Nutrient Composition and Utilization of Edible Termites (*Macrotermes Subhylanus)* and Grasshoppers (*Ruspolia Differenes*) from Lake Victoria Region of Kenya

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A thesis submitted in partial fulfillment for the degree of Master of Science in Food Science and Technology in the Jomo Kenyatta University of Agriculture and technology

2009

DECLARATION

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

Signature………….. ………… Date………………….

John Ndung'u Kinyuru

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

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DEDICATION

This work is dedicated in its entirety to *Mbari ya Kibugi* clan of Murang'a South District, Central Province, Kenya, as an epitome of excellence and dedication to a cause. May the work be an inspiration to the young generation to soar even higher and a reflection of a well-mentored life to the older generation.

ACKNOWLEDGEMENT

I acknowledge Jomo Kenyatta University of Agriculture and Technology for facilitating this research as well as VicRes for the financial support without which the work would not have been possible. I would like to acknowledge my supervisors, Prof. G. M. Kenji and Prof. S. M. Njoroge for their assistance and guidance throughout the entire research work. I also wish to acknowledge Mr. P. N. Karanja and Mr. D. M. Votha of Food Science and Technology Department for their devoted dedication in assisting me during the laboratory work. Mr. P. Kahenya, my worthy colleague and office-mate for the encouragement when it seemed too difficult to move on, is also acknowledged. The teaching and technical staff in the Department of Food Science and Technology is acknowledged, as well as the entire M.Sc. class of 2006 in Food Science and Technology Department. Finally, I wish to acknowledge Almighty God for showering me with wisdom and insight throughout the research work.

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
BEED	Biomechanical and Environmental Engineering Department
CFU	Colony Forming Units
FAO	Food and Agriculture Organisation
GC	Gas Chromatography
HPLC	High performance Liquid chromatography
IMCSF	International Microbiological Commission Specification of Foods
JKUAT	Jomo Kenyatta University of Agriculture and Technology
NRC	National Research Council
RDI	Recommended Daily Intake
SPE	Solid Phase Extraction
UV-VIS	Ultra Violet- Visual
WHO	World Health Organisation

ABSTRACT

Insects have been consumed as part of a traditional diet in Lake Victoria region. However, with modernization, there has been a decline in this practice yet the insects can provide beneficial nutrients to the human body. The aim of this research was to assess the nutrient content of the selected edible insects and their utilization in food product development.

The termite (*Macrotermes subhylanus*) and the green and brown grasshoppers (*Ruspolia differens)* samples were collected from Siaya District in 2007 and 2008 during the short and long rainy seasons. Nutrient composition analysis were performed on each of the species (n= 6) in triplicates. The termite showed a protein content of 45.9 ± 0.2% while the green grasshopper showed a fat content of 48.2 ± 0.2%. Potassium was the most abundant macro mineral in the green grasshopper with a value of 370.60 ± 1.10 mg/100g while iron was the most abundant trace mineral in the termite with a value of 11.52 ± 0.92 mg/100g. The brown grasshopper had a retinol content of 2.4 ± 1.48 μg/g while the termite had a riboflavin content of 3.39 ± 1.76 mg/100g. Oleic acid was the main fatty acid in the termite with a value of 53.07 ± 1.21% while the brown grasshopper showed linoleic content of 31.21 ± 0.25%. The neutral lipids formed the major lipid fraction in termite with a value of 64.73 ± 1.59% and 89.42 ± 1.80% in the green grasshopper.

A serving of 100g of fried dried termite was found to contribute 13% retinol RDI and 150% of riboflavin RDI. The mushroom-termite composite soup with

2.5% and wheat-termite composite buns with 5% termite had the highest consumer preference rating. The mushroom-termite composite provides 61.24% of the protein RDI while the wheat-termite composite buns provide 34.73% of the protein RDI on consumption of 100g of the product. The research has shown that the insects and their products are a rich source of nutrients required by the body.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Insects have played an important part in the history of human nutrition in Africa, Asia and Latin America. There are more than 400 known species of edible insects (Allotey and Mpuchane, 2003) in the world. In Africa, many species of insects have been used as traditional foods among indigenous people and have played an important role in the history of human nutrition. Insects were an equally important resource for the Indians of western North America who like other indigenous groups, expended much organization and effort in harvesting them (Sutton, 1988).

Some of the important groups of insects used as human food include grasshoppers, caterpillars, beetle grubs and (sometimes) adults, winged termites (some of which are very large in the tropics), bees, wasps and ant brood (larvae and pupae) as well as winged ants, cicadas, and a variety of aquatic insects. Termites are eaten in several parts of western Nigeria. The winged adults are usually caught while on their nuptial flight or collected from the mound after they have shed their wings and then roasted for eating (Banjo *et al*, 2006). 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.

In Kenya, the most common edible insects are the termites, which are highly appreciated across the country. The sexual winged forms (reproductive forms) of termites are frequently caught for food (Yagi, 1998). In Lake Victoria region of Kenya, the local communities can easily distinguish the species and the emergence patterns of termites.

The long-horned grasshopper is a delicacy in some parts of Lake Victoria region. During certain times of the year, usually after the first rains following a dry season, the grasshoppers swarm (Bailey and McRae, 1978). The grasshopper is non-destructive, as it causes no major damage to crops and vegetation and this makes them unique from locusts. They are attracted to light in the evenings and are thus easily collected. During their swarming season, children and their parents use saucepans, baskets and polythene bags, which they fill with thousands of the insects, which swarm around streetlights every evening (Odhiambo, 1978).

Edible insects are prepared in various ways for eating. It is popular to fry them lightly in their own fat over low heat, add a little salt and sometimes remove the wings. Fried termites are tasty after being dried in the sun and they can be consumed for a rather long period of time. Raw termites are also frequently eaten. In some areas of western Kenya, sun-dried termites are packed in various containers and sold in the local food markets. They are sometimes transported over long distances to urban markets of East African large cities such as Kisumu, Kampala or even Nairobi (Yagi, 1998). The long-horned grasshoppers are eaten raw, after the wings have been pulled off, but more often they are fried. They are said to be protein-rich and add important nutrients to the diet (Kent, 2002).

Certainly, edible insects have come along way regarding their acceptance as food resource nationally, regionally and perhaps internationally. However, entomophagy is decidedly conditioned by cultural biases. Many areas of the world have and still derive part of their protein from insect sources. Why then have we not taken advantage of the presence of many and diverse species of insects on the continent by promoting their incorporation into diets? Considering the chronic or seasonal shortage of vertebrate protein reserves in sub-Saharan Africa, utilization of insects, as alternative food source on a wide scale should be encouraged. Nutrients useful in the maintenance of human health may be obtained by direct consumption of insects. Food supplies in many countries in Africa are inadequate both in quantity and quality, contributing to the widespread malnutrition on the continent (Kent, 2002; Allotey and Mpuchane, 2003).

Since insects are so abundant and contain many useful nutrients, including proteins and calories, they may help in alleviating malnutrition in Africa. Malnutrition continues to kill many children, act as a catalyst in various childhood diseases, exacerbate rates of illiteracy, unemployment and impede overall socio-economic progress in Kenya (Oniang'o and Mutuku 2001).

1.2 Problem Statement

Entomophagy has been an indigenous practice among African communities. In East Africa, it has been practiced in the Lake Victoria region where various insects form part of the food culture. There are claims by the consumers that the insects provide essential nutrients to the human diet. There is need therefore to understand whether these insects actually provide the essential nutrients required by the body for a healthy growth. In addition, information is needed in order to understand whether the insects can be incorporated into a food product so as to be of economic value.

1.3 Justification

Termites and grasshoppers have been a delicacy in the diet of communities living in Lake Victoria region of East Africa for centuries. These insects are also a source of income as they are harvested and sold in local markets, though on a small scale. Nutrients essential in the maintenance of human health may be obtained by direct consumption of these insects. However, with modernization, there has been a decline in entomophagy, as the practice is perceived as primitive although the insects have an economic and nutritive potential to humans. This study was therefore geared towards assessing the nutrient composition of selected edible insects consumed in the Lake Victoria region of Kenya hence enhance consumption by utilizing the insects in modern foods. This was by their utilization in formulation and improvement of food products that have a commercial potential.

1.4 Hypothesis

The hypothesis tested was: -

Insects consumed in the Lake Victoria region of Kenya contains nutrients required by the human body and the insects can be utilized in food product development.

1.5 Objectives

1.5.1 Main Objective

To assess the nutrient composition of selected types of insects consumed in Lake Victoria region of Kenya and utilize them in formulation and improvement of food products that have a commercial potential

1.5.2 Specific Objectives:

The specific objectives were to:

1. Determine the insects commonly consumed in the region.
2. Determine the nutrient composition of the edible insects and characterize the insects' oil.
3. Assess the influence of processing methods of the insects on their nutrient content.
4. Develop food products by incorporating the insects and evaluate the sensory, acceptability and nutritional quality of the new food products.

CHAPTER TWO

LITERATURE REVIEW

2.1 Entomophagy

Entomophagy is the habit of eating insects as food. The term is used to describe human insect-eating habits that are common in some cultures in parts of the world including Africa, Central and South America, Asia and Australia, but uncommon and even taboo in some societies. Entomophagy is also found in a large number of taxonomic groups including insects (that eat other insects), birds and mammals (Kumar, 2001).

Throughout human history, insects have served as a supplementary food source. All the major insect orders, including *Lepidoptera* (moths and butterflies), *Hymenoptera* (bees and ants), *lsoptera* (termites), *Coleoptera* (beetles), *Hemiptera* (true bugs), and *Ortboptera* (locusts and grasshoppers) have been eaten in Africa (Ohiokpehai *et al*, 1996). The consumption of insects in sub-Saharan Africa is widespread either as delicacies or as important components of the daily diet (DeFoliart, 1999). However, it is purely a cultural practice (Allotey and Mpuchane, 2003).

Insects are regarded as the largest group within the animal kingdom with over 80% of all living animals being insects. About one million species of insects are known and over 7,000 new species are described every year (Allotey and Mpuchane, 2003). Prominent reasons for their success are: ability to live in and adapt to diverse habitats, high reproductive capacity, ability to consume different kinds and qualities of food, and the ability to escape quickly from their enemies (Kumar, 2001). Insects play both negative and positive roles in the lives of humans. They may destroy crops as pests and transmit diseases to man as vectors. However, not all insects are pests or vectors. The majority are harmless and many are beneficial (Allotey and Mpuchane, 2003) to mankind.

DeFoliart (1995) has established that 1000 insect species have been used as traditional foods by humans and they still form an important part of the nutritional intake and economy of many societies. In addition, over the last two decades the importance of insects as a commodity has been increasingly recognized (Hardouin, 1995; Illgner and Nel, 2000) and are now regarded as a class of mini-livestock (which includes large land snails, guinea pigs and lizards). These activities are attracting increasing attention and support from international organizations such as the Centre Technique de Cooperation Rurale et Agricole (CTA) and the Food and Agricultural Organizations (FAO) (Hardouin, 1995).

Various methods for collecting edible insects are applied. In one method, a tent-like structure is built consisting of branches and leaves to cover some of the emergence holes. By closing the other holes, the termites have to emerge from the holes in the tent structure, which has an opening on one side to which the flying termites are attracted by sunlight, artificial light or moonlight. Near this opening, a receptacle is placed to collect the termites. In another method, a light source introduced inside a bucket lined with wet slippery banana leaves, and then the bucket is placed near an active mound. Attracted by the light, termites drop in the bucket. In the dry season, termites can be induced to come out when stimulated by fumes of smoke from burnt dried leaves of specific wild plants or the slow rhythmic vibrations created by striking stones or by beating a large piece of wood with two sticks (Yagi, 1998).

2.2 Termites

Termites belong to the order *Isoptera* and are small, pale, soft insects primarily cellulose feeding. They are divided into castes, the most numerous caste are relatively undifferentiated and perform much of the colony work, there is a specialised soldier caste with head and jaw structures differentiated with stronger features and often mouthparts more suited to defense than feeding. The reproductive caste, known as alates (winged), are produced when nymphs matures to develop wings and a generally darker colouring (Illgner and Nel, 2000). Colonies of some termite species build huge earthen mounds, called termitaria, which may be up to 6m high. Periodically, the winged adults emerge in huge swarms, mate while in flight, and then start new colonies. Termites are collected throughout most of Africa as a kind of snack, but in some places, especially in the semi-arid savannah zones, termites do provide an essential element of the diet among the non-livestock keeping groups (Odhiambo, 1978). Traditionally, the insects are harvested soon after the rains begin by digging a hole near the base of their mound, then knocking the mound over and lighting a fire near the hole. The emerging winged termites are stupefied by the smoke and fall into the hole, from which they are scooped and stuffed into leather bags to suffocate. They are then dried in the sun, the wings are removed, and the bodies pounded into a paste, which is either eaten alone, or with honey (Yagi, 1998).

Various species of termites are consumed around Lake Victoria region of Kenya, which are distinct in their features and behaviors (Christensen *et al,* 2006). At the onset of a rainy season, male and female long winged reproductive fly off from their nest in large numbers. This is called ‘Swarming’ (Illgner and Nel, 2000). During this flight, known as the nuptial (wedding) flight, pairs of male and female termites isolate themselves from the others and fall to the ground. Their wings then break off and each pair goes its own way to form a nest in a suitable spot. They begin by making a few tunnels in the ground, which eventually forms the nest. In a new nest, the male is the potential king while the female is the potential queen (Ekpo and Onigbinde, 2007).

Winged termites emerge with the first rains at the ends of the dry season. The termite is usually attracted to sources of illumination at nights and may be found, also, in the early hours of the mornings and it is a highly relished food item in Nigeria, especially among children (Ekpo and Onigbinde, 2007). At elevations of about 1800m the termites are smaller and do not build tall mounds. They are harvested differently. A hole is dug about 20cm in diameter and 20cm deep, about a metre from where the termites are expected to emerge. It is lined with smooth, neatly overlapping leaves. A piece of hide, to exclude the sunlight, is supported by twigs from the termite exit hole to the pit that has been dug. The emerging sexuals, unable at first to use their wings, crawl toward the light at the end of the hide-covered tunnel and fall into the pit, from which they are unable to escape because of the smooth leaf lining. They are gathered in bags and taken away to dry. Termites from yet higher elevations are not collected, as they are very small and said to be bitter (Illgner and Nel, 2000).

The usual steps in the processing of the insect include dewinging, roasting and salting, or grinding into flour. They are usually consumed as part of a meal or as a complete meal with tapioca, bread, roast corn, or simply eaten as snack food. They provide important protein, fat and vitamins. In East Africa, termite mounds are considered so important that they are owned by individuals and sometimes form part of his inheritance when he dies (Banjo *et al*, 2006).

2.3 Grasshopper

Grasshopper belongs to the order orthoptera. Tetigoniidae is a sub-order of herbivorous insects of the order orthoptera, commonly called grasshoppers and it includes short-horned grasshoppers, long-horned grasshoppers and locusts. The tetigoniidae have antennae that are shorter than the body, and short ovipositors. Those species that make easily heard noises usually do so by rubbing the hind femurs against the forewings or abdomen, or by snapping the wings in flight. Tympana, if present, are on the sides of the first abdominal segment. The front femora are long and strong, fitted for leaping. Generally they are winged, but hind wings are membranous while front wings (tegmina) are coriaceous and unfitted for flying. Females are normally larger than males, with short ovipositors.

During the rainy season, large swarms fall in the fields on grasses and shrubs and they are then collected for food in the early morning before they can be able to fly. The grasshoppers are consumed in many forms during their swarming seasons. The usual steps in the processing of the insect include dewinging, roasting and salting, or grinding into flour though they are also consumed raw. They are usually consumed as part of a meal or as a complete meal with tapioca, bread, roast corn, or simply eaten as snack food (Bailey and McRae, 1978). Bailey and McRae (1978) further reported that the long-horned grasshopper has not been previously associated with damage to crops in the east Africa region. Illigner and Nel (2002) reported that in Nigeria, grasshoppers are collected by children while in South Africa women play a major role in collecting them.

2.4 General nutritional value of various insects

2.4.1 Protein

In the dried form most frequently found in village markets of the developing world, insects are very high in crude protein, many species ranging above 60%. Finke *et al*, (1989) reported that the house cricket [*Acheta domesticus* (L.)], when fed to weaning rats, was superior to soy protein as a source of protein at all levels of intake. On the other hand, whole insects as a source of protein are of somewhat lower quality than vertebrate animal products because of the indigestibility of chitin (Phelps *et al,* 1975; Dreyer and Wehmeyer, 1982). Despite this, Dreyer and Wehmeyer (1982) concluded that, the consumption of mophane caterpillars (*Gonimbrasia belina)* can to a substantial degree supplement the predominantly cereal diet with many of the protective nutrients'. Removal of chitin increases the quality of insect protein to a level comparable to that of products from vertebrate animals. The weaver ant common in China contains 42% - 67% protein and is rich in amino acids (Chen, 1994).

2.4.2 Lipids

Insects vary widely in fat content. Isoptera (termites) and lepidoptera (caterpillars) rank among insects with the highest fat contents. Oyarzun, *et al,* (1996), reported that in the reproductive caste of the termites (Nasutitermes), mature alates have a fat content of 40.2% while Banjo *et al,* (2006) reported 19.7- 24.1 % for immature alates (nymphs) of four other termite species. Cholesterol levels in insects vary from as low as none in the edible leaf-cutter ant (*Atta cephalotes* L.) to approximately the levels found in other animals (1 mg sterol/g tissue), depending on species and diet (Ritter, 1990). Insect fatty acids are similar to those of poultry and fish in their degree of unsaturation, with some groups being rather higher in linoleic and/or linolenic acids, which are the essential fatty acids (DeFoliart, 1991). Palmitic and oleic acids are the major fatty acids in the insect oil (Ekpo and Onigbinde, 2007). Some researchers have also reported significant amounts of linoleic and linoleic acids (DeFoliart, 1991) while others have small amounts of arachidonic acid (Ekpo and Onigbinde, 2007).Significant amount of triacylycerides and diacylglycerides have also been reported in edible termites as well as phospholipids and glycolipids (Banjo *et al*, 2006).

2.4.3 Vitamins and minerals

The caterpillar, *Usta terpsichore* M. & W. (Satumiidae) from Angola was found to be a rich source of iron, copper, zinc, thiamin and riboflavin (B2); 100g of cooked insect provided > 100% of the daily requirement of each of these minerals and vitamins (Oliveira *et al*, 1976). Winged adults of the termite, *Macrotermes subhyalinus*, are high in magnesium and copper, and the palm weevil larva, *Rhynchophorus phoenicis* F., in zinc, thiamin and riboflavin. In each case, 100g of these insects were found to provide more than the minimum daily requirement (Banjo *et al*, 2006).

In Zaire, Kodondi *et al*, (1987) analysed three species of caterpillars prepared by the traditional techniques of smoking and drying, and found them to be high in riboflavin and niacin, but low in thiamin and pyridoxine (B6). Feeding trials confirmed that, except for thiamin and pyridoxine, the vitamins supplied by the caterpillars are sufficient to allow proper growth of young rats. The caterpillars studied by Kondodi *et al,* (1987), also in Zaire, proved an excellent source of iron; of 21 species tested, 100 g of insects provided (average value) 335% of the minimum daily requirement. *Sphenarium* grasshoppers are high in niacin, while *axayacatl* is a rich source of iron.

The high content of iron and zinc in many edible insects is of particular interest. Iron deficiency is a major problem in women's diets in the developing world particularly among pregnant women, and especially in Africa (Orr, 1986). Barker *et al,* (1998) reported retinol content in insects ranging from undetectable in fruit flies to 0.7 µg/g in wild-caught earthworms and a-tocopherol levels ranging from of 46.9 µg/g in wild – caught earthworms to 153.6 µg/g in commercially raised earthworms.

2.4.4 Fibre

Chitin comprises approximately 10% of whole dried insects (Oyarzun *et al*, 1996). It is a carbohydrate polymer found in invertebrate exoskeletons, protozoa, fungi and algae, and is being referred to as the polymer of the future because of its abundance, toughness and biodegradability (Goodman, 1989). Numerous applications of chitin and its derivatives (especially chitosan) are being found in medicine, agriculture and industry. Chitin from shells of lobsters, crabs and crayfish has been approved by the Japanese for use in cereals as a source of fibre and calcium. Cellulose and lignin has also been detected in digestive components of termites as they feed on wood, a cellulose and lignin-rich material (Oyarzun, *et al,* 1996).

2.5 Economic potential of edible insects

Since the 1970s insects have been evaluated as a potential feedstuff for poultry and other food-producing animals. The insects evaluated include house flies, Mormon crickets, silkworm larvae, house crickets and several other species (Finke, 2002;). Attempts have been made to apply industrial methods to the production of insects as food (Kok *et al*, 1991). Commercially grown insects available in the developed countries include the cricket, *Acheta domesticus*, the mealworm, *Tenebrio molitor* L. (a beetle grub), and the greater waxmoth larva, *Galleria mellonella* (L.) (Finke, 2002).Several processed insects are commercially available in Japan (Mitsuhashi, 1988; Kantha, 1988).

2.6 Potential hazards associated with consumption of insects

Some insects secrete toxins, produce toxic metabolites or sequester toxic chemicals from food plants (Duffey, 1980; Wirtz, 1984). Defensive secretions that may be reactive, irritating or toxic include carboxylic acids, alcohols, aldehydes, alkaloids, ketones, esters, lactones, phenols, 1,4-quinones, hydrocarbons and steroids, among others. Phytochemicals sequestered by various insects include simple phenolics, flavin, tannins, terpenoids, polyacetylenes, alkaloids, cyanogens, glucosinolates and mimetic amino acids. Insects are also a source of injectant, ingestant, contactant and inhalant allergens (Wirtz, 1984; Gorham, 1991), and some insects serve as vectors or passive intermediate hosts of vertebrate pathogens such as bacteria, protozoa, viruses or helminths (Gorham, 1991). More attention should be directed toward assessing these risk factors in the edible insect groups. The long history of human use suggests, however, with little evidence to the contrary, that the insects intentionally harvested for human consumption do not pose significant health problem.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental design

The project was a cross-sectional study set in a complete randomized design. The insects were randomly collected from the farmer's fields and the species analyzed separately during laboratory analysis and product development.

3.2 Sampling area

The study area consisted of Kanyaboli and Kadenge Sub-locations of West and Central Alego Locations, Siaya District, Nyanza Province of Kenya. The area was selected due to the large availability of the insects.

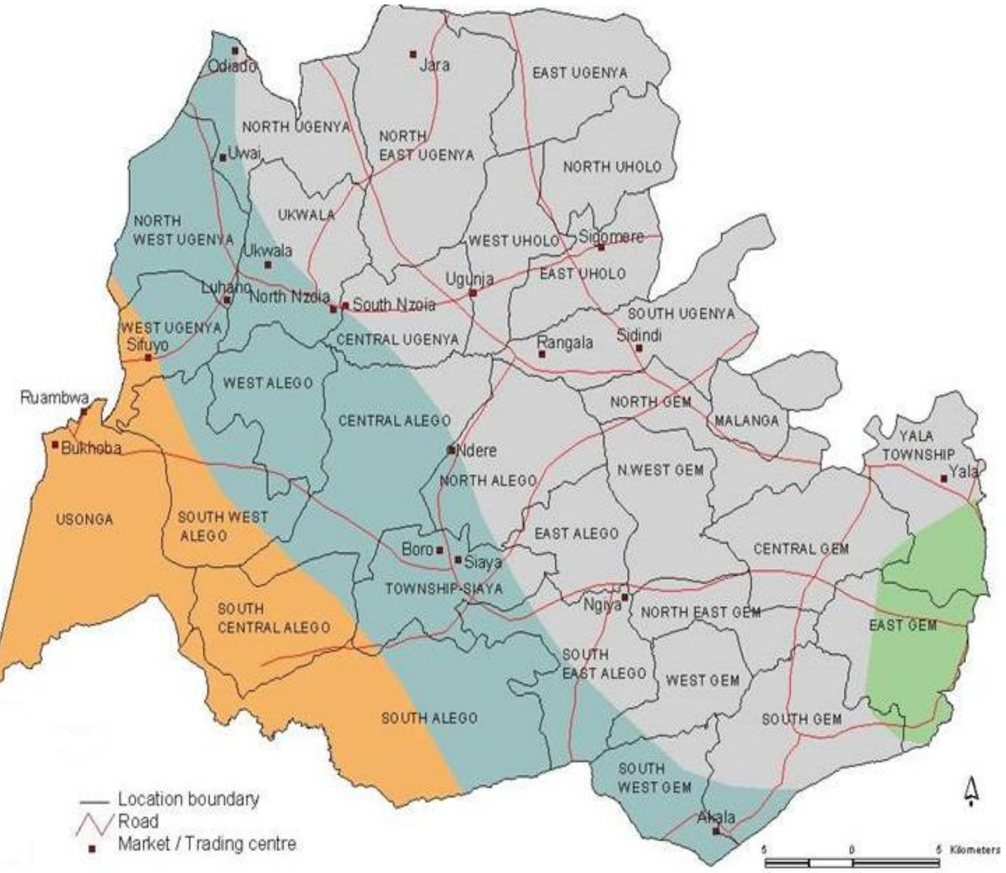


Fig. 1: Map of Siaya district; Source: Ministry of Finance & Planning, Siaya District Development Plan (2002 – 2008).

Fig. 2: Map of Kenya showing Siaya District, bordering Lake Victoria
Source: www.bushdrums.com- 2006

3.3 Identification, collection and characterisation of edible insects

The information on the insects was acquired through personal observation and informants living in Kanyaboli and Kadenge Sub-location of Siaya District. A total of six villages were selected namely; Urembo, Uradi, Wang', Chieny, Nyaseda and Liganwa. A total of twelve families were selected and one adult from each family was a key informant. Guiding questions (Appendix 1) were used to get information with regard to the insects consumed in the village and identification of the collection sites in the fields. The insects collected were available during the time of the fieldwork during the short rains season from March through May, and the long rain season from September through December. The samples were collected in triplicates in 2007 and a replication done in 2008.

Termites were attracted by light from a lantern lamp causing them to fall in large swarms that were collected and put in a clean container. Three nests were identified in each village and 0.5 kg of insects were collected from each nest.
The green and brown grasshoppers were collected early in the morning between 6.00 and 7.00 am when they were inactive and therefore could not fly. A sample weight of 0.5kg was collected for each grasshopper.

Each of the insect sample were divided into two equal groups and one group sent to the Department of Entomology at Maseno University for taxonomic identification by an entomologist. The other group was then put in plastic bags and kept cool boxes with dry ice and transported to the Food Biochemistry laboratory, Jomo Kenyatta University of Agriculture and Technology for laboratory analysis within 12 hours of collection. The analysis commenced immediately upon arrival in the laboratory. The wings of the whole insects were plucked off manually before laboratory analysis. Both insect species were analysed for their proximate composition, minerals, fatty acids and selected fat and water soluble vitamins in triplicates before frying and/or drying.

3.4 Determination of the proximate composition of the insects

The samples were analyzed for moisture content by drying method, crude fat by Soxhlet method, crude protein by semi-micro-kjeldhal method, crude ash by incinerating in a muffle furnace at 550^{o}C and crude fibre determined by Hennenberg-Stohman method (AOAC, 1996).

3.5 Quantification of the minerals in the insects

The quantification of calcium, magnesium, potassium, phosphorous, sodium, iron, zinc, copper and manganese was done by atomic absorption spectrometry using AAS (Shimadzu AA-6200) according to AOAC methods (AOAC, 1984).

3.6 Analysis of fatty acid composition of the insects' lipids

The fatty acid profile was determined by gas chromatography. The extraction of the lipids was done by a modification of the Bligh and Dyer method (1959). Samples of each 1g, were crushed and placed in a glass stoppered centrifuge tube and denatured at 100^{o}C for 3 minutes. Then 2ml of water and 7.5ml of methanol-chloroform (2:1 v/v) was added and the mixture shaken overnight at room temperature. The samples were centrifuged and the supernatant decanted and the residue resuspended in 9.5ml of methanol-chloroform-water (2:1:0.8), the homogenate was centrifuged. Then 7.5ml each of chloroform and water were added to the supernatant and the mixture centrifuged. The chloroform phase was extracted and dried in a vaccum rotary evaporator at 40^oC. The residue was completely dried in a dessicator over KOH pellets. Then the lipid was methylated by placing 2mg of the sample in a flask and refluxing with 2ml of 95% methanol-HCl for 1 hour. The methyl esters were extracted with 3 portions of hexane (1ml) and then washed with distilled water (3ml). The hexane layer was dried in vaccum rotary evaporator and the residue redissolved in a small drop of hexane. Then 0.2ul was injected into the GC (Shimadzu GC-9A) with a capillary column, supelcowax 30m x 0.53mm; injection/detection temperature, 220^oC under a flame ionization detector. Identification of the fatty acid methyl esters was by comparison of retention times with standards and expressed as percentages of total methyl esters.

3.7 Determination of retinol and α-tocopherol content in the insects

Retinol and α-tocopherol were analyzed by HPLC using a modification of the method of Barker *et al* (1998). Duplicate tissue samples (2.0g) were homogenized with 4ml of 95% ethanol and 1ml of 50% KOH. The mixtures were saponified by heating in a 70°C water bath for 15 minutes and then cooled in an ice bath. Fat-soluble vitamins were extracted with 1ml hexane containing 0.2% BHT, and a 1ml aliquot of the hexane layer was evaporated under nitrogen. Saponification, extraction, and evaporation procedures were performed under yellow light. Samples were then reconstituted with 0.25ml ethanol containing 0.1% BHT. A Shimadzu LC-10A VP Series liquid chromatograph equipped with a 250 x 4.0 mm stainless steel ODS reversed-phase column was used to quantify α-tocopherol, and retinol as measures of vitamins E and A, respectively. The mobile phase was 96:4 methanol: water, for α-tocopherol detection, and 90:10 methanol: water for retinol separation at a flow rate of 1 ml/min. The α-tocopherol was monitored at 285 nm wavelength and retinol at 325 nm on a UV-VIS detector (Shimadzu LC-10A). External standards were compared to sample extracts for determination of vitamin concentrations.

3.8 Determination of water-soluble vitamins in the insects

The water-soluble vitamins were determined according to the method by Ekinci and Kadakal (2005). The sample was prepared using solid-phase extraction (SPE) in order to remove components that may cause interference with vitamins. For this reason the sample treatment proposed consists of SPE with sep-pak C_{18} (500mg) cartridges that enable separation of water-soluble vitamins and remove most of interfering components. Twenty grammes of deionised water were added into 5g. The mixture was homogenized using a homogenizer at medium speed for 1 minute. The homogenized samples were centrifuged for 10 minutes at 14 x 10^3g. The stationery phase was flushed with 10ml methanol and 10ml water adjusted to pH 4.2 to activate the stationery phase. Homogenized and centrifuged samples (10ml) were then loaded. The sample was eluted with 5 ml water (pH 4.2) then 10ml methanol at a flow rate of 1ml/min. The eluent was collected in a bottle and evaporated to dryness in a rotary vacuum evaporator. The residue was dissolved in the mobile phase. Before HPLC analysis, all the samples were filtered through 0.45 µm pore size. Samples (20µl) of solutions of the water-soluble vitamins were injected into the HPLC column and monitored on a photo-diode detector (Paris Waters 2996). The column eluate was monitored with a photodiode-array detector at 265 nm for ascorbic acid, 234 nm for thiamine, 266 nm for riboflavin, 234 nm for pyridoxine, 282 nm for folic acid and 261 nm for niacin. The mobile phase was filtered through a 0.45 um membrane and degassed by sonication before use. The mobile phase was 0.1 mol/ L KH_2PO_4 (pH 7): methanol (90:10) and reverse phase C-18 column. The flow rate was 0.7ml/min at room temperature (25^{o}C). Identification and quantification of compounds was achieved by comparing their retention times with those of standards of known concentrations.

3.9 Extraction and physicochemical characterization of the insects' oil

The samples were ground and the oil extraction done by solvent extraction method (AOCS, 1997). The extract was the filtered and the solvent evaporated in a vacuum rotary evaporator at 40^{o}C. The resultant oil was then subjected to determination of iodine value, saponification value, peroxide value, total cholesterol, physical properties and lipid classes.

3.9.1 Determination of chemical characteristic

Iodine value, saponification value and peroxide value were determined by AOAC (1996) methods.

3.9.2 Determination of total cholesterol

Total cholesterol was determined by Liebermann-Buchard method as described by Barreto (2005). Samples (0.5ml) of lipid were measured into a test tube and 5.0ml of Liebermann-Buchard reagent added. The test tubes were vortex and placed in a water bath at 35ºC for 10 minutes after which the absorbance measured at 550 nm using a Spectronic 20 photometer. Quantification of the total cholesterol in the samples was done by external standards and comparing the absorbance.

3.9.3 Determination of physical characteristics

The specific gravity of the oil was determined by pycnometry, refractive index was determined using Abbe refractometer (AOAC, 1996). The solidification temperature was determined by open capillary method (AOAC, 1996).

3.10 Analysis of the lipid classes of the insects' oil

The lipids were extracted according to Bligh and Dyer method (1959) and the lipid fractions in the samples determined by thin layer chromatography. For neutral lipids, hexane: ethyl ether: acetic acid (80:20:1) solvents were used while for the polar lipids, chloroform: methanol: water (65:25:4) solvents were used for separation in a 250cm^3 separation tank after spotting on TLC plates (silica gel 250 µm layer flexible plates). Then 50% H_2SO_4 was sprayed on the plate after separation and the plates heated on a hot plate. All the lipid spots were charred and appeared as black spots and the various classes of lipids identified by spotting standards. The intensity of the spots was quantified by densitometry using a flying spot scanning densistometer (Shimadzu CS: 9000) and the values obtained used to give the fraction of the lipid classes.

3.11 Determination of the influence of frying and solar drying on the protein digestibility

Digestibility of protein in the insects was determined by the method outlined by Mertz *et al*, (1984). Initial protein content of the samples was determined using semi-micro-kjeldahl nitrogen determination method. The second stage involved pepsin digestion, where 0.2g of the sample was weighed into centrifuge tubes. Then 20ml buffered pepsin was added and mixed. A blank was prepared in the same way but without a sample. The tubes were placed in a water bath at 37ºC for 2 hours with gentle shaking after every 20 minutes. The tubes were then placed in an ice bath for 30 minutes to attain a temperature of 4ºC followed by centrifugation at 6000 rpm for 15min. The supernatant was discarded and 10ml of buffer solution added, then shaking and centrifugation was done again. The supernatant was discarded and the residue filtered using a filter paper. The filter paper was rolled and inserted into a Kjeldahl flask and dried for 15 minutes in the oven. Ten milliliters of concentrated sulphuric acid, 1g potassium sulphate and 1ml of 10% copper sulphate solution were added to the Kjeldahl flask containing the dried filter paper and sample. Then digestion, distillation and titration were done according to the micro-Kjeldahl nitrogen determination.

Protein digestibility (%) = (A - B)/A

Where A = % protein content in the sample before pepsin digestion

B = % protein in the sample after pepsin digestion

3.12 Determination of the influence of frying and solar drying on the vitamins and fatty acids of the insects.

The influence of frying and drying on the vitamins and fatty acids was done. The fresh samples (200g) were fried without addition of oil in a stainless steel cooking pan over an open flame for 5 minutes. The frying was done according to the traditional practice at the villages where the insects are fried in their own oil. Then 100g of fried samples was cooled to room temperature and subjected to solar drying immediately. Solar drying was done in solar drier at the BEED at JKUAT using a solar drier (Plate 1). The samples were evenly spread on the wire mesh trays on the drier and turned severally to dry. The drying temperature was approximately $30^{o}C$ with a relative humidity of approximately 40% and the samples were dried until the moisture content was below 10% as determined by the AOAC method (AOAC,1996).

Plate 1: Solar drier used in the study

3.13 Preparation of termites for product development

The process for preparation of termites for development of products is shown below (Fig. 3).

Fresh termites were washed in chilled water to maintain freshness and remove dirt.

↓

Termites were spread on perforated plastic trays to drip and dry.

↓

The trays were put on drying racks in the solar drier and turned twice a day, in the morning and in the evening, during the drying period to constant weight (<10%).

↓

Dried termites were removed from the drier and scattered in a room to cool (23^oC –25^oC).

↓

A hand operated electric blower producing a constant draft was used to blow away the wings. The wings detach upon drying.

↓

Termites were ground and sieved with a 250 µm mesh sieve. Particle sizes <250 µm were used for product development.

↓

Ground termites were packaged in polythene bags.

Fig. 3: Flow chart showing the preparation of insects for product development

3.13.1 Development of a mushroom-insect composite flour

The mushroom-termite composite was prepared according to the formulations shown in (Table 1), where products B – F were prepared. The product A formulation for mushroom soup was obtained from Biosafe Co. Ltd which is an already existing product and this study sought to supplement mushroom powder with ground termite at the ratios of 2.5% to 50%.

Table 1: Formulation for the mushroom-termite composite flour

	Product					
Ingredient (%)	A	B	C	D	E	F
Ground insect	0.0	2.5	5.0	10.0	20.0	50.0
Mushroom powder	75.0	72.5	70.0	65.0	55.0	25.0
Corn starch	10.0	10.0	10.0	10.0	10.0	10.0
Skimmed milk powder	10.0	10.0	10.0	10.0	10.0	10.0
Salt	1.5	1.5	1.5	1.5	1.5	1.5
White pepper	3.0	3.0	3.0	3.0	3.0	3.0
Monosodium glutamate	0.5	0.5	0.5	0.5	0.5	0.5

Mushroom powder with ≤ 250 μm particle size was obtained from a local producer, Biosafe Co. Ltd. All the other ingredients were purchased in a ready to use form from the local market. The process for development of mushroom-termite composite soup is shown (Fig. 4).

Each of the ingredients was weighed using an

analytical weighing balance.

↓

The ingredients were mixed as in the ratios shown in Table 1

↓

The composite was packaged in polythene bags

for use in product development.

↓

Microbiological analysis was carried out immediately.

Fig. 4: Flow chart showing the preparation of mushroom-termite composite flour

3.13.2 Preparation of mushroom-termite composite soup

The preparation of the soup was done according to the recommendation by Biosafe Co. Ltd of mushroom soup preparation (Fig. 5).

Formulated product (20g) was weighed using an analytical weighing balance.

↓

Product was mixed with 200ml of water at room temperature and mixed by stirring with a cooking spoon to form a slurry.

↓

Mixture was heated in an aluminum cooking pot until boiling and held at boiling temperature for 5 minutes.

↓

Cooked product was cooled to room temperature and subjected to microbiological analysis as well as sensory evaluation.

Fig. 5: Flow chart showing the preparation of mushroom-termite composite soup

3.13.3 Microbiological analysis of the developed mushroom-termite composite product

Microbiological analyses of the developed mushroom-termite composite as well as the prepared soup were done. The microbiological procedures used were as recommended by AOAC (1996). Microbiological analyses included total aerobic plate count using nutrient agar, fungal count using potato dextrose agar, *E-coli* count using red violet bile agar and *salmonella* sp. count using salmonella shigella agar.

3.13.4 Sensory evaluation of mushroom-termite composite soup

Sensory evaluation of the mushroom-termite composite soup in relation to aroma, taste, appearance, texture and overall consumer preference were carried out using a questionnaire (Appendix 2) by twenty five untrained panelists recruited from staff and graduate students of the university. Samples of about 20 ml of the cooked product were placed in plastic cups at room temperature, coded with three-digit random numbers. A 7-point hedonic scale (1—dislike extremely to 7—like extremely) with equivalent intervals between the categories was used (Ihekoronye and Ngoddy, 1985). The assessment was carried out under natural light at a room temperature.

3.14 Development of wheat-termite composite bun

A wheat-termite bun was developed with substitution of the wheat flour with the dried and ground termite at 0%, 5%, 10% and 40% substitution. The formulation for the wheat bun is as shown (Table 2).

Table 2: Formulation for the wheat-termite composite buns

	Product			
Ingredient	0%	5%	10%	20%
Ground insect (g)	0.0	12.5	25.0	50.0
Bakers flour (g)	250.0	237.5	225.0	200.0
Fat (g)	12.0	12.0	12.0	12.0
Salt (g)	5.0	5.0	5.0	5.0
Yeast (g)	5.0	5.0	5.0	5.0
Water (ml)	142.0	142.0	142.0	142.0

The formulation for wheat bun was obtained from the Food Processing workshops at JKUAT. Baking was done using method of AACC (1995) and the specific volume of the product determined.

3.14.1 Determination of specific volume of the baked buns

Specific volume was determined by a modification of the method by Mepba *et al*, (2007). Bun volume was measured after cooling overnight using the millet seed displacement method. A box of fixed dimensions (14.00 x 5.70 x 8.95 cm) with an internal volume of 714.21 cm^3, was half filled with millet seed, shaken vigorously 4 times and then filled till slightly overfilled such that the overspill fell into the tray. The box was shaken again twice and then a straight ruler was used to press across the top of the box once to give a level surface. The seeds were emptied from the box into a receptacle and weighed. The procedure was repeated three times and the mean value for seed weight was noted. A bun was placed in the box and weighed seeds were used to fill the box and leveled off as before. The seeds were then emptied on another container and weighed (L). Specific bun volume was obtained by dividing the bun volume by its corresponding weight.

Volume of bun (V) = L x 714.21cm^3

Specific volume of bun = V/wt (cm^3/g)

3.14.2 Sensory evaluation of the prepared mushroom-termite composite bun

Sensory evaluation of the wheat-termite bun was performed 12 hours after baking. Size, crust color, texture, taste, aroma and overall consumer preference were evaluated using a questionnaire (Appendix 3) by twenty five untrained panelists recruited from staff and graduate students of the university. Samples wheat-termite composite bun were presented in plastic plates at room temperature, coded with three-digit random numbers. A 7-point hedonic scale (1—dislike extremely to 7—like extremely) with equivalent intervals between the categories was used (Ihekoronye and Ngoddy, 1985). The assessment was carried out under natural light at room temperature.

3.15 Statistical analysis

Descriptive analysis of the data was done. Analysis of variance (ANOVA) between samples and treatments was also done. Where significant differences were found, means were separated using Duncan's Multiple Range test (SAS, 2001).

CHAPTER FOUR

RESULTS AND DISCUSSION

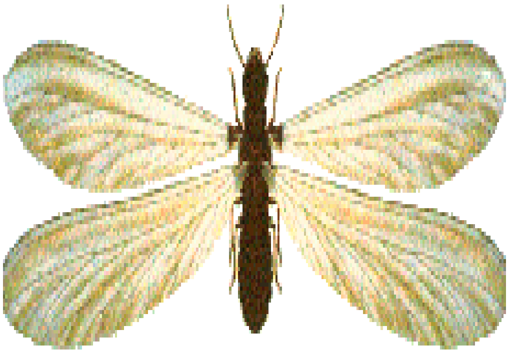
4.1 Selected edible insects from Lake Victoria region

The species of commonly consumed insects around Lake Victoria region were selected. They were identified taxonomically as shown in Table 3.

Table 3: Selected species of the edible insects

Order	Family	Scientific name	Common name	Local name	Edible stage
Isoptera	Termitidae	*Macrotermes subhylanus*	Termite	*Agoro*	Winged adults
Orthoptera	Acrididae	*Ruspolia differens*	Long horned grasshopper (Green)	*Senesene*	Adult
Orthoptera	Acrididae	*Ruspolia differens*	Long horned grasshopper (Brown)	*Senesene*	Adult

Macrotermes subhylanus is a gregarious insect, which in the Lake Victoria region is most common during the rainy season. The winged reproductives (alates) are dark brown and are the largest of all types of alates. They are usually attracted to sources of illumination at night and may also be found in the early hours of the mornings. The edible insects selected for this study are shown in plates 2 and 3.



A

B

Plate 2: Edible winged reproductive termite (A) and de-winged reproductive termite (B), *Macrotermes subhylanus*

Ruspolia differens is a grasshopper that is common in the Lake Victoria region. It expresses color polymorphism with green and brown coloration being the most common. However, more color forms such as purple have been reported (Bailey and McRae, 1978). The insects are collected during the early hours of the morning on the bushes and grass before they can be able to fly away. They are also attracted to illumination at night and can therefore be easily harvested.

A

B

Plate 3: Edible green (A) and brown (B) grasshopper (*Ruspolia differens*) on grassland

4.2 Proximate composition of the edible insects

The proximate composition of reproductive caste of termites and grasshoppers expressed on dry matter basis are presented in Table 4.

Table 4: Proximate composition of termite and grasshoppers

Parameter	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Moisture content (%)	58.3 ± 1.2^{c}	66.4 ± 0.5^{b}	71.2 ± 1.0^{a}
Ash (%)	2.6 ± 0.5^{b}	2.8 ± 0.5^{b}	2.6 ± 0.4^{b}
Crude Protein (%)	45.9 ± 0.2^{a}	43.1 ± 0.5^{b}	44.3 ± 0.7^{b}
Crude fat (%)	47.6 ± 0.1^{a}	48.2 ± 0.2^{a}	46.2 ± 0.3^{b}
Crude fibre (%)	2.9 ± 0.6^{c}	3.9 ± 0.1^{b}	4.9 ± 0.1^{a}

All values except moisture content expressed as means ± SE on dry weight basis of triplicates. Values on the same row followed by the same letter are not significantly different ($p>0.05$)
n = 6

There was a significant difference between the moisture contents of the termite and grasshoppers ($p \leq 0.05$) (Table 4). The difference may be attributed to non-uniform moisture loss from the insects due to the waxes on the surface that prevents water loss and the waxes may vary depending with species (Patel *et al,* 2001; Nelson *et al,* 2001).

The analysis of ash content of the termites (alates) gave an ash value slightly lower than the values indicated for alates of *Nasutitermes spp.* (3.7%) as reported by Oyarzun *et al,* (1996). There was no significant difference between the termite and the grasshoppers ($p>0.05$) in ash content. The values obtained from the insects were however consistent with the values of 2.1% to 6.8% of different species of termite alates and other edible insects as reported by Christensen *et al,* (2006).

The termites had significantly higher protein content than the grasshoppers ($p \leq 0.05$, Table 4) but was no significant difference between the green and brown grasshoppers ($p > 0.05$). Crude protein content of alates (45.85%) was lower than the values for other alates as reported by Oyarzun, *et al* (1996) at 48.8%. The values were however significantly higher than those for *Macrotermes bellicosus* (20.4%) and *Macrotermes notallensis* (22.1%) as reported by Banjo *et al*, (2005). The protein content of the grasshoppers was lower than the values reported for first instar stage (53.10%) and fourth instar stage of variegated grasshopper, *Zonocerus variegatus* L. (52.50%) (Adedire and Aiyesanmi, 1999). The protein content exhibited by the insects was significantly higher than that of red meats (Williams, 2007) and therefore insects may offer an affordable source of protein to counteract the protein malnutrition in Kenya (Kariuki and White, 1991).

The fat content of the termites (47.6%) shown in Table 4 was considerably higher than the fat values reported by Banjo *et al*, (2006) for immature alates (nymphs) of four other termite species (19.7 - 24.1%). The values were also slightly higher than those of *Nausitermes spp.* reported by Oyarzun *et al*, (1996) at 40.23%. Insects can offer a high fat content in the human diet among the communities practicing entomophagy. Fat is essential in the human diets because it increases the palatability of foods by absorbing and retaining their flavors (Aiyesanmi and Oguntokun, 1996). Fat is also vital in the structural and biological functioning of cells. However, one implication of the high fat content in the insects is that it may increase susceptibility of the undefatted insect to storage deterioration via lipid oxidation (Ekpo and Onigbinde, 2007).

There was a significant difference between the crude fibre content between the termite and grasshopper ($p \leq 0.05$) as shown in Table 4. However, the grasshoppers had higher values than the termites. The crude fibre values (2.7 – 2.9%) obtained were consistent with the values obtained by Banjo *et al,* 2006 for *Macrotermes bellicosus* and *Macrotermes notallensis,* 2.70% and 2.20% respectively. These fiber fraction values may represent a measure of the true fiber values of the termites' digestive contents, as these insects feed on wood, a cellulose and lignin-rich material. The alates are not considered as serious eaters as their main daily work is reproduction and therefore they may report a lower crude fibre than grasshoppers (Oyarzun *et al*, 1996).

4.3 Mineral contents of edible insects

The mineral profile of the insects is as shown in Table 5. Potassium was the most abundant among the macro minerals. Calcium was approximately ten times less than the amount of potassium. This is distinctive from vertebrate species with higher calcium and phosphorous contents. This could be logical as invertebrates have no bony skeleton. There was however a significant difference between the values from the insect species ($p \leq 0.05$). In addition, the green grasshoppers had significantly higher potassium content than the termites ($p \leq 0.05$). The termites had a slightly lower potassium value than the value reported by Oyarzun *et al*, (1996) {370 mg/100 g} but far much higher than what Ekpo and Onigbinde (2005) reported (26.65 mg/100 g) in larva of palm weevil (*Rhynchophorus phoenicis*) consumed in Nigeria.

Table 5: Mineral contents of the termite and grasshoppers

Mineral (mg/100g)	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Calcium	22.00 ± 0.90[c]	27.40 ± 0.01[a]	24.50 ± 0.01[b]
Magnesium	42.63 ± 1.50[a]	33.87 ± 0.02[b]	33.06 ± 0.29[b]
Potassium	259.60 ± 1.10[b]	370.60 ± 0.02[a]	259.70 ± 0.05[b]
Sodium	123.60 ± 1.20[c]	358.70 ± 0.01[a]	229.70 ± 0.02[b]
Phosphorous	182.30 ± 0.87[a]	140.90 ± 0.01[b]	121.00 ± 0.01[c]
Iron	11.52 ± 0.92[c]	16.63 ± 0.09[a]	13.01 ± 0.16[b]
Zinc	10.23 ± 0.20[c]	17.32 ± 0.01[a]	12.38 ± 0.02[b]
Manganese	3.29 ± 0.80[b]	5.26 ± 0.03[a]	2.46 ± 0.03[c]
Copper	1.70 ± 0.50[a]	0.63 ± 0.01[b]	0.47 ± 0.03[b]

All values expressed as means ± SE of triplicates on dry weight basis of triplicates; Values on the same row followed by the same letter are not significantly different ($p>0.05$); n = 6

There was significant difference in calcium content between the termite and the grasshoppers ($p\leq0.05$) as well as between the green and brown grasshopper. The values for the termites were however comparable with the values reported by Banjo et al, 2006 for *Macrotermes bellicosus and Macrotermes notallensis*, 21.0 mg/100 g and 18.0 mg/100 g respectively. The termite had a higher phosphorous value than the grasshoppers. There was a significant difference between the termite and grasshoppers ($p\leq0.05$) in the content of phosphorous

(Table 5) as well as between the green and brown grasshoppers. The values reported were also slightly higher than the values reported by Banjo et al, 2006 for *Macrotermes bellicosus* and *Macrotermes notallensis*, 136.0 mg/100 g and 114.0 mg/100 g respectively but approximately 50% less than values reported by Oyarzun *et al*, (1996). Unlike plant based phytate-phosphorus, the phosphorus in insects is likely to be readily available, as has been shown for facefly pupa (Finke, 2002).

Iron was consistently the most abundant mineral among the trace minerals among the insect species as shown in Table 5. The values obtained were significantly different ($p \leq 0.05$) between the termite and grasshoppers with the termites showing lower iron content than the grasshoppers. The values were however lower than the 24.6 mg/100g reported by Oyarzun *et al*, (1996) for *Nasutitermes spp.* of termites. This may be possible due to difference in species as well as possible soil contamination thus raising the iron content. There was significant difference ($p \leq 0.05$) between the termite and the grasshoppers in manganese content as shown in Table 5 even though the green grasshopper showed the highest amount of manganese than the other insects.

The concentrations of zinc (10.23 mg/100g) obtained from the termites were consistent with the values reported by Christensen *et al*, (2006) {8.1 to 14.3 mg/100 g} for termites consumed by Luo community from Bondo District of Kenya (Christensen *et al*, 2006). The termite showed the highest concentrations of copper 1.70 mg/100 g, being more than twice the values for green and brown grasshopper. Oyarzun *et al,* (1996) reported an average of 1.8 mg/100 g of copper in *Nasutitermes* spp. of termites a value that compared well with the value obtained from the samples in this research.

In general, mineral composition probably largely reflects the food sources of the insect (Oyarzun *et al,* 1996). Studies of wild insects also show variations between different populations of the same species living in the same general area (Finke, 1984). This may explain the difference between the green and brown grasshoppers in mineral contents. Contrary to the high potassium, zinc and iron contents in insects, calcium content has been reported to be relatively low compared to the Recommended Daily Intake (FAO/WHO, 2001) for all age categories (Onigbinde and Adamolekun, 1998). Even so, as long as insects are eaten for supplementation to the main meals, they will still provide an important additional calcium source. However, other means of supplementing the traditional diets with calcium in many African communities are important as well, since calcium deficiency and even rickets is a problem among many communities in Africa (Bwibo and Neumann, 2003).

Favourable potassium to sodium ratio renders the insects a potential component of the diets for the management of hypertension. Potassium intake as opposed to sodium intake has been found to lower blood pressure by antagonizing the biological effect of sodium (Einhorn and Landsberg, 1988; Akinnawo and Ketiku, 2000). The levels of minerals present indicate that the insects may be good sources of minerals for pregnant and lactating mothers. Iron and zinc deficiency is widespread in developing countries, especially in children and women of reproductive age (Christensen *et al,* 2006). Iron deficiency leads to anemia, reduced physical activity and increased maternal morbidity and mortality (Hallberg *et al,* 2000). Zinc deficiency causes impaired growth and contributes considerably to the high infectious disease burden (Walker and Black, 2004). Zinc deficiency has also been known to cause poor growth and impairment of sexual development in children (Chaney, 1997). The cereal based diets used for feeding infants and young children in most third world countries could receive a boost with the addition of the insects to the diets.

4.4 Vitamins in edible insects

A range of both water and fat soluble vitamins were detected from the insect samples analyzed (Table 6). The brown grasshopper had the highest retinol content while the termite had lowest retinol content. There was no significant difference ($p>0.05$) in retinol content between the termite and grasshoppers nor between the two grasshopper species. The levels of retinol observed in this study were higher than the values reported by Oyarzun *et al* (1996) who reported 0.65µg/g of retinol in alates of *Nasutitermes* spp. The authors however reported that the figure was unlikely, considering the high fat content of the alates and retinol being a fat-soluble vitamin. In addition, Oyarzun *et al* (1996) reported a high variability in retinol content between the termite castes, ranging from 0.65 µg/g for alates to 20.64 µg/g in worker caste of *Nasutitermes* spp of termite. The observed values were within the range reported by Banjo et al (2006), of 2.89

μg/g and 2.56 μg/g for the termites *Macrotermes bellicosus* and *Macrotermes notallensis*, respectively consumed in Southern Nigeria.

Table 6: Vitamin content of the termite and grasshoppers

Vitamin	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Retinol (μg/ g)	2.41 ± 1.48[a]	2.12 ± 1.92[a]	2.75 ± 0.38[a]
α-tocopherol (μg/ g)	62.41 ± 0.36[c]	201.01 ± 0.36[a]	152.0 ± 0.07[b]
Niacin (mg/100 g)	1.07 ± 0.77[c]	2.12 ± 0.05[b]	2.36 ± 0.80[a]
Riboflavin (mg/100 g)	3.39 ± 1.76[a]	1.20 ± 0.24[b]	1.36 ± 0.63[b]
Ascorbic acid (mg/100 g)	0.59 ± 0.03[a]	0.07 ± 0.01[b]	0.14 ± 0.12[b]
Folic acid (mg/100 g)	0.18 ± 0.02[b]	0.99 ± 0.35[a]	0.92 ± 0.04[a]
Pyridoxine (mg/100 g)	0.27 ± 0.07[b]	0.44 ± 0.2[a]	0.16 ± 0.1[b]
Thiamin (mg/100 g)	nd	nd	nd

nd - Not detected; All values as means ± SE on triplicates dry weight basis of triplicates; Values on the same row followed by the same letter are not significantly different (p>0.05); n = 6

The retinol contents reported for the termite represent 803.9 IU of vitamin A (conversion factors: 0.3 μg retinol = 1 IU) (Barker et al, 1998) while the retinol reported for grasshoppers represent 707.2 IU and 923.0 IU for green and brown grasshoppers respectively. These insects have been found to contribute significantly to the recommended daily intake (RDI) of infants, adult female and lactating females of 1249 IU, 2664 IU and 4329 IU respectively (NRC, 1989) per 100g serving. This shows that insects can be a good source of retinol to supplement the widely consumed plant food sources in the Lake Victoria region of Kenya (Ruel and Levin, 2000).

The grasshoppers had a higher α-tocopherol content than the termites as shown in Table 6. There was also variability between the two species of grasshoppers with a significant difference ($p \leq 0.05$) for both green and brown grasshopper. Oyarzun *et al*, (1996) reported 40.44 µg/g of α-tocopherol in alates of *Nasutitermes* spp. of termite while the soldiers had 84.45µg/g. The values found in this research compared closely with previous reports, with the termite showing 62.41µg/ g of α-tocopherol. The grasshoppers showed much higher values of α-tocopherol in the levels of 201.01µg/g and 152.00µg/g for the green and brown grasshoppers, respectively. The α-tocopherol detected in the termite represents 10.0 IU (conversion factors: 1mg α-tocopherol = 1.49 IU) (Barker *et al*, 1998) while the grasshoppers represent 29.95 IU and 22.64 IU for the green and brown grasshoppers respectively, on consumption of 100 g of the insect. These insects may contribute significantly to the RDI for humans, which ranges from 50 IU to 100 IU (National Research Council, 1989) per 100g serving.

The niacin content was relatively high in the brown grasshoppers while the green grasshopper had a lower value as shown in Table 6. The results obtained were comparable to the values for commercially raised adult crickets (3.84 mg/100g) and mealworm larvae (4.07 mg/100g) reported by Finke (2002). In addition, Rao (1994) reported that dried silkworm pupae contained 1.17 mg nicotinic acid/100g. This compares well with the values obtained for termite even though the grasshoppers reported a slightly higher value for the green and brown grasshoppers respectively. Consumption of 100g of termite was found to contribute 6.23% of RDI in adult males while the green grasshopper contributed 13% and the brown grasshopper 13.5% of niacin RDI. Niacin is an important component of the diet since it forms nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are important co-enzymes in energy metabolism. It also plays a role in fat synthesis and fat breakdown (McClenahan and Driskell, 2000). The termite showed a significantly a higher riboflavin content than the green and brown grasshoppers. There was, however no significant difference ($p>0.05$) between the green and brown grasshoppers in riboflavin contents. The values reported for the termite were higher than the values reported by Banjo *et al* (2006) for *Macrotermes bellicosus* and *Macrotermes notallensis* of 1.98 mg/100g and 1.54 mg/100g, respectively. The values obtained for the termite and grasshoppers were lower than for most of the insects reported by Finke (2002), which had less than 1.0 mg/100g in mealworm, wax worm, silk worm except adult cricket which was shown to contain 3.4 mg/100g of riboflavin. Consumption of 100g of termite was found to contribute 226% of RDI in adult males while the green grasshopper contributed 80% and the brown grasshopper 91% of niacin RDI. The termites showed a higher amount of ascorbic acid than grasshoppers as shown in Table 6. There was significance difference between the insect species ($p\leq0.05$) in ascorbic acid content. Oyarzun *et al* (1996) reported that no detectable amounts of ascorbic acid were found in *Nasutitermes* spp of termite collected in Venezuela. Finke (2002) reported a varied content of ascorbic acid ranging from less than 1.0 mg/100 g in silkworm to 5.4 mg/100 g in adult mealworm. Banjo e*t al* (2006) reported a comparable ascorbic acid content of 3.41 mg/100 g and 3.01mg/100 g for *Macrotermes bellicosus* and *Macrotermes notallensis* termites respectively. These values were much higher than the amounts observed in this study. This could however be attributed to the dietary intake of the insects. Folic acid reported in this study was consistent with value reported by Finke (2000) with most of the insects analyzed showing <1.0mg/100g of folic acid. However, no detectable amounts of folic acid were found in the earthworm analyzed by Finke (2002). Low amounts of pyridoxine were found in termite and both grasshoppers (<1.0 mg/100 g). A thiamine assay was also done but no detectable amounts were observed. This was consistent with the results reported by Finke (2002) for most of the edible insects analyzed. The insects analyzed were therefore lacking these two vitamins.

4.5 Fatty acid profile of the edible insects

The fatty acid profiles of the lipid fraction in the termite and grasshopper samples are as shown in Table 7. The results indicate that the grasshoppers' oil contained more saturated fatty acids than the oil from the termites.

Table 7: Fatty acid composition of the lipid fraction of the termites and grasshoppers

Fatty Acid (%)	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Capric acid (C10:0)	nd	0.36 ± 0.08[a]	0.22 ± 0.14[a]
Lauric acid (C12:0)	nd	nd	nd
Myristic acid (C14:0)	1.12 ± 0.11[a]	0.97 ± 0.63[a]	0.77 ± 0.41[b]
Palmitic acid (C16:0)	22.56 ± 0.90[b]	31.52 ± 0.68[a]	32.12 ± 0.23[a]
Palmitoleic acid (C16:1)	1.36 ± 0.23[b]	1.98 ± 1.11[a]	1.42 ± 0.05[b]
Stearic acid (C18:0)	7.24 ± 1.40[a]	5.49 ± 0.19[b]	5.99 ± 0.13[b]
Oleic acid (C18:1)	53.07 ± 1.21[a]	24.58 ± 1.50[b]	24.87 ± 1.22[b]
Linoleic acid (C18:2)	12.96 ± 0.96[c]	31.21 ± 0.25[a]	29.54 ± 0.20[b]
Linolenic acid (C18:3)	1.53 ± 0.02[b]	3.21 ± 0.23[a]	4.23 ± 0.54[a]
Total saturated [1]	30.92 ± 0.81[b]	38.34 ± 0.39[a]	39.10 ± 0.23[a]
Total unsaturated [2]	68.92 ± 0.80[a]	60.98 ± 0.77[b]	60.06 ± 0.50[b]
Monounsaturated [3]	54.43 ± 0.72[a]	26.56 ± 1.31[b]	26.29 ± 0.61[b]
Polyunsaturated [4]	14.49 ± 0.96 [b]	34.42 ± 0.24[a]	33.77 ± 0.37[a]

All values as means ± SE on triplicates dry weight basis. Values on the same row followed by the same letter are not significantly different (p>0.05); nd- not detected; Fatty acid content expressed as percentages of total methyl esters. Fatty acids are denoted by their common name, number of carbons and number of double bonds.

[1] Sum total percentage of 10:0, 12:0, 14:0, 16:0, 18:0

[2] Sum total percentage of 16:1, 18:1, 18:2, 18:3;

[3] Sum total percentage of 16:1, 18:1

[4] Sum total percentage of 18:2, 18:3

n = 6

Oleic acid was the predominant fatty acid in the lipid fraction in the termite species. This was in agreement with the findings of Oyarzun *et al* (1996) who reported that oleic acid was the major fatty acid (51.1%) in *Nasutitermes spp.* of termites collected in Venezuela. Palmitic acid was the major saturated fatty acid in the green and brown grasshopper. Linoleic acid was only slightly lower in content.

Other researchers have reported small amounts of arachidonic acid in insects (Oyarzun *et al*, 1996; Ekpo and Onigbinde, 2005; Ekpo and Onigbinde, 2007). Bozkus (2002) reported that arachidonic acid among other polyunsaturated fatty acids, occur in low proportions, and are reported in only a few instances. They are probably routinely present in the lipids of most insects, but are often overlooked because they are not present in detectable quantities. The grasshoppers had higher concentration of polyunsaturated fatty acids (PUFA), with values of 34.42% and 33.77% for the green and brown grasshopper respectively. The PUFA in grasshoppers was dominated by linoleic acid (C18:2) at 31.21% and 29.54% while linolenic acid was 3.21% and 4.23% for green and brown grasshopper respectively.

The termite contained linoleic acid as the major PUFA at 12.96% while linolenic acid was found in smaller amounts (1.53%). The presence of these essential fatty acids points to the high nutritional value of the insect oil. Ekpo and Onigbinde (2005) reported a content 17.70% of PUFA in edible larvae of *Rhynchophorus phoenicis* (F) and a 61.10% degree of unsaturation of the fatty acids. This was lower than the PUFA content found in grasshoppers but higher than that in termites. Ekpo and Onigbinde (2005) also reported 33.08% PUFA content in *Macrotermes bellicosus* species of termites analysed in Nigeria.

The fatty acids in insects are similar to those of poultry and fish in their degree of unsaturation, with some insects being higher in linoleic and linolenic acids (DeFoliart, 1991). In terms of degree of saturation, DeFoliart (1991) reported 35.5% and 29.5% for poultry and fish respectively and this was found to be comparable to the saturation in insects. The insects' oil had a lower degree of saturation than beef and pork, which reported 52.0% and 44.1%, respectively (DeFoliart, 1991). Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders (Williams, 2007). The ratio of polyunsaturated to saturated fatty acids (P/S) has been used widely to indicate the cholesterol lowering potential of a food. A P/S ratio of below 0.2 has been associated with high cholesterol level with high risk of coronary heart disorders (Mann, 1993). The P/S ratio of 0.5 for termites as well as the P/S ratio of 0.89 and 0.86 for green and brown grasshoppers suggest that the insects can be associated with a lower risk for certain coronary heart diseases.

4.6 Physicochemical characterization of the insect oil fraction

The physical and chemical characteristics of termites and grasshoppers oil are shown in Table 8.

Table 8: Physicochemical characteristics of the insects' oil

Parameter	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Solidification Temperature (^oC)	8 – 12	10 – 15	10 – 15
Specific gravity	0.93 ± 0.01^a	0.94 ± 0.02^a	0.94 ± 0.03^a
Refractive index	1.35 ± 0.02^a	1.38 ± 0.69^a	1.34 ± 0.81^a
Iodine value	83.51 ± 1.93^b	86.97 ± 1.85^a	86.97 ± 0.54^a
Saponification value	160.61 ± 1.82^c	229.67 ± 1.91^b	234.40 ± 1.78^a
Peroxide value	0.19 ± 0.01^a	0.13 ± 0.07^b	0.14 ± 0.02^b
Cholesterol (mg/100 ml)	38.77 ± 0.48^a	27.51 ± 0.43^c	31.40 ± 1.03^b

All values as means ± SE on triplicates dry weight basis of triplicates. Values on the same row followed by the same letter are not significantly different ($p>0.05$); n = 6

The oil from the termite showed a solidification temperature of 8 - 12^oC while the grasshoppers' oil had a solidification temperature of 10 – 15^oC. These values were comparable to the values reported by Ekpo and Onogbinde (2007) for *Macrotermes bellicosus* species (10 - 14^oC) of termite analysed in Nigeria. The specific gravity and refractive index were comparable to 0.90 and 1.20, reported by Ekpo and Onogbinde (2007) for *Macrotermes bellicosus* species of termite. The observed values were however lower than those reported for linseed and olive oils (Pearson, 1976). This implies that the oil from these insects is lighter than these oils that have been considered to be of high quality and as such find much use even in the pharmaceutical industries.

The iodine value was found to be 83.51 for the termite oil while the green and brown grasshoppers' oil showed an iodine value of 86.97. These values were lower than values previously reported for other insects where a high iodine value is a common feature of most insect lipids as reported for silkworm oil 117, *Lepidopterous* larvae between 112-159 and 108.6-118 in *Phytophagous chrysomelids,* 123.6 and 140 in *Rhynchophorus pheonicis and Oryctes rhinoceros* larval oils respectively (Ekpo and Onogbinde, 2007). The low peroxide values in the oils are an indication that they could be less susceptible to rancidity (Wigglesworth, 1976). These values can be attributed to the high levels of α-tocopherol found in the insects. The grasshoppers showed a higher level of PUFA than the termites. However, the peroxide value in grasshoppers was lower than in termites and this can be attributed to the fact that grasshoppers had a far much higher content of α-tocopherol than the termites as observed in the study. This implies that α-tocopherol may have had a higher effect as an antioxidant in stabilizing the oil. In his study on cashew kernel oil, Ojeh (1981) showed that oils with high peroxide values are unstable. Sterols especially cholesterol, are essential in insects for normal growth, metamorphosis and reproduction (Ritter, 1990). The total cholesterol value for the termite oil was found to be 38.77mg/100ml lipid while green and brown grasshoppers had 27.51mg/100ml and 31.40mg/100ml lipid respectively. The values were slightly lower than the values reported for *Macrotermes bellicosus* (Ekpo and Onigbinde, 2007). These values are also low compared to values reported for some conventional foods of animal origin which includes beef 50mg/100g, veal 51mg/100g, lamb 66mg/100g, mutton 66 mg/100g (Williams,

2007) and turkey 63.6mg/100g (Serdaroulu, 2005). Ritter (1990) attributed the level of sterol in insects to species differences and diet. Thus, these insect species whose tissues are low in cholesterol, either naturally or due to special feeding, may be an especially useful addition to the human diet.

4.7 Lipid classes

Lipid classes from lipid fractions of termite and grasshoppers were obtained (Table 9)

Table 9: Lipid classes of the insects' oil

Lipid Fraction (%)	*Macrotermes subhylanus*	*Ruspolia differens* (Green)	*Ruspolia differens* (Brown)
Neutral lipids	64.73 ± 1.59[c]	89.42 ± 1.80[a]	84.32 ± 0.22[b]
Phospholipids	21.39 ± 0.29[a]	7.39 ± 0.67[c]	9.29 ± 0.85[b]
Glycolipids	13.76 ± 0.17[a]	3.21 ± 0.25[c]	6.41 ± 0.42[b]

All values as means ± SE on triplicates dry weight basis of triplicates. Values on the same row followed by the same letter are not significantly different ($p>0.05$); n = 6

The neutral lipids were the major fraction, followed by the phospholipids. These results are in agreement with observed results for other insect oils (Buckner and Hagen, 2003; Ekpo and Onigbinde, 2007). Gary (1995) reported that the neutral lipid fraction comprises mainly of triacylglycerols and this is an indicator of the high caloric value of the insect oil. Forte *et al,* (2002) reported significant levels of monoglycerides, diglycerides and triglycerides in codling moth. He further reported that triacylglycerides (TAG) formed a major portion of neutral lipids in the moth samples studied. The termite was found to contain more than double the phospholipids content in grasshoppers hence a better source of phospholipids than the grasshoppers (Table 9). The major constituents of phospholipids in the insects are phosphatidylcholine, phosphatidylethanolamine and phosphatidyinositol (Forte *et al,* 2002). However, adult insects report a low level of level of total phospholipids and this may be due, partly, to their use as energetic material through the actions of phospholipases that could generate free fatty acids to the fact that they could be precursors for the biosynthesis of triacylglycerides (Forte *et al*, 2002) as evidenced by the high level of neutral lipids. In the human body, phospholipids function as the principle components of cell membranes therefore they are essential for all vital cell processes. Phospholipids are the most important membrane building compounds that occur in human, animal and plant cells (Gary, 1995). Studies on nutmeg and clove showed that glycolipid content was higher than phospholipids content though neutral lipids, mainly triacylglycerides, were the major fraction (Suzuku *et al,* 2000). Although the human body synthesises phospholipids, dietary supplementation has been found to enhance performance in sportsmen (Suzuku *et al,* 2000).

4.8 Influence of processing on digestibility of proteins present in the insects

After the insects were fried and solar dried, the products developed are as shown in the plates 4 and 5.

Plate 4: Fried termites *(Macrotermes subhylanus)*

Plate 5: Fried and dried dewinged grasshoppers *(Ruspolia differens)*

Change of frying and solar drying on digestibility of the insects' protein was evaluated and is shown in Fig. 6. Frying and solar drying of insects are traditional practices in the Lake Victoria region. There was a general decline in digestibility of the protein in the three insect species with the fresh samples having the highest digestibility. However, the decline in *Macrotermes subhylanus* was not significant ($p>0.05$) but it was significant in the green and brown *Ruspolia differens.* The level of digestibility observed in the fresh insects compared well with the values reported in conventional animal and plant protein sources. Bodwell *et al,* 1980 reported protein digestibility values of 89% for beef, 90% for pork, 78% for turkey and 85% for salmon. Processing reduced the protein digestibility though the values were still found to be comparable to some reported of some food proteins.

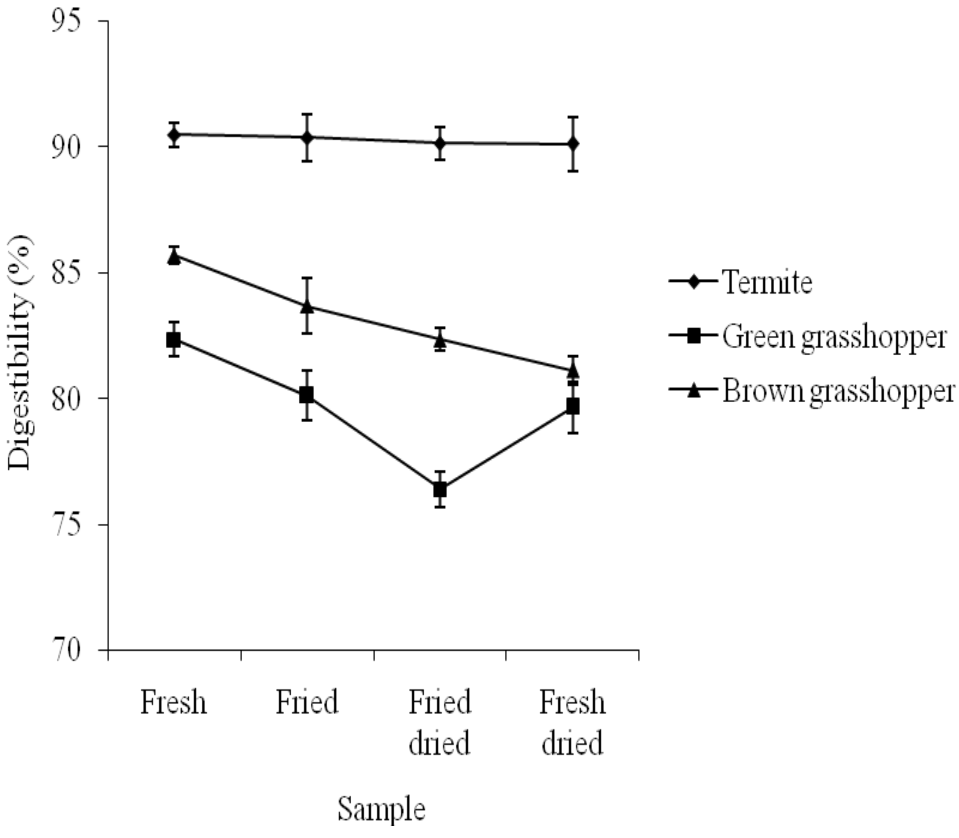


Fig. 6: Changes in digestibility of proteins from termite (*Macrotermes subhylanus)* and grasshopper (*Ruspolia differens)* on frying and solar-drying.

Depending on processing conditions, heat processing may reduce or increase protein digestibility. Exposure to denaturation temperatures may increase digestibility of native proteins by unfolding the polypeptide chain and rendering the protein more susceptible to digestive enzymes (Opstvedt *et al,* 2003). On the other hand, when proteins are exposed to higher temperatures, digestibility is reduced due to reactions between amino acids and other compounds and intramolecular reactions between amino acids within the protein molecule that cannot be split by digestive enzymes (Stanley, 1998). Insects' exoskeleton is composed of the indigestible chitin and this in turn lowers protein digestibility (Oyarzun *et al*, 1996). According to Solomon *et al,* (2002), protein digestibility of giant grasshopper was found to reduce with heating. This was attributed to binding of proteins with indigestible phytates thus are inaccessible to digestive enzymes. Solomon *et al*, (2008) had earlier reported high levels of phytates in the grasshopper and this may also explain the reduced protein digestibility in the samples analyzed in this study.

4.9 Influence of processing on the water-soluble vitamins of the insects

The influence of frying and solar drying on the water-soluble vitamins from the insects was evaluated as shown in Fig. 7, 8 and 9. There was a general decrease in all the vitamins evaluated from the fresh, fried and solar dried samples. There was a significant ($p \leq 0.05$) reduction in riboflavin content on frying and subsequent solar drying in the two species of insects as compared with the fresh samples. Frying reduced the riboflavin content. Subsequent solar drying on the fried sample led to a much higher loss in riboflavin content as compared to the fresh dried sample. Riboflavin is relatively heat and light labile and therefore during drying, it becomes supersaturated and precipitates from solution (Ana and Lia, 1997) thus the reported reduction. There was a significant reduction ($p \leq 0.05$) in niacin content in the termite and grasshoppers on processing (Fig. 5, 6 and 7). Niacin is known to be relatively stable to light, heat and oxidation and its major loss is therefore due to leaching. Therefore, loss of moisture content during frying and drying has a reducing effect on niacin (Onyeike and Oguike, 2003).

Ascorbic acid is highly susceptible to heat and light, therefore frying and solar drying reduced it significantly ($p \leq 0.05$) to low levels compared to the fresh sample. Ascorbic acid has been shown to be destroyed at high temperatures under conditions that permit access to oxygen from the atmosphere (Onyeike and Oguike, 2003). Some factors which readily oxidize and destroy the biological activity of ascorbic acid includes oxygen, neutral or alkaline pH, metallic ions and light. However, the rate of destruction is accelerated by increase in temperature. A recent study has shown that cooking significantly decreases the concentration of ascorbic acid in the seeds of African oil bean, melon, castor oil and fluted pumpkin (Onyeike and Onwuka, 1999). These results indicate that heat processing and solar drying of insects can result in significant reduction in levels of water-soluble vitamins. Such losses during drying have also been reported for other foods (Mziray *et al,* 2000).

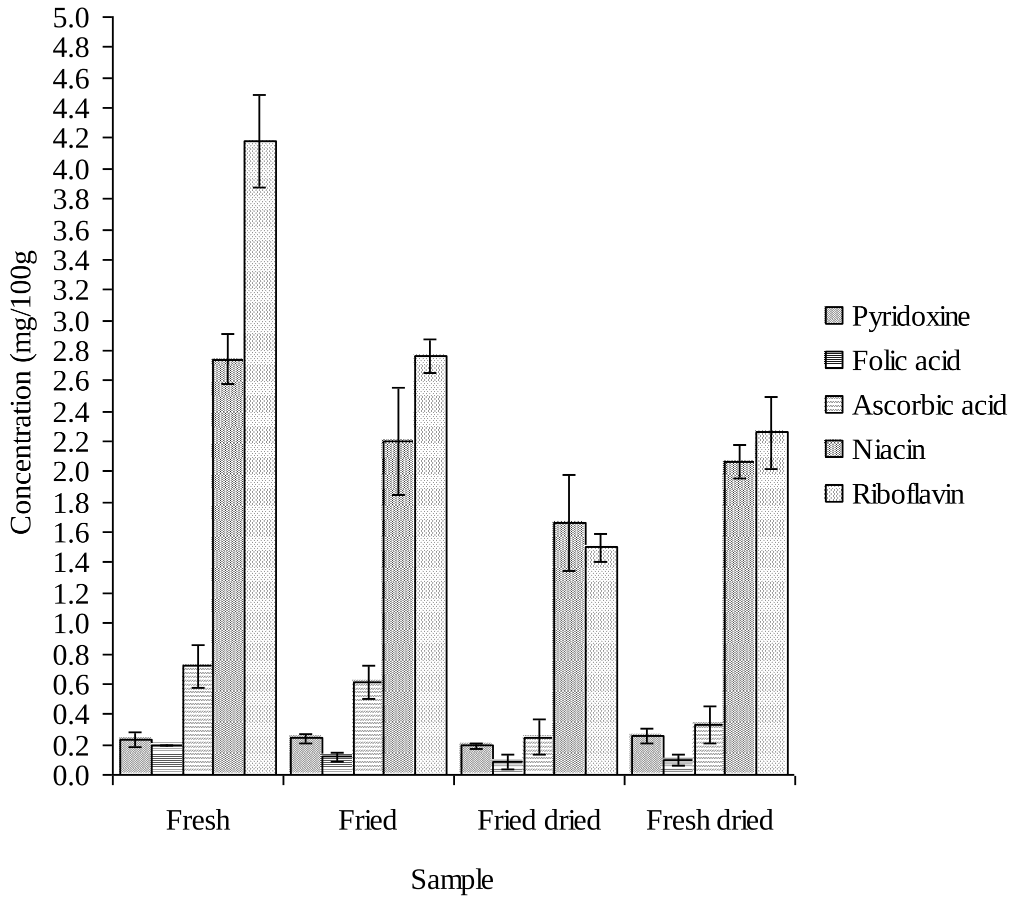


Fig. 7: Changes in water-soluble vitamins content in the termite (*Macrotermes subhylanus)* on frying and solar-drying

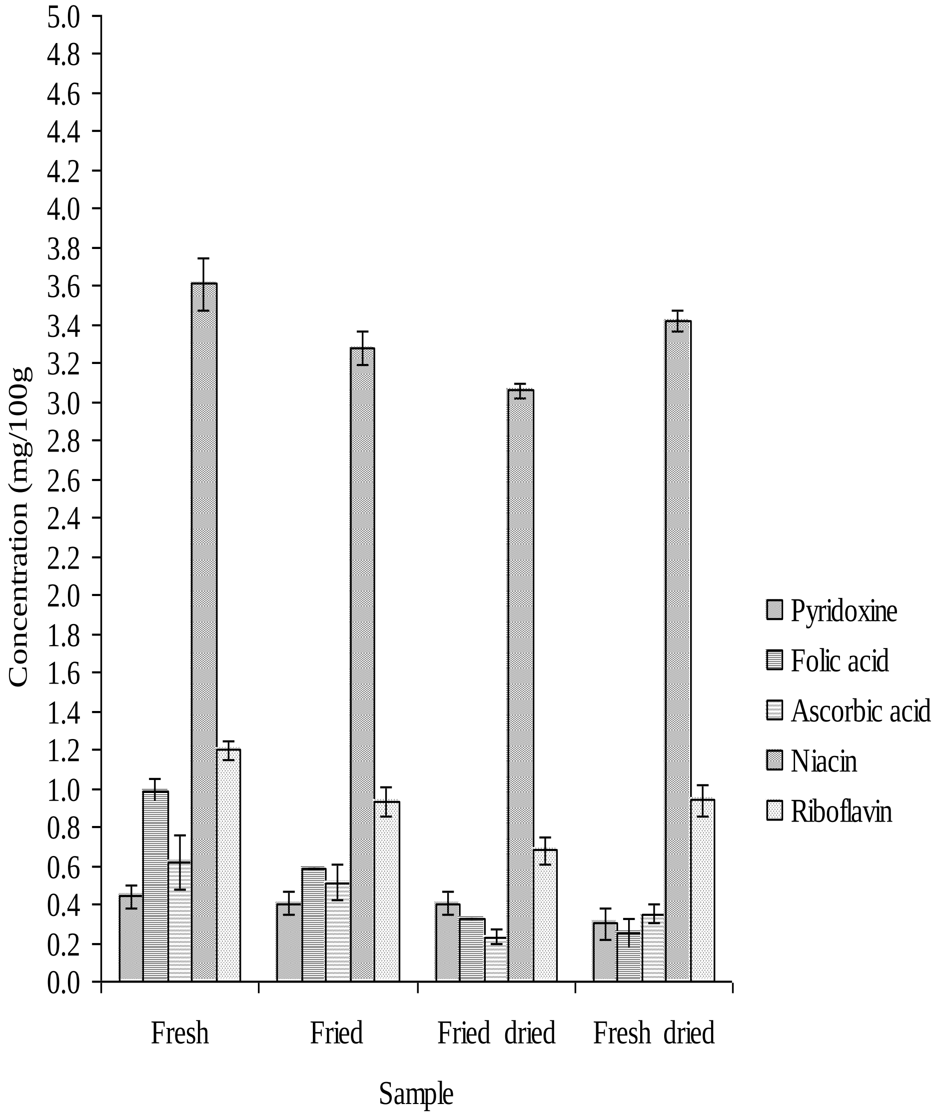


Fig. 8: Changes in water-soluble vitamins content in the green grasshopper (*Ruspolia differens)* on frying and solar-drying

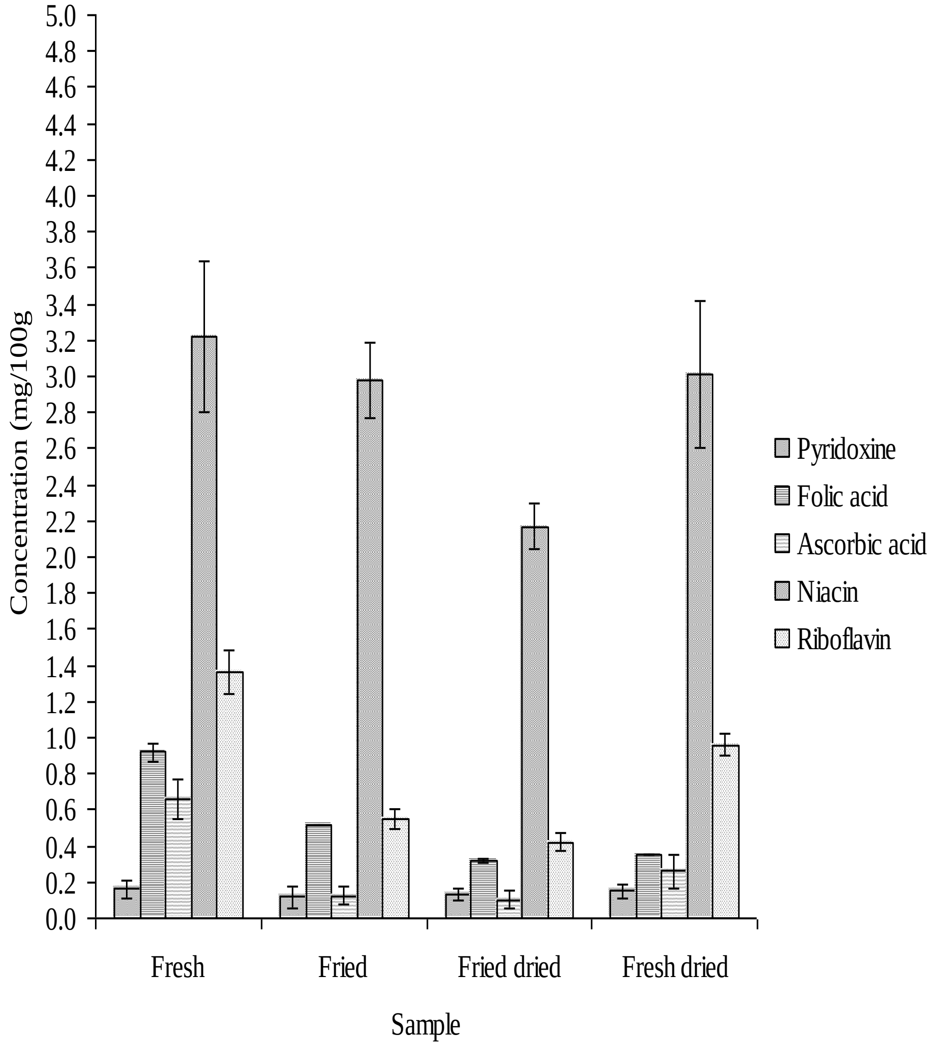


Fig. 9: Changes in water-soluble vitamins content in the brown grasshopper (*Ruspolia differens)* on frying and solar-drying

It is generally recognized that dehydration of foods results in loss of water-soluble vitamins depending on the type of foods (Garg *et al*, 1990). The loss in the vitamins content in this study may have resulted from the effects of the processing temperatures or due to enzymatic and chemical degradation especially in the presence of traces of heavy metal ions (Ana and Lia, 1997) which positively catalyses the chemical degradation as the moisture content reduces.

Frying and subsequent solar-drying of the insects is the common practice before consumption in the Lake Victoria region, because frying is culturally perceived to add a desirable flavor to the insects. Consumption of 100g of the fried and subsequently dried termites in a day was found to provide 150% of the RDI for riboflavin, 25% of the RDI for folic acid, 13% of the RDI for pyridoxine and 10% RDI for niacin in male adults. Consumption of 100g of fried and dried green and brown grasshopper was found to provide of the 11% and 7% RDI for niacin, 63% and 37% of RDI for riboflavin, 88% and 80% of RDI for folic acid in adults (National Research Council, 1989).

4.10 Influence of processing on the fat-soluble vitamins of the insects

Influence of frying and solar drying on retinol from the insects was evaluated and reported as shown in the Fig. 10 below. There was a general decrease in retinol content on processing from frying to solar drying in the termite and grasshoppers.

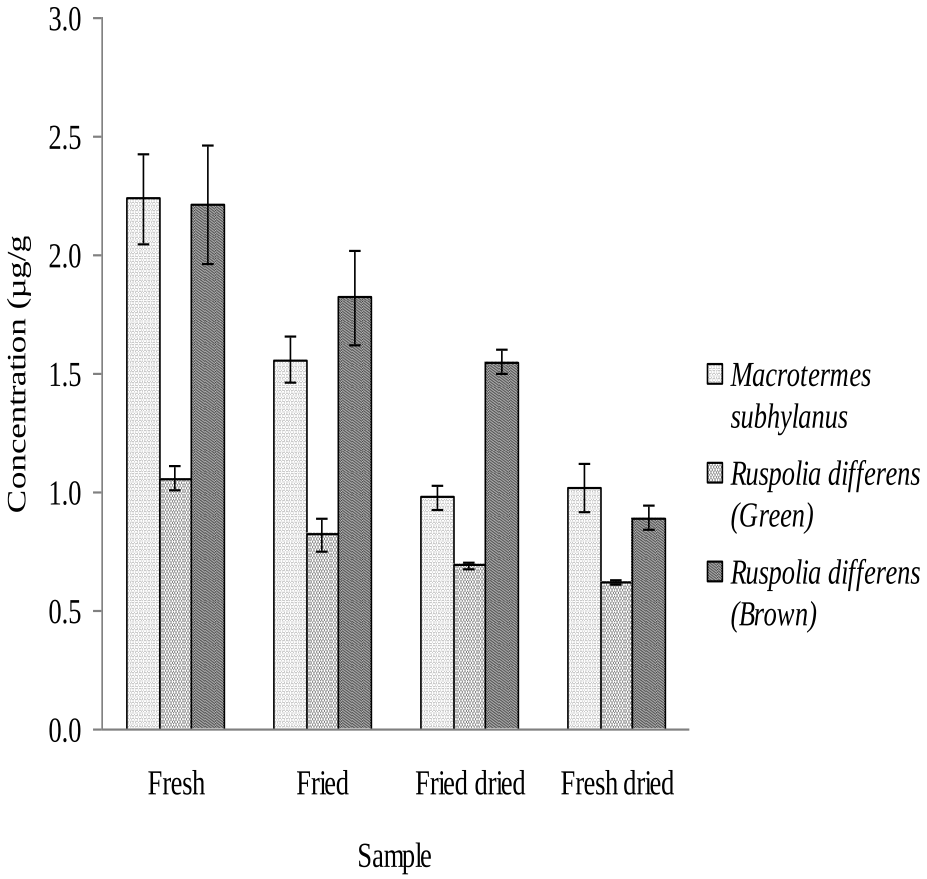


Fig. 10: Changes in retinol content in the termite (*Macrotermes subhylanus*), green and brown grasshoppers (*Ruspolia differens*) on frying and solar-drying.

The retinol content in the fried, fried dried and fresh dried samples were significantly different ($p \leq 0.05$) in termite samples though there was no significant difference ($p > 0.05$) between fried dried and fresh dried samples as shown in Fig. 11. There was significant reduction of retinol content during heat processing and solar-drying on the grasshoppers studied. Reduction in retinol content has been similarly reported by Onyeike and Oguike (2003) who reported reduction on heat processing of groundnuts. Consumption of fried and subsequently dried 100g of the termite (conversion factors: 0.3 μg retinol = 1 IU) was therefore found to contribute 13% of RDI for retinol while the green and brown grasshoppers were found to provide 8% and 11% of RDI respectively in adult males (Barker *et al*, 1998; National Research Council, 1989).

The influence of frying and solar drying on α-tocopherol from the insects was also evaluated and reported (Fig. 11). There was a general decrease in α-tocopherol content on processing from frying to solar drying in the two insect species. There was no significant difference ($p>0.05$) in α –tocopherol between fresh and fried termites as well as between fried dried and fresh dried. Due to the presence of methyl ($-CH_3$) group in its ring, vitamin E is stable to heat, alkali or acid. However, in the presence of oxidizing agents or UV light, this vitamin undergoes degradation and isomerization and yields four major polymers (i.e. α, β, γ, and σ- tocopherols). Three of these isomers lower biological activities i.e., 30, 15, and 5% for β, γ, and σ-tocopherols, respectively, while α -tocopherol has 100% biological activity (Mannan, 1994). In addition, vitamin E has been found to be an antioxidant (Soon-nam *et al,* 2003) and this property may be responsible for its insignificant decrease between the fresh and fried samples. Biological activity of vitamin E is believed to be due to its antioxidant action by inhibiting lipid peroxidation in biological membranes (Emmons *et al*, 1999).

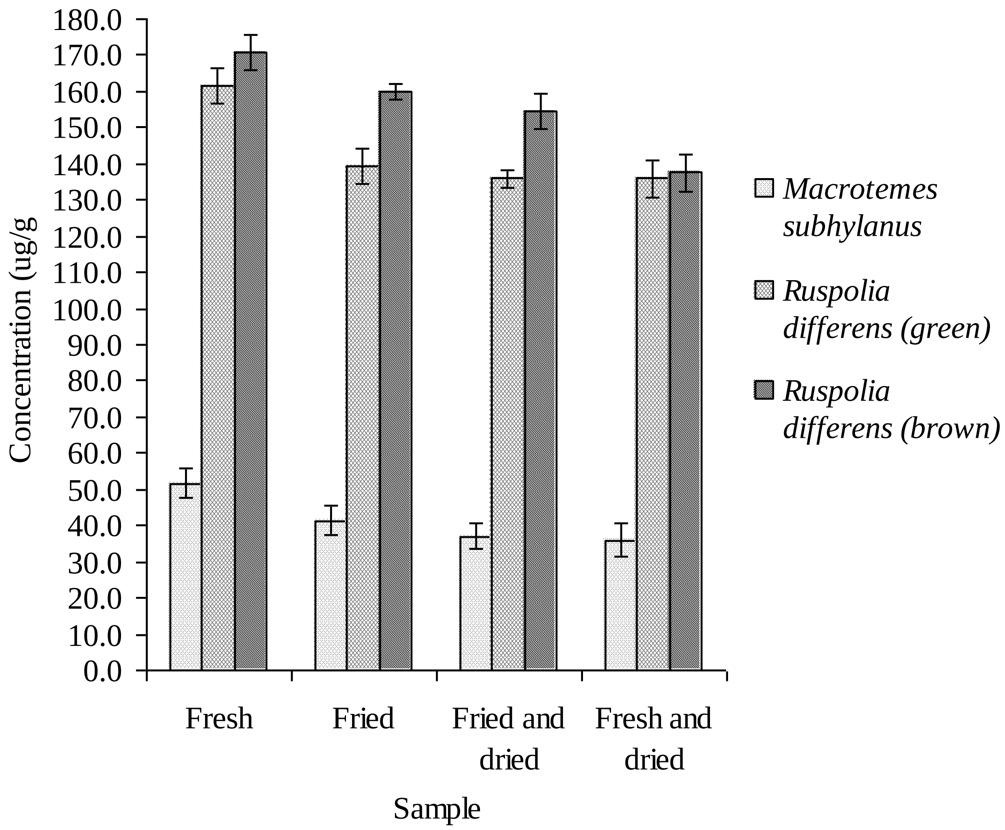


Fig. 11: Changes in α- tocopherol content in termite (*Macrotermes subhylanus*), green and brown grasshoppers (*Ruspolia differens*) on frying and solar-drying.

Consumption of 100g of fried then dried termite (conversion factors: 1 mg = 1.49 IU) was therefore found to contribute 6.0 IU of vitamin E while the green and brown grasshoppers were found to provide 22.0 and 25.0 IU of vitamin E respectively (Barker *et al*, 1998; National Research Council, 1989).

The processing resulted in a decrease in fat-soluble vitamins. Fat-soluble vitamins are mostly contained within the dry matter of the food and are not therefore concentrated during drying. However, water is a solvent for heavy metal catalysts that promote oxidation of unsaturated nutrients. As water is removed, the ions become less mobile leading to a higher level of lipoxidation. Fat-soluble vitamins are destroyed by interaction with the peroxides produced by fat oxidation. However, losses during heat processing and drying can be reduced by low oxygen concentration and process temperatures and also by exclusion of light (Ana and Lia, 1997).

4.11 Influence of processing on the fatty acid profile of the insects lipid fraction

The influence of frying and solar drying on the fatty acid composition of the termite and grasshoppers lipid fraction is shown in Fig. 12, 13 and 14. Oleic acid the major fatty acid in all the termite (*Macrotermes subhylanus)* samples (Fig. 12), while palmitic acid was almost equal in proportion to oleic acid in the grasshopper (*Ruspolia differens*) samples (Fig. 13 and 14). The level of saturated fatty acids increased and the level of unsaturated fatty acids decreased in termite oil on frying and solar drying, though the change was not significant ($p>0.05$). A possible explanation for this observation could not be found. This trend was also observed in the green grasshopper.

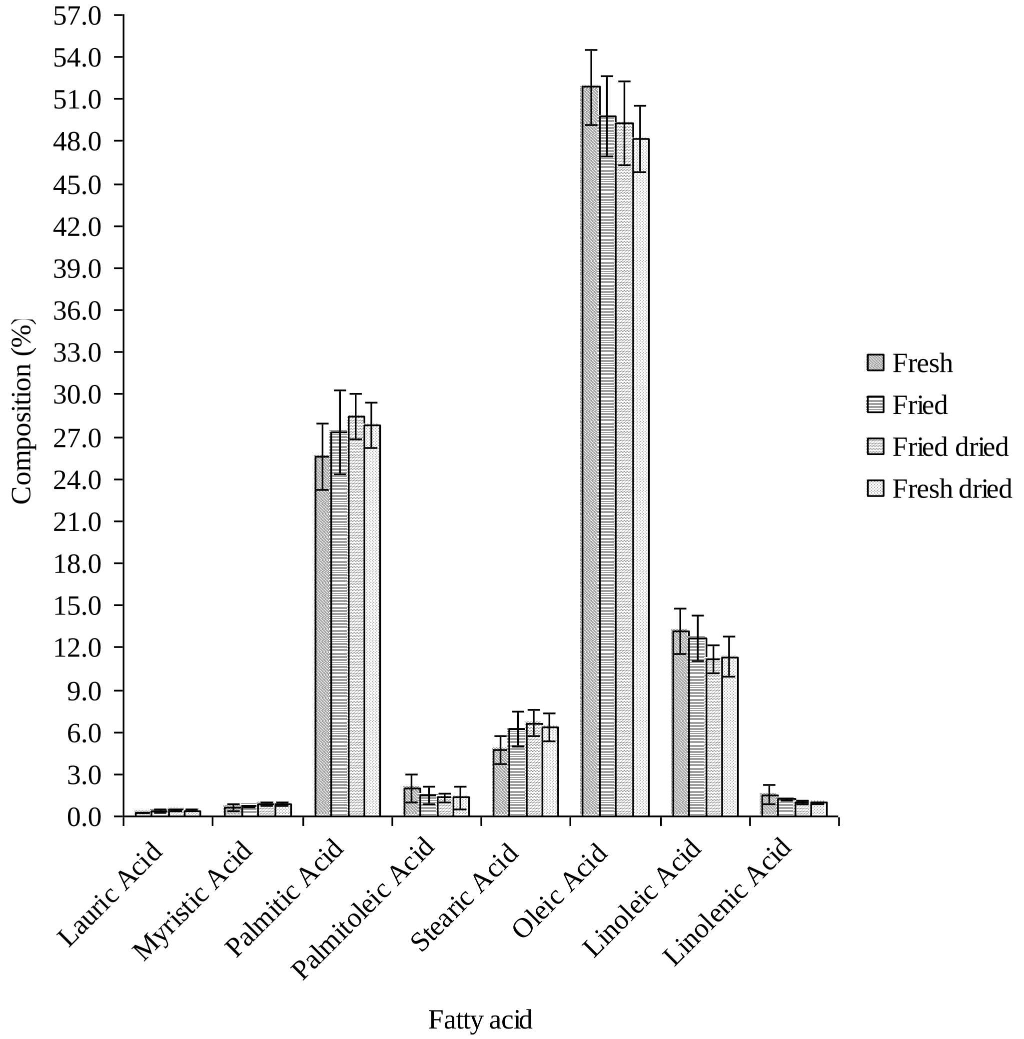


Fig. 12: Changes in fatty acid composition of the termite (*Macrotermes subhylanus*) on frying and solar-drying

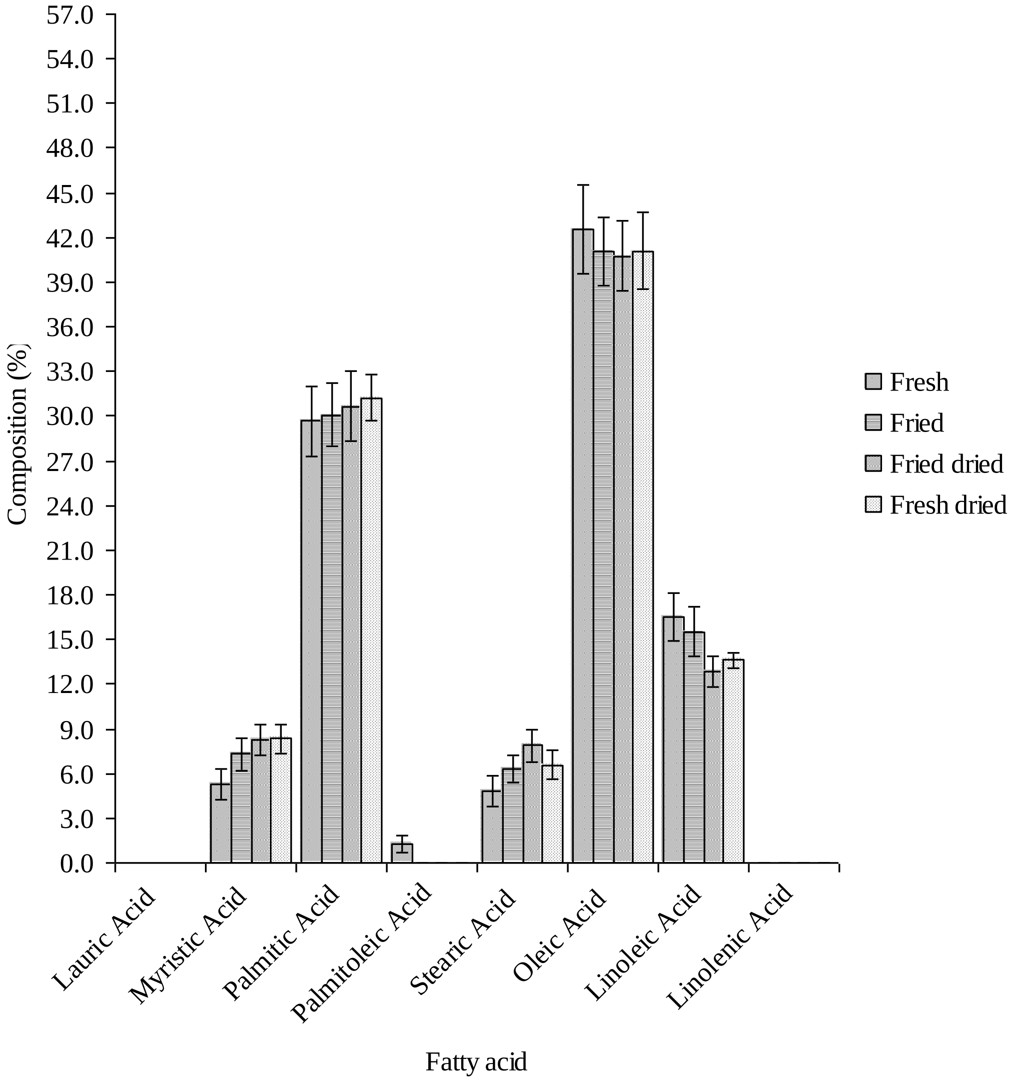


Fig. 13: Changes in fatty acid composition of green grasshopper (*Ruspolia differens*) on frying and solar-drying.

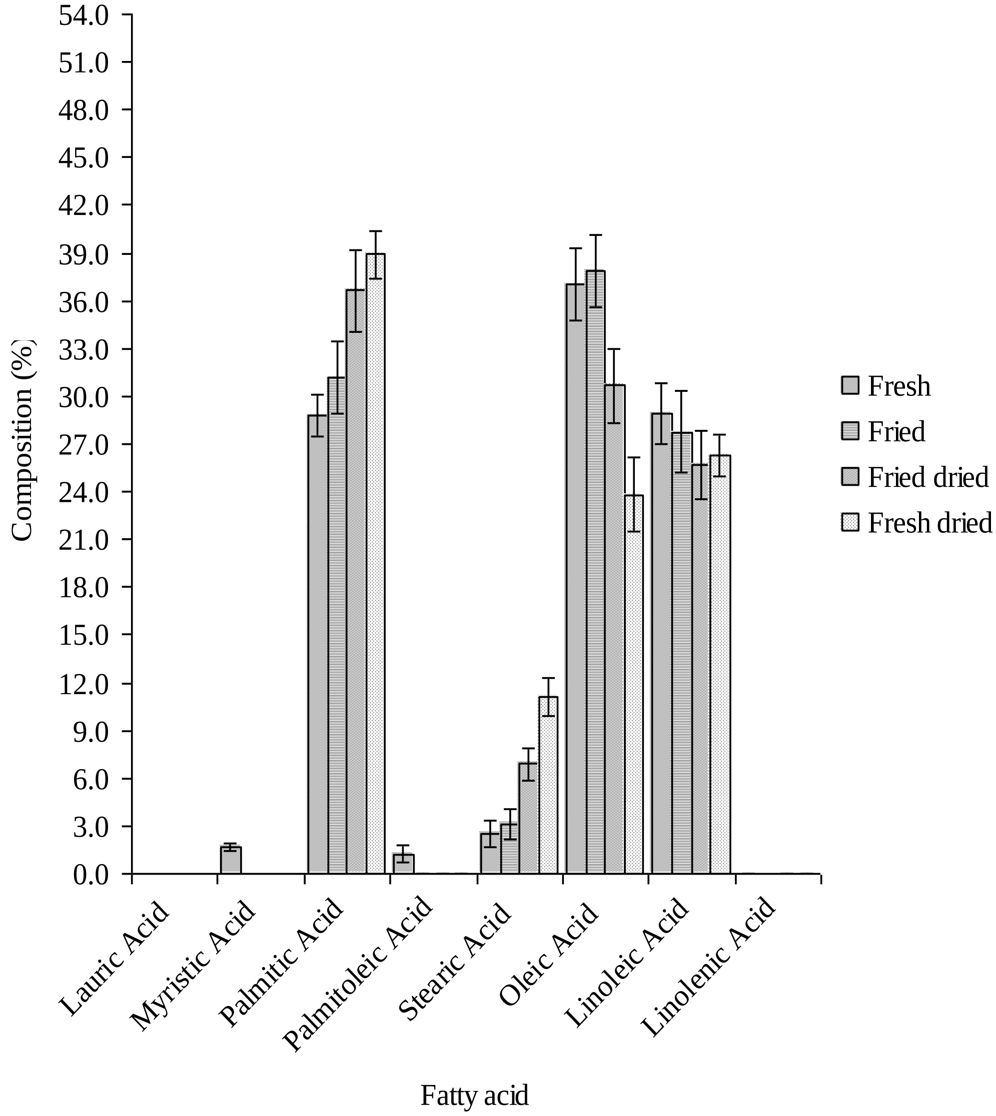


Fig. 14: Changes in fatty acid composition of the brown grasshopper (*Ruspolia differens*) on frying and solar-drying.

It is postulated that changes in free fatty acids may be attributed to interaction and association with other constituents of the lipid system as well as thermal decomposition (Onyeike and Oguike, 2003). Therefore relatively changes observed in unsaturated fatty acids on processing resulted in relative changes in saturated fatty acids within the same lipid system. However this does not mean that these are absolute increases in the saturated fatty acids as this study was qualitative and not quantitative.

It has also been previously reported that the nutritional value of a lipid depends, in some aspects on the free fatty acid composition (Onyeike and Oguike, 2003). Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders (Ekpo and Onigbinde, 2007). In general, heat processing and drying did not significantly affect the fatty acid composition of termites and green grasshopper. However, there is need to optimize the traditional processes in order to minimize the breakdown of unsaturated fatty acids especially as observed in the brown grasshopper. This would ensure that the nutritionally superior quality of the insects' oil is maintained since the processing is necessary in order to improve the flavor quality of the insects.

4.12 Mushroom-insect composite flour

Six mushroom-insect soup composite products were developed and packaged as shown (Plate 6).

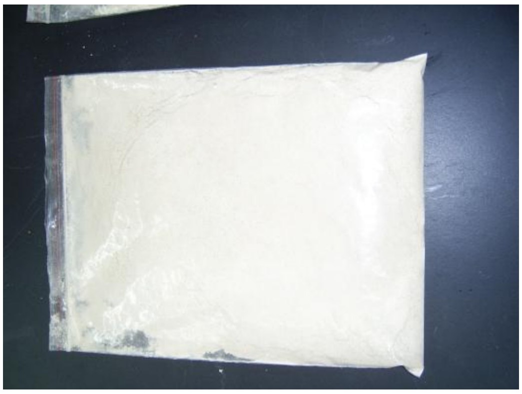


Plate 6: Packaged mushroom-termite composite with 2.5% termite

4.12.1 Microbiological quality of the uncooked mushroom-termite composite flour

The microbiological quality of the uncooked mushroom-insect soup composite packaged the consumer is presented in Table 10. There was a significant (p≤0.05) increase in total plate count, yeasts and *E-coli* as the percentage of incorporated insects increased.

Table 10: Microbiological quality of the uncooked mushroom-termite composite

Product code	Termite composition (%)	Total plate count (cfu/g)	Yeasts (cfu/g)	Molds (cfu/g)	*E.coli* (cfu/g)	*Salmonella* (cfu/g)
A	0.0	$2.0 \times 10^{4\ f}$	$2.8 \times 10^{3\ d}$	nd	$8.0 \times 10^{2\ e}$	nd
B	2.5	$1.3 \times 10^{5\ e}$	$1.2 \times 10^{2\ c}$	nd	$1.2 \times 10^{3\ d}$	nd
C	5.0	$5.6 \times 10^{5\ d}$	$2.0 \times 10^{2\ c}$	nd	$1.6 \times 10^{3\ d}$	nd
D	10.0	$1.7 \times 10^{6\ c}$	$3.6 \times 10^{2\ b}$	nd	$3.1 \times 10^{3\ c}$	nd
E	20.0	$4.6 \times 10^{6\ b}$	$4.2 \times 10^{2\ b}$	nd	$4.2 \times 10^{3\ b}$	nd
F	50.0	$2.6 \times 10^{7\ a}$	$5.8 \times 10^{2\ a}$	nd	$5.8 \times 10^{3\ a}$	nd

Values in the same column followed by the same letter are not significantly different (p>0.05); nd - Not detected; n = 6

This trend also applied for yeasts though molds and *salmonella* were not detected in any of the samples. *Salmonella spp.* and *E-coli* are common inhabitants of the gastrointestinal tract of mammals. Their presence has been associated with feacal contamination (Gillian *et al,* 1999; Ehiri *et al*, 2001). Contamination of food with these microorganisms is mainly through the handlers, where hygiene is not observed. If the raw materials are improperly handled, it may render the product more suitable for growth and survival of spoilage and pathogenic microorganisms (Brackett, 1994; Gillian *et al,* 1999).

The presence of molds in a food product is undesirable, especially *Aspergillus flavus* and *Aspergillus niger* which are sporadic. Toxigenic strains of *A. flavus* have been known to produce aflatoxins, which are potent hepatotoxic and carcinogenic agents (Babajide *et al*, 2006). The fungi have been implicated in food poisoning and are also known as spoilage microorganisms. Products A, B and C were within the required limits for aerobic total plate count (10^6cfu/g) and complete absence of *salmonella* and fungi. All the products met the required limits for fungi (10^5cfu/g) required for products that require heating to boil before consumption (ICMSF, 1996). Products A, B and C were therefore selected for sensory and nutritional analysis. There is need however to investigate processing methods that are likely to reduce the microbial content in termites in order to be able to utilize a higher quantity of the insects without compromising on the microbiological quality of the product.

4.12.2 Microbiological quality of the cooked mushroom-termite composite soup

The microbiological quality of the cooked mushroom-insect composite soup is presented (Table 11) however, only products A, B, C were cooked as they are the only ones that had met the ICMSF (1996) requirements. There was a significant reduction ($p \leq 0.05$) in microbial content of the cooked products. The results of microbial safety tests show that this type of food posed little/no microbiological health risk to humans as no investigated pathogenic microbes were detected.

Table 11: Microbiological quality of the cooked mushroom-termite composite soup

Product code	Termite composition (%)	Total plate count (cfu/g)	Yeasts (cfu/g)	Molds (cfu/g)	*E.coli* (cfu/g)	*Salmonella* (cfu/g)
A	0	3.6×10^{2} [a]	4.0×10^{1} [c]	nd	nd	nd
B	2.5	1.5×10^{3} [b]	1.0×10^{1} [d]	nd	nd	nd
C	5.0	9.0×10^{2} [c]	1.0×10^{1} [d]	nd	nd	nd

Values in the same column followed by the same letter are not significantly different ($p > 0.05$); nd- Not detected; n = 6

In light of information presented in this investigation, the food products should not to be consumed raw but processed as previously described in the cooking method. The cooked products met all the requirements according to ICMSF (1996).

4.12.3 Sensory attributes of the cooked mushroom-termite composite soup

The sensory evaluation was performed on the products A, B, C and the scores obtained are shown (Table 12). These are the products that met the ICMSF (1996) requirements. The attractive brownish appearance of the products was maintained though it gradually changed to a darker colored product as the insect concentration increased. This reduced consumer acceptability of the product in terms of appearance.

Table 12: Sensory attributes of the cooked mushroom-termite composite soup

Product code	Termite composition (%)	Attributes				
		Aroma	Taste	Appearance	Texture	Consumer Preference
A	0.0	4.2 ± 0.8^{b}	3.7 ± 0.9^{b}	5.3 ± 0.2^{a}	4.2 ± 0.1^{b}	4.7 ± 0.9^{b}
B	2.5	4.8 ± 0.8^{a}	4.3 ± 0.1^{a}	5.5 ± 0.1^{a}	5.1 ± 0.9^{a}	5.7 ± 0.3^{a}
C	5.0	4.5 ± 0.7^{a}	4.6 ± 0.7^{a}	5.4 ± 0.5^{a}	5.1 ± 0.7^{a}	5.3 ± 0.6^{a}

Values in the same column followed by the same letter are not significantly different (p>0.05); Panelists = 25

There was an increase in aroma scores on the products containing insects as an ingredient. This trend was similar to the taste and texture. The product with 2.5% termite had the highest score for the consumers' preference though there was no significant difference (p>0.05) between the product with 2.5% and that with 5.0% of termite in its composition. The results indicate that addition of insects improved most of the attributes evaluated signifying a potential for insects utilization.

4.13 Buns baked from wheat-termite composite flour

4.13.1 Physical characteristics of the wheat-termite composite buns

There was an increase in specific volume of the buns on incubation and baking (Fig. 15). The results showed that the specific volume of the buns varied depending on the percentage of termite in the composite flour.

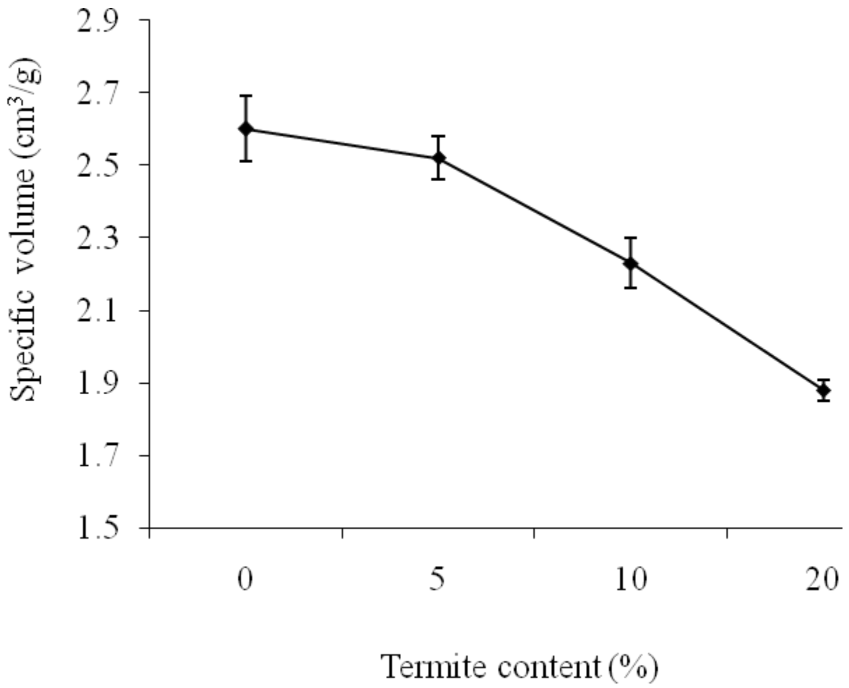


Fig. 15: Change in specific volume of wheat-termite composite buns with increasing termite content

The specific volume of the buns reduced as the termites' concentration increased from 0% to 20%. There was no significant difference ($p>0.05$) in specific volume between the buns with 0% and 5% termite concentration but the buns containing 10% and 20% were significantly different at the same level of significance.

Similar results on decrease in product volume have been reported on incorporation of non-gluten flours in wheat-baked products (Mepba *et al,* 2007).

Increase in size is a function of the gluten content in the wheat flour and therefore reducing it has an effect on the size of the product. Increase in volume is as a result of the ability of the dough to hold gas produced from the fermentation within the dough. The gas leads to formation of void spaces or crumb cells in the product after baking. In a wheat product the crumb cells should be even in size and evenly distributed within the product. This was observed in the control product with 0% termite content. However, as the termite content increased, there was an increase in unevenness of the void spaces (Plate 7) and this led to a reduction in volume of the buns.

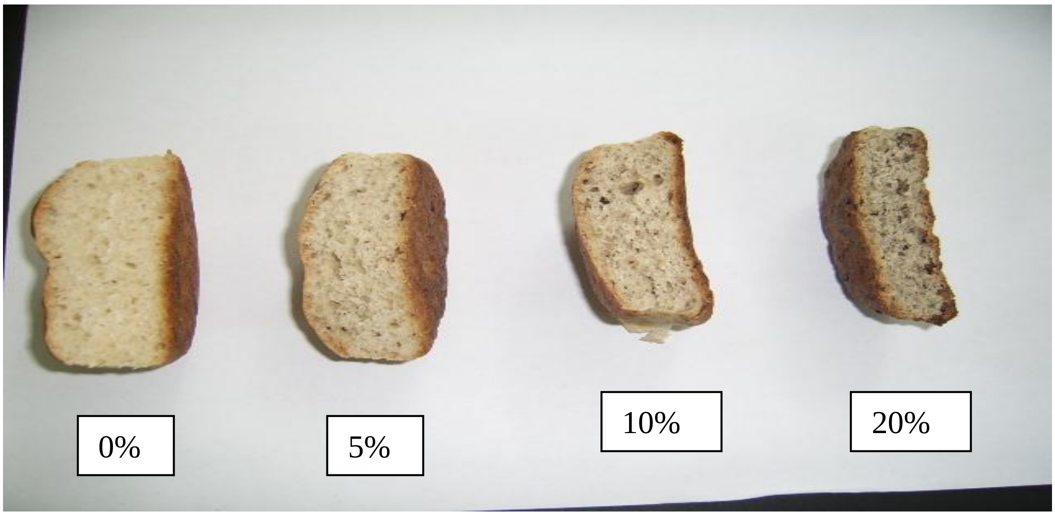


Plate 7: Changes in bun size and crumb structure with increase in termite content

4.13.2 Sensory attributes of the wheat-termite composite buns

The sensory attributes of wheat-termite composite buns are presented in Table 13.

Table 13: Sensory attributes of the wheat-termite composite buns

Termite content (%)	Attribute					
	Size	Color	Texture	Aroma	Taste	Consumer preference
0.0	6.3 ± 0.1^{a}	6.6 ± 0.1^{a}	6.2 ± 0.2^{a}	5.6 ± 0.2^{a}	5.4 ± 0.2^{a}	5.9 ± 0.2^{a}
5.0	5.4 ± 0.1^{b}	5.8 ± 0.2^{b}	5.6 ± 0.2^{a}	5.4 ± 0.2^{ab}	5.5 ± 0.2^{a}	5.7 ± 0.1^{a}
10.0	5.1 ± 0.2^{b}	4.5 ± 0.3^{c}	4.3 ± 0.2^{b}	4.9 ± 0.2^{bc}	4.9 ± 0.2^{b}	4.9 ± 0.2^{b}
20.0	2.9 ± 0.3^{c}	2.9 ± 0.3^{d}	3.4 ± 0.3^{c}	4.6 ± 0.2^{c}	5.0 ± 0.3^{b}	4.2 ± 0.3^{c}

Values in the same column followed by the same letter are not significantly different ($p>0.05$); Panelists = 25

Taste panel ratings of sensory properties of the bun samples decreased significantly ($p\leq0.05$) with increased contents of termite concentration in the composite flour. For the control (0%) and composite up to 5% of termite, the scores for bun texture, aroma, taste and overall consumer preference were not significantly different ($p>0.05$). However, the size and color were significantly different ($p\leq0.05$). Differences in size, aroma and taste scores for the 5% bun and the 10% substitution were non-significant. Scores obtained at 20% level of substitution were less acceptable in all the attributes tested except aroma which scored above 5.0. In terms of consumer preference, there was no significant difference ($p>0.05$) between the control (0%) and 5% substitution with both scoring above 5.0 (like slightly). The bun with 20% substitution scored below score 4.0 with regard to size, color and texture. The high substitution reduced the gluten content, which in turn reduced the size of the baked buns significantly. The color of the buns darkened with increase in termite concentration and at 20% substitution it was unacceptable to the consumer (Plate 8).

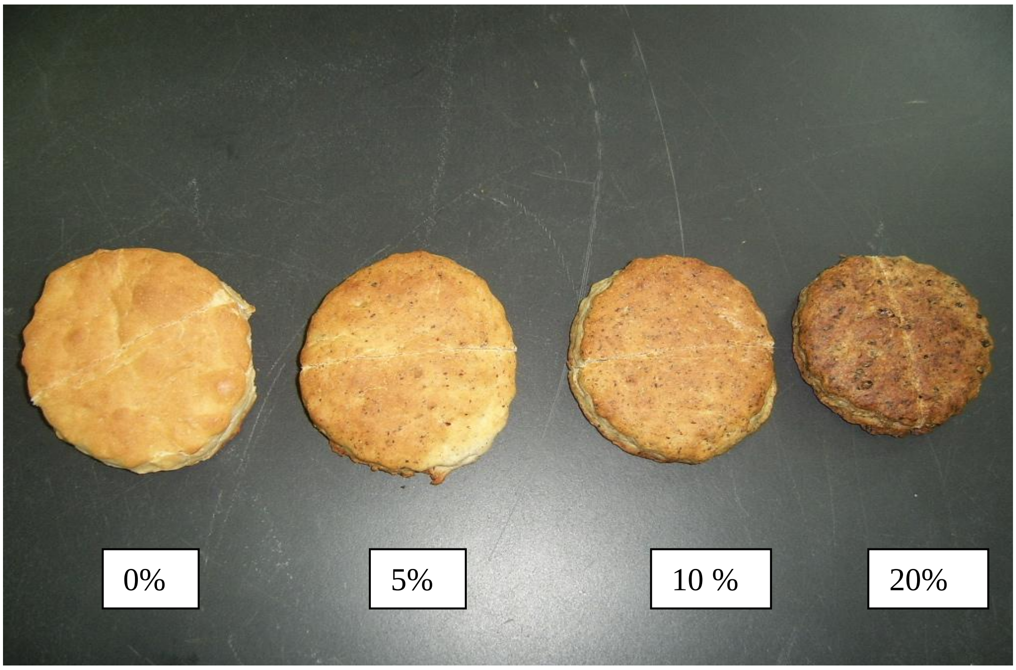


Plate 8: Baked buns at different concentrations of termite incorporated into wheat flour

During baking, Maillard reactions occur between sugar and the amino acids, peptides or proteins from other ingredients in the baked products, causing the browning (Rickard and Sjodin, 1984). The intense brown color was observed with increase in termite concentration meaning an increase in protein content. The Maillard reactions also result in the taste and aroma associated with the baked products (Sugar Association Inc., 2005). This may explain the average score in aroma and taste on 20% substitution.

4.14 Nutrient composition of the developed products

The nutritional composition of the mushroom termite composite and wheat-termite buns was evaluated and the results are shown in Table 14. These are the products that showed high consumer acceptability on sensory evaluation.

Table 14: Nutrient composition of mushroom termite composite and wheat-termite composite buns

Parameter	Mushroom-termite composite soup		Wheat-termite composite bun	
	Control (0% termite)	Product (5% termite)	Control (0% termite)	Product (5% termite)
Moisture content (%)	7.27 ± 0.56	8.41 ± 0.34	26.79 ± 0.87	28.19 ± 0.58
Protein (%)	20.61 ± 0.83	27.56 ± 0.84	10.60 ± 0.90	15.63 ± 1.24
Fat (%)	2.96 ± 0.43	7.76 ± 0.48	8.11 ± 1.20	9.46 ± 0.14
Ash (%)	7.61 ± 0.72	8.76 ± 0.09	0.29 ± 0.00	0.57 ± 0.19
Retinol (µg/g)	0.10 ± 0.01	0.20 ± 0.00	nd	0.10 ± 0.00
α-tocopherol (µg/g)	1.28 ± 0.11	11.91 ± 0.59	99.43 ± 1.10	117.94 ± 0.75
Riboflavin (mg/100g)	2.11 ± 0.68	3.72 ± 0.28	0.17 ± 0.01	0.52 ± 0.87
Niacin (mg/100g)	23.54 ± 0.87	18.04 ± 0.89	0.90 ± 0.10	1.11 ± 0.17
Folic acid (mg/100g)	nd	nd	0.30 ± 0.01	0.33 ± 0.01
Calcium (mg/100g)	56.23 ± 1.49	49.67 ± 0.42	10.00 ± 1.00	10.83 ± 0.02
Phosphorous (mg/100g)	25.23 ± 1.49	29.09 ± 0.11	23.99 ± 1.57	30.11 ± 0.10
Magnesium (mg/100g)	1.93 ± 0.04	2.49 ± 0.00	1.21 ± 0.23	1.81 ± 0.06
Iron (mg/100g)	8.34 ± 0.50	9.33 ± 0.16	1.20 ± 0.10	1.80 ± 0.22
Zinc (mg/100g)	5.68 ± 0.79	11.26 ± 1.21	2.78 ± 0.60	3.23 ± 0.29

Values are mean ± SE on dry weight basis of triplicates; nd- Not detected; n = 6

It was observed that addition of 5% termite content led to an increase in most of the nutrients analyzed except calcium and niacin content in mushroom-termite composite. Oyster mushroom utilized in this study has been reported to contain high amounts of the niacin and calcium (Bernas' *et al,* 2006) and so addition of termites led to a reduction. The moisture content of the mushroom-termite composite was within the recommended range for flour samples (7-13%) according to Babajide *et al*, 2006 who reported that there is a linear relationship between moisture content and incidence of different types of microorganisms in foods.

The mushroom-termite composite provided 61.24% of the protein RDI while the wheat-termite buns provided 34.73% of the RDI on consumption of 100g of the product (National Research Council, 1989). On the whole, the mushroom-termite composite was found to provide RDI values of 2.02% retinol, 12.49% α-tocopherol, 105.88% niacin, 248.00% riboflavin, 77.75% iron, 75.07%, zinc and 2.42% phosphorous for a male. The wheat-termite bun was found to provide RDI values of 0.1% retinol, 35.15% α-tocopherol, 6.53% niacin, 34.66% riboflavin, 15.00% iron, 21.53%, zinc and 2.51% phosphorous for a male adult (National Research Council, 1989). The products were found to be a rich source of nutrients especially those that are of public health concern.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This research provides an overview of the nutrient composition of termite (*Macrotermes subhylanus*) and grasshoppers (*Ruspolia differens*). It confirmed the empirical knowledge of local people in a scientific way. The insects were found to contain significant proportion of proteins, fats and other nutrients. The insects' protein was also found to be highly digestible and compared well with conventional animal proteins. The research showed that consumption of 100 grams of traditionally processed insects contributes significantly to the recommended daily requirements of the respective vitamins/minerals contained.

The insects' oil was found to be of high quality and with lower cholesterol values as compared to most conventional animal fats and oils. It was also found to provide significant quantities of phospholipids and therefore nutritionally important. Processing methods of frying and solar drying were found to reduce the vitamin content in the insects. They were also found to reduce the unsaturated fatty acid in the insects' oil.

The mushroom-termite composite flour with 2.5% termite had the highest consumer acceptability. It was microbiologically safe for human consumption and nutritionally rich. The wheat-termite baked bun with 5% termite scored highest in terms of consumer acceptability. It was also found to be nutritionally rich.

The findings of this research show that edible insects need be considered more seriously as potentially viable in formulation and improvement of food products that have a commercial potential. This is in the efforts to improve food security and to alleviating poverty in sub-Saharan Africa.

5.2 Recommendations

- In this study, only two species of insects were characterized. There is need therefore to characterize other types of insects consumed in this region. Also there is need to promote the insects as a conventional food source.
- The findings of this research also show that the insects' oil is of high quality. Its potential in development of functional foods needs to be evaluated.
- Other conventional forms of processing need to be evaluated and their influence on quality of the final products evaluated.
- High quantities of the insects could not be used in baked buns as this was found to compromise the bun quality. Therefore, other types of baked products that do not require very quality wheat flour need to be evaluated so as to utilize a higher quantity of the insects.
- A value-chain for commercialization of the insects needs to be eveloped so as to realize their full potential.

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APPENDICES

Appendix 1: Guiding questions on the identification and collection of edible insects

The following guiding questions will be used to gather information on the type, collection and consumption of insects in Kandenge and Kanyaboli Sub-Locations of Siaya District, Kenya.

1. Do you/ your family consume insects?
2. Which are the commonly insects consumed?
3. On which time of the year is each of the insect available?
4. Which are the collection sites of the insects?
5. What are the different methods used to collect the insects?

Appendix 2: Sensory evaluation questionnaire for mushroom-termite composite soup

Name:________________________________ Gender (M/F):_______

You are provided with three (3) coded samples of soup. Please rate the samples (1-7) according to the scale provided below by filling in the table against each sample and attribute with 1, for Disliking extremely and 7, for liking extremely.

7. Like extremely
6. Like moderately
5. Like slightly
4. Neither like nor dislike
3. Dislike slightly
2. Dislike moderately
1. Dislike extremely

Sample Code	Appearance	Texture	Aroma	Taste	Overall consumer preference
681					
211					
448					

Any other comments:____
____
____

Appendix 3: Sensory evaluation questionnaire for wheat-termite composite buns

Name:________________________________ Gender (M/F):_______

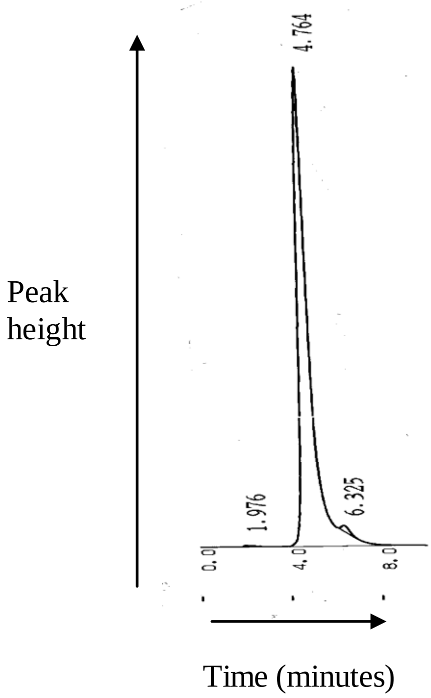
You are provided with four coded samples of buns. Please rate samples (1-7) according to the scale provided below by filling in the table against each sample and attribute with 1, for Disliking extremely and 7, for Liking extremely.

7. Like extremely
6. Like moderately
5. Like slightly
4. Neither like nor dislike
3. Dislike slightly
2. Dislike moderately
1. Dislike extremely

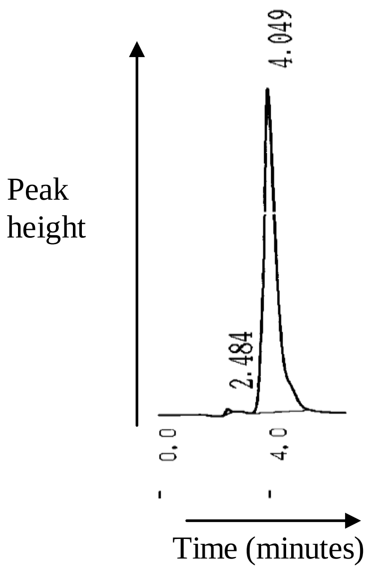
Sample Code	Size	Crust color	Texture	Aroma	Taste	Overall consumer preference
425						
181						
767						
212						

Any other comments:____
____
____

Appendix 4: HPLC chromatogram showing α-tocopherol peak in fresh termite (Macrotermes subhylanus) sample (a) and α-tocopherol standard (10ppm) peak (b).

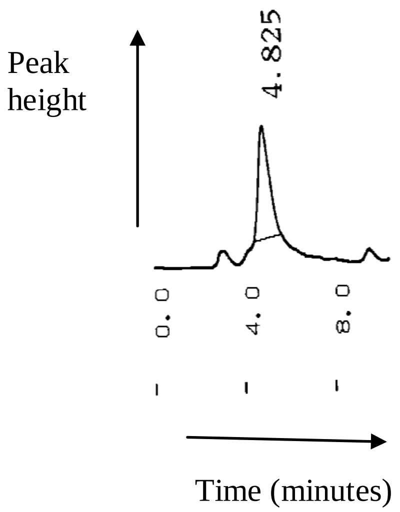


(a)

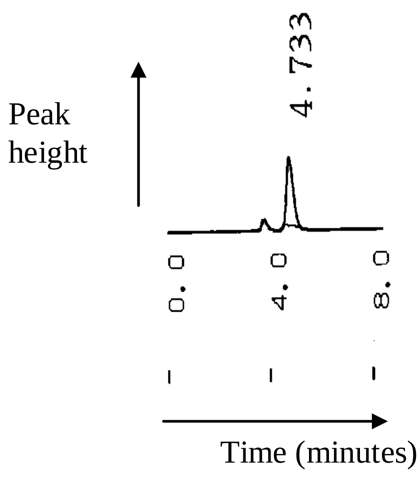


(b)

Appendix 5: HPLC chromatogram showing retinol peak in fresh termite (*Macrotermes subhylanus*) sample (a) and retinol standard (1.0 ppm) peak (b).

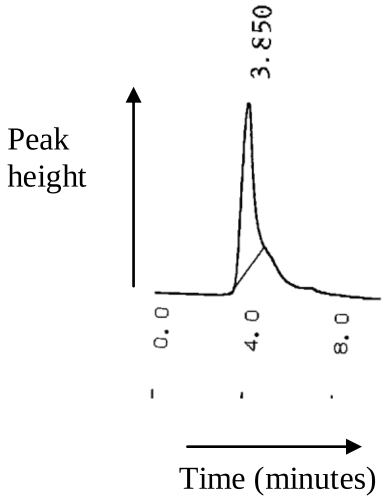


(a)

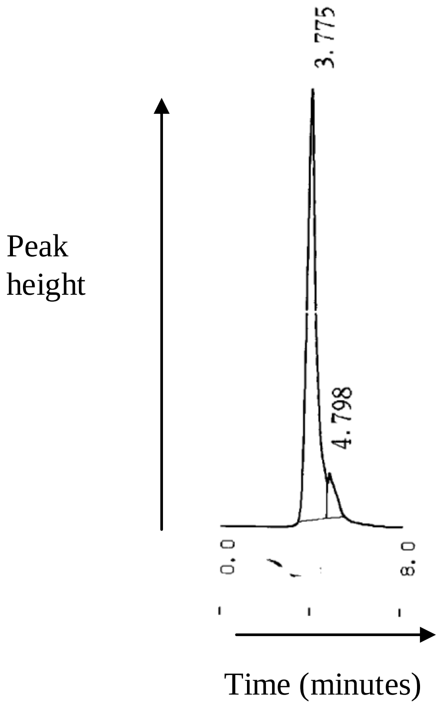


(b)

Appendix 6: HPLC chromatogram showing riboflavin peak in fresh green grasshopper (*Ruspolia differens*) sample (a) and riboflavin standard (2.0ppm) peak (b).



(a)



(b)

Appendix 7: GC Chromatogram showing fatty acid composition of fresh termite (*Macrotermes subhylanus*) oil sample.