218	2	1.23	≥ 0.10
Error	4	0.356	4		
Total	8	9.179	100		

Appendix 4.11 Analyses of variance for arginine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	7.498	98	101.16	≤ 0.01
Location	2	0.030	0	0.42	≥ 0.10
Error	4	0.148	2		
Total	8	7.677	100		

Appendix 4.12 Analyses of variance for aspartic acid under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	4.72276	55	3.13	≥ 0.10
Location	2	0.86536	10	0.57	≥ 0.10
Error	4	3.01791	35		
Total	8	8.60602	100		

Appendix 4.13 Analyses of variance for glycine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	8.478	94	43.82	≤ 0.01
Location	2	0.149	2	0.77	≥ 0.10
Error	4	0.386	4		
Total	8	9.014	100		

Appendix 4.14 Analyses of variance for glutamic acid under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	13.36	68	5.38	≥ 0.10
Location	2	1.301	7	0.52	≥ 0.10
Error	4	4.968	25		
Total	8	19.631	100		

Appendix 4.15 Analyses of variance for histidine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	1.808	98	106.82	≤ 0.01
Location	2	0.006	0	0.39	≥ 0.10
Error	4	0.033	2		
Total	8	1.849	100		

Appendix 4.16 Analyses of variance for proline under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	10.353	97	99.84	≤ 0.01
Location	2	0.0746	1	0.72	≥ 0.10
Error	4	0.2074	2		
Total	8	10.635	100		

Appendix 4.17 Analyses of variance for serine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	3.068	98	139.99	≤ 0.01
Location	2	0.010	0	0.14	≥ 0.10
Error	4	0.043	2		
Total	8	3.123	100		

Appendix 4.18 Analyses of variance for calcium under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2316.51	69	17.06	≤ 0.01
Location	2	748.76	23	5.51	≥ 0.10
Error	4	271.59	8		
Total	8	3336.87	100		

Appendix 4.19 Analyses of variance for chlorine under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	4746.01	74	8.96	≤ 0.05
Location	2	616.23	10	1.16	≥ 0.10
Error	4	1059.07	16		
Total	8	6421.32	100		

Appendix 4.20 Analyses of variance for cobalt under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	19630.0	25	1.00	≤ 0.05
Location	2	19602.1	25	1.00	≥ 0.10
Error	4	39317.0	50		
Total	8	78549.1	100		

Appendix 4.21 Analyses of variance for copper under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	4180.83	75	9.86	≤ 0.05
Location	2	531.84	10	1.25	≥ 0.10
Error	4	848.34	15		
Total	8	5561.01	100		

Appendix 4.22 Analyses of variance for iron under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	7706.56	92	139.21	≤ 0.01
Location	2	504.71	6	9.12	≥ 0.10
Error	4	110.72	2		
Total	8	8321.99	100		

Appendix 4.23 Analyses of variance for magnesium under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	6987.27	84	20.44	≤ 0.01
Location	2	637.29	8	1.86	≥ 0.10
Error	4	683.84	8		
Total	8	8308.40	100		

Appendix 4.24 Analyses of variance for manganese under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	2638.42	93	69.54	≤ 0.01
Location	2	122.29	4	3.22	≥ 0.10
Error	4	75.88	3		
Total	8	2836.59	100		

Appendix 4.25 Analyses of variance for phosphorus under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	249616	92	29.69	≤ 0.01
Location	2	5976	2	0.71	≥ 0.10
Error	4	16812	6		
Total	8	272404	100		

Appendix 4.26 Analyses of variance for potassium under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	515114	92	28.49	≤ 0.01
Location	2	10493	2	0.58	≥ 0.10
Error	4	36162	6		
Total	8	561769	100		

Appendix 4.27 Analyses of variance for sodium under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	5811.24	88	15.53	≤ 0.01
Location	2	29.82	1	0.08	≥ 0.10
Error	4	748.32	11		
Total	8	6589.38	100		

Appendix 4.28 Analyses of variance for selenium under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	0.720	97	94.46	≤ 0.01
Location	2	0.009	1	1.26	≥ 0.10
Error	4	0.015	2		
Total	8	0.744	100		

Appendix 4.29 Analyses of variance for zinc under different drying methods and locations of *Sternocera orrisa*

Sources of variation	DF	SS	Percent	F-value	P-value
Drying	2	10665.8	87	14.54	≤ 0.01
Location	2	64.6	1	0.09	≥ 0.10
Error	4	1467.5	12		
Total	8	12198.0	100		