Chapter- II
INTRODUCTION

Nutrition is pivotal to every aspect of human health. In physical and mental development and from conception to death, good nutrition ensures optimum human performance in all areas of life. The consequences of poor nutrition are clearly seen in developed and developing countries alike, in the types of illness that prevail and the most common cause of premature death. The factors responsible for this were suggested: the negligence of agriculture relevant to very poor people by government and international agencies; the current world wide economic crisis and the significant increase of food prices. Taking into consideration that insects have a high fecundity, can be multivoltine, have a high feed conversion efficiency, low space requirement, and are omnivorous in addition to their nutritive value, edible insects can contribute to world food security and represent an interesting food and feed alternative, especially to meat products and fish meal.

The main components of insects are protein, fat and fibre; nutritional values are expressed in this chapter as dietary energy, proteins, fatty acids, fibres, dietary minerals and vitamins. Rumpold and Schlüter (2013) compiled nutrient compositions for 236 edible insects, as published in the literature (based on dry matter). Although significant variation was found in the data, many edible insects provide satisfactory amounts of energy and protein, meet amino acid requirements for humans, are high in monounsaturated and/or polysaturated fatty acids, and are rich in micronutrients such as copper, iron, magnesium, manganese, phosphorous, selenium and zinc, as well as riboflavin, pantothenic acid, biotin and, in some cases, folic acid.

The nutritional values of edible insects are highly variable, not least because of the wide variety of species. Even within the same group of edible insect species, values may differ depending on the metamorphic stage of the insect (in particular, for species with a complete metamorphosis – known as holometabolous species – such as ants, bees and beetles), and their habitat and diet. Like most foods, preparation
and processing methods (e.g. drying, boiling or frying) applied before consumption will also influence nutritional composition. A few scattered studies analyse the nutritional value of edible insects; however, these data are not always comparable due to the above-mentioned variations between insects and because of the varying methodologies employed to analyse the compounds. Moreover, where commonly consumed, insects comprise only a part of local diets. For example, in certain African communities insects form 5–10 percent of the protein consumed (Ayieko and Oriaro, 2008). Nevertheless, because of their nutritional value they are still a highly significant food source for human populations.

About 80 grasshopper species are consumed worldwide, and the large majority of grasshopper species are edible. The edible grasshopper (*Ruspolia differens*), formally known as *Homorocoryphus nitidulus vicinus*, is a long-horned grasshopper of the Tettigoniidae family. It is a common food source in many parts of eastern and southern Africa. In the Lake Victoria region of East Africa, where the grasshoppers are known as nsenene, they form a major part of food culture (Kinyuru, *et al.*, 2010). The Bahaya ethnic group in Tanzania’s Bukoba district considers grasshoppers a delicacy. In Uganda, nsenene are traditionally collected by women and children.

During the past few decades increasing awareness that insects are traditionally and nutritionally important food items for many nonwestern cultures has spurred renewed interest in the use of insects as food (Meyer-Rochow, 1973, 1975; DeFoliart, 1992; Menzel and D’Alusio, 1998; Durst *et al*., 2010; Sah and Jung, 2012; Jung, 2013; Kim and Jung, 2013; van Huis *et al*., 2013). It has been postulated that insects could be an important source of high quality and easily digestible proteins (Ramos-Elorduy *et al*., 1997; Malaisse, 2005; Zhou and Han, 2006). An analysis by Ramos-Elorduy *et al*. (1997) of the nutrient composition of 78 different Mexican edible insect species revealed contents of protein ranging from 15 to 81% and of fat ranging from 4.2 to 77.2%; carbohydrate content could reach 77.7% on a dry-matter
basis. Blasquez et al. (2012) specifically analyzed the nutrient compositions of 25 Orthopteran species and reported values ranging for protein from 43.9 to 77.1%, for fat from 4.22 to 34.2% and for crude fiber from 3 to 12.17%; considerable amounts of minerals were also reported. Earlier analyses, based on 11 species of African Orthoptera and mentioned in Malaisse (2005), showed ranges for protein of 13.7–69.5%, for fat of 5.3–22.2%, for carbohydrates of 11.3–13.5% and for ash of 1.1–2.8%; appreciable amounts of calcium, phosphorous and iron were also reported and the average caloric value was given as 423 kcal. Besides of being of high nutritive value, some species of edible insects can successfully be cultured and traded (Meyer-Rochow et al., 2008; Young-Aree, 2010; Hanboonsong et al., 2013). The FAO is now actively involved in developing a programme designed to find alternative sources of food, which includes insects, to feed the world's still growing human population (FAO, 2010), and declared insect as the priority alternative with minimum environmental hazards (FAO, 2013). Even in Korea, there is so-called “insect industry legislation” supporting R&D and commercialization of insect resources (MOLEG, 2010).

As far as India is concerned, except for a few rural tribes of North East India, the full potential of insects as food and/or raw material for medicines is still far from being appreciated (Meyer-Rochow and Chakravorty, 2013). A perusal of the literature has revealed scanty and fragmentary information about edible insects in India. Yet, as early as 1945, Das (1945) analyzed the locust Schistocerca gregaria for use as human food and fertilizer in India and concluded that locusts were high in crude protein and fat.

Recent documentations of edible insects among tribes of Arunachal Pradesh, NE India have revealed 102 species belonging to at least 12 different orders of insects (Chakravorty et al., 2011a, 2013) The analysis of the nutrient composition of Asponopus nepalensis, (a hemipteran bug) Oecophylla smaragdina (ant), Odontotermes sp (termite). Chondacris rosea (short-horned grasshopper,) and Brachytrupes
orientalis (mole cricket) considered the most popular among tribals of Arunachal and beyond (Chakravorty et al., 2011b; Chakravorty et al., 2014; Chakravorty et al., 2016) has demonstrated these species' value as a source of useful fatty acids, minerals and vitamins.

Research to date has shown that Arunachal tribes consume comparatively greater numbers of Orthopterans than do other insect-consuming tribals in India (Singh et al., 2007; Singh and Chakravorty, 2008). The most popular species of Orthopterans, appreciated by all members of the ethnic Galo, Nyishi, Deori, Wancho, Singpho, Chaknma and other inhabitants of North-East India, are Chondacris rosea (De Geer, 1773) and Brachytrupes orientalis (Burmeister, 1883) (Chakravorty et al., 2011a, 2013). Additionally, from the present study on edible insects apperceived by Adi tribes, revealed that they also prefer mostly different groups of orthopteran species about 16 different species has been documented. Also in this study, the ortopteran species outreach the other insects order in their insect food list. It is for this reason that the present study focuses on seven edible insects such as Conocephalus sp., Mecopoda sp., Hexacentrus sp., Schistoserc sp., Ducetia japonica, Phyllozetus sp., Oxya fuscovittata, as well as assorted sample (mixture of grasshopper, locust etc.) collected during a single harvest by women folk while they work in the field throughout the day for their consumption on the same day or next as one of the side dish). Since reliable and detailed information on the nutrient composition of any food/feed stuffs is a pre-requisite for their effective utilization, this study to examine proteins and amino acids, fats and fatty acids, mineral content of those eight orthopteran sample as food items for people living in Arunachal Pradesh and perhaps beyond.
MATERIALS AND METHODS

SAMPLE COLLECTION

Seven edible insects such as Conocephalus sp., Mecopoda sp., Hexacentrus sp., Schistoserc sp., Ducettia japonica, Phyllozeltus sp., Oxyla fuscovittata, and Assorted sample* were considered for nutritional content analysis.

*Mixture of grasshopper, locust etc. frequently hand picked by women folk during their work in the field through out the day for one meal. This mixture of samples are devoid of the above mentioned seven grass hopper species and designated as “assorted sample” through out the thesis)

The entomological sweep net was used for sampling orthopteran species from various habitats, the agricultural fields of rice, sugarcane, forests, fruit orchards, hills, trees, shrubs, herbs and grasses. Sampling period extended for through out the year for two consecutive years, 2013 and 2015. All the collected insects were adults; the sexes were not separated, because no taboo had been reported regarding the sex of the selected insects. In the field they were kept in insect envelopes. All the samples were taken back to the Biochemical Nutrition Laboratory, Rajiv Gandhi University in chilled freeze- boxes. They were later identified scientifically in the laboratory and confirmed with the help of Zoological Survey of India, Kolkata. The specimens were identified using valid key characters (Arrow, 1949; Atkinman, 1974; Vazirani, 1984; Gahan, 1988). One part of the collected specimens was transferred in bottles for killing that contained cotton soaked with ethyl acetate covered with paper. The other part of the samples, once in the laboratory, counted, sorted. were first washed
thoroughly. Wings and appendages of the orthopteran species and has been discarded. dried and kept in individual labeled glass vials in the laboratory. These vials could be stored in freezer for a year with no apparent damage to the specimens then bottled.

SAMPLE PREPARATION

Once in the laboratory, the sampled insects were first washed thoroughly, wings/ appendages/ antennae were discarded and then bottled dry. The samples were oven dried (50° C to 60° C), ground to powder and then stored in deep freeze (-20° C, NSW 152-L, Narang Scientific Works Pvt. Ltd., India) for further analyses. All the analyses were completed within a month after collection. All the solvents and chemicals used in the study were of analytical grade and care was taken that the glass wares were meticulously clean.

CHEMICAL ANALYSES

All the analysis were performed on dry matter of the respective samples except for the moisture content, where the fresh samples had to considered for analysis. All the analyses were performed in triplicate and the methods were verified for reproducibility by repeating additional pilot experiment.

A. PROXIMATE CONTENT ANALYSES

a. Moisture: Moisture content was determined following standard procedure of AOAC (1990). Sample was dried in hot air oven (Narang Scientific Works Pvt. Ltd., India) at 105° C for 2 hours. Moisture content was calculated using following formula:

\[
\% \text{ of moisture} = \frac{(W_1 - W_2) \times 100}{W}
\]
Where, $W_1=$ weight of sample with moisture plate (g)

$W_2=$ weight of sample after drying with moisture plate (g)

$W=$ weight of sample before drying (g)

b. Ash:

Ash content was determined following the standard procedure of AOAC (1990). Sample was kept in muffle furnace (NSW 101, Narang Scientific Works Pvt. Ltd., India) at 550$^\circ$ C for 2 hours and ash content was calculated using following formula:

$$\% \text{ of ash} = \frac{(W_1 - W_2) \times 100}{W}$$

Where, $W_1=$ weight of crucible with ash (g)

$W_2=$ weight of empty crucible (g)

$W=$ weight of sample (g)

c. Crude fat:

Crude fat content of insects was determined following the standard procedure of AOAC 1990. The fat from the samples was extracted by solvent extraction in petroleum ether (boiling point 60$^\circ$ C to 80$^\circ$ C) using Soxhlet apparatus (Rivotek, Reviera Glass Pvt. Ltd., India). Percentage of crude fat was calculated using following formula:

$$\% \text{ of crude fat} = \frac{(W_1 - W_2) \times 100}{W}$$
Where, $W_1 =$ weight of flask along with fat extracted (g)

$W_2 =$ weight of empty flask (g)

$W =$ weight of sample (g)

Crude fat content was expressed as percentage of dry weight (g/100g sample). The values were compared with Recommended Dietary Allowance according to ICMR (2009).

d. **Crude protein:**

The standard procedure of AOAC (1990) was followed to estimate crude protein content. Nitrogen was determined by micro-Kjeldahl method where, sample was digested with concentrated sulfuric acid ($\text{H}_2\text{SO}_4$) at 400$^\circ$ C for 2 hours (Kel Plus KES-04L, Pelican Equipments Pvt. Ltd., India) followed by distillation (Kel Plus Distyl-EM, Pelican Equipments Pvt. Ltd., India) and titration with 0.1 N Hydrochloric acid (HCl) and the total protein content was calculated as the amount of total N determined multiplying by the specific nitrogen-to-protein conversion factor of 6.25. Nitrogen content was calculated as follows:

$$\% \text{ of nitrogen} = \frac{14.01 \times (S - B) \times 0.1 \times 100}{W \times 1000}$$

Where, $S =$ 0.1N HCl required for titration of sample (mL)

$B =$ 0.1N HCl required for titration of blank (mL)

$W =$ weight of sample (g)
The values were compared with Recommended Dietary Allowance according to ICMR (2009).

e. **Crude fiber:**

Crude fiber content was determined following the standard procedure of AOAC(1990). Defatted samples were digested, first with \( \text{H}_2\text{SO}_4 \) (0.255N) and then with NaOH (0.313N). The resulted content was dried overnight in hot air oven at 80\(^\circ\) C to 100\(^\circ\) C and kept in muffle furnace (600\(^\circ\) C) for ashing. Percentage of crude fiber was calculated as follows:

\[
\% \text{ of crude fiber} = \frac{[\text{We} - \text{Wa}] \times 100}{\text{W}}
\]

Where, \( \text{We} \) = weight of dried sample after overnight drying (g)
\( \text{Wa} \) = weight of ash (g)
\( \text{W} \) = weight of sample

f. **Nitrogen free extracts (NFE):**

Nitrogen free extracts (NFE) was estimated by subtracting the sum of percentage of crude protein, crude lipid, crude fiber and ash from 100.

g. **Calorific Value:**

Calorific value (kcal/100g) was computed by multiplying the factors for carbohydrate and protein by 4 each and that of fat by 9, excluding crude fiber and then taking the sum of the products.
B. AMINO ACIDS ANALYSIS

Amino acid analyses of the dried samples were performed following the standard method of AOAC (1990). 20 mg of dried sample was taken for hydrolysis by adding 10 ml 6N hydrochloric acid (HCl), the samples were flushed with nitrogen, evacuated, sealed and placed in hot air oven (Oswald laboratory oven) for 18 hours at 115°C. Following hydrolysis, aliquot of 10μl was subjected to derivatization with AccQ borate buffer and AccQ flore reagent solution (6-aminquinolyl-N-hydroxysuccinimidyl carbamate). The amino acid composition of the samples was determined by HPLC (Agilent 1100 series) where AccQ-Tag column was used. Cysteine and tryptophan were completely lost by acid hydrolysis and methionine was destroyed to varying degree by this procedure. Hydrolysates were suitable for analysis of all other amino acids. Among non-essential amino acids, glutamine and asparagines was converted into glutamic acid and aspartic acid by acid hydrolysis therefore, they were detected as glutamine+glutamic acid and aspargine+aspartic acid. Standard amino acids used for the analysis was from Waters. In order to determine the quality of protein, individual amino acid was calculated and expressed as percentage of total amino acids.

To determine the quality of insects’ protein, the chemical score for EAA was done following FAO/WHO/UNU (2007) and they were calculated as follows:

\[
\text{Chemical score} = \frac{\text{(Essential amino acid in the protein of test sample)} \times 100}{\text{Same essential amino acid in the reference protein}}
\]

The amino acid having the chemical score below 100% was considered as limiting amino acid.
C. FATTY ACIDS ANALYSIS

The dried powdered samples were extracted with chloroform–methanol (2:1, v/v) and the solid non-lipid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then redissolved in anhydrous chloroform–methanol (19:1, v/v) and clarified by centrifugation at 10,000 Xg for 10 min.

The methyl ester of the fat of samples was prepared according to Longvah and Deosthale (1991). Transmethylation was performed using 14% (w/v) boron trifluoride (BF₃) in methanol. After removal of the solvent by nitrogen gassing, the sample was mixed with 0.5 ml of the BF₃ reagent, placed in a warm bath at 100 °C for 30 min and cooled. After the addition of saline solution, the trans-methylated fatty acids were extracted in hexane. The fatty acid composition of the methyl ester samples were determined by gas chromatography- Flame Ionization detector (GC-FID, Shimadzu) following the standard method of IS 548:Part III (1976 Reaff. 2006). The standard methyl ester of fatty acids used for the analysis was from Sigma- Aldrich. In order to determine the quality of fat, individual fatty acid was calculated and expressed as percentage of total fatty acids.

D. MINERALS ANALYSES

The mineral compositions (Na, K, Ca, Mg, Mn, Fe, Zn and Cu) were determined using the standard method of AOAC (1990). Samples obtained through ashing were digested with hydrochloric acid. The digests were diluted with 100 ml of distilled water and then filtered through Whatman’s no. 1 filter paper. The extracts were stored in dry clean plastic sample bottles for mineral analysis. The concentrations of minerals were determined using an atomic absorption spectrophotometer (Shimadzu AA-7000).
Recommended Dietary Allowance (RDA) for individual minerals were calculated considering the intake of 100g of respective insect species considering the recommended values suggested by ICMR (2009).

E: EXPRESSION OF RESULTS

All the analyses were performed in triplicate and expressed as mean ± standard error. Moisture, Ash, crude fat, crude protein, crude Fiber, Carbohydrate (Nitrogen free extract) contents were expressed as percentage (g/100g sample) Energy, the caloric value as kcal/100g sample. The composition of Fatty acid and Amino acid were expressed as percentage (g/100g sample). The concentration of individual Amino acid and fatty acid was also expressed as % of total amino acid and % total fatty acids content respectively. Mineral content was expressed as mg/100g of sample.
Plate 1: Selected Orthopteran species: *Schistocerca sp.*, *Oxya fuscovittata*, *Hexacentrus sp.*, *Phyllozetus sp.*, *Mecopoda sp.*, *Conocephalus sp.* and *Ducetia japonica* analysed in this study.