AN INVESTIGATION ON SOME EDIBLE INSECTS AS SOURCE OF HUMAN FOOD AND ANIMAL FEED

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
AMIRABBAS AMIRI

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

NICOSIA, 2017

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Approval of the Director of Graduate School of Applied Sciences

Prof. Dr. Nadire Çavuş

We certify this thesis is satisfactory for the award of the degree of Master of Science in Food Engineering

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I hereby declare that, all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name:
Signature:
Date:

To the entire humanity…

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ABSTRACT

With the world population growing and expensive reliance of conventional protein sources and other foodstuff on land and water use, humanity must be in search of other sources of nourishment, which are easier to (re-)produce, more economically rewarding and have lesser environmental imprints. Insects have shown to be a potential source of human food and animal feed. They can be more easily (re-)produced and their feed conversion ratio is greater than that of other livestock. In addition, insect farming has lesser environmental footprints. The reasons are enough for entomophagy to be taken more seriously by the governments, international organizations and academics.

In this thesis, some of the nutritional aspects and microbiological analysis of three commercially available insects (*Locusta migratoria, Tenebrio molitor* and *Zophobas morio*) has been studied. The results are satisfactory to consider further studies and to implement insect as a source of nourishment for humans and animals.

Keywords: Edible insects; entomophagy; food security; food safety; Locusta migratoria; Tenebrio molitor; Zophobas morio

ÖZET

Dünya nüfus artışı nedeniyle ve geleneksel ve pahalı olan protein kaynaklarına bağımlılık ve su kullanımından dolayı, insanların daha ekonomik, kolay üretilebilen ve çevreye daha az zararlı etkileri olan gıda ürünlerine ihtiyacı ortaya çıkmıştır. Yenilebilen böceklerin, insan ve hayvanların gıda ihtiyaclarını karşılayabileceği ortaya çıkmıştır. Böcekler daha kolay üretilebilir ve onların yem dönüşüm oranı, geleneksel hayvan bazlı gıdalardan daha fazladır. Buna ek olarak, böcek yetiştiriciliğinin çevreye tahribatı daha azdır. Entomofajinin gelişimi ve yaygınlaştırılması için, uygun nedenler vardır ve devletler, uluslararası kuruluşlar ve bilim insanları yenilebilen böcekler konusuna yeterli ilgiyi göstermelidir.

Bu tezde, üç ayrı çeşit böceğin (*Locusta migratoria*, *Tenebrio molitor* ve *Zophobas morio)* bazı temel besinsel ve mikrobiolojik değerlendirilmesi yapılmıştır. Elde edilen veriler araştırmanın genişletilmesine neden olacak sonuçlar çıkartmış ve böceklerin insan ve hayvan gıda kaynağı olarak kullanabileceğini göstermiştir.

Anahtar kelimeler: Yenilebilen böcekler; Entomofaji, Gıda güvenliği; Gıda güvencesi; Locusta migratoria; Tenebrio molitor; Zophobas morio

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LIST OF ABBREVIATIONS

UN:	United Nations
FAO:	Food and Agriculture Organizations.
BC:	Before Christ
NFE:	Nirtogen-free Exctract
GWP:	Global Warming Potential
FDA:	Food and Drug Administration
FCDBs:	Food Compositional Databases
EU:	European Union
NPN:	Non-Protein Nitrogen
AOAC:	Association of Analytical Communities
FAMEs:	Fatty Acid Methyl Esters
AACC:	American Association of Cereal Chemists
BCR:	Bureau Communataire de Reference
EC:	European Community
ISO:	International Standards Organization
BHI:	Brain Hear Infusion
BD:	Becton Dickinson
APW:	Alkaline Peptone Water
TCBS:	Thiosulfate-citrate-bile Salts-sucrose
CDC:	Center for Disease Control
SDA:	Sabouraud Dextrose Agar
EMB:	Eosin Methylene Blue
SS:	Salmonella Shigella
CIN:	CefsulodinIrgasanNovobiocine
APC:	Aerobic Plate Count
LECO:	Laboratory Equipment Corporation
EDTA:	Ethylenediaminetetraacetic acid
USDA:	United States Department of Agriculture

RTE: Ready-to-Eat

CHAPTER 1
INTRODUCTION

Today, the world population stands at 7.3 billion. It is predicted that by 2030 that number will be around 8.5 billion and by 2050 the earth will host 9.7 billion people (UN, 2015).

Out of today's 7.3 billion population, about 793 million people are living undernourished globally. Although this number has declined from 960 million in the last decade, and a little more than 1 billion around the year 1990-1992, still hindrances exist in developing regions such as Central Africa, Western Asia, and some part of Latin America (FAO, 2015).

World Summit on Food Security held in November 2009 declared their mission to reduce the number of people suffering from hunger, and to increase food production by 34-70% -higher than what it is today- for a population surpassing 9.1 billion in 2050 (FAO, 2009).

An increase in food production needs an increase in land and water use, two vital natural resources that are under pressure by the increase in population, economic growth, and environmental challenges (Schneider, 2011). It is predicted that by 2050 there will be an increase in meat demand by 76% mostly in developing countries, which is an increase from over 200 million tons to 470 million tons annually (FAO, 2009).

Climate change and greenhouse gas emissions, in addition to insufficient drinkable water are some of the problems that global organizations are dealing with, because they have a direct link with the conventional food sources consumed today by humankind (Baker, et al., 2016)

Consumption of livestock products dominates environmental impacts begging changes in utilization patterns and reductions in consumption levels (Röös, et al., 2014). In the light of introducing new sources of food, whether animal-based or plant-based, insects are one of the sources of animal-based protein (van Huis, 2012).

Food and Agriculture Organization of United Nations (FAO) promotes entomophagy as the act of eating insects, practiced by at least 2 billion people globally (FAO, 2013).

1.1 Geography, History and Culture of Entomophagy

Insect-eating is a norm in many parts of Africa, Asia, Latina America and Australia (Bukkens, 1997). It is mistakenly believed that entomophagy is solely practiced in tropical regions, but in countries which are partially or fully in temperate zones, such as the Netherlands, China, Japan and Mexico, insect species are consumed as a source of nutrition and nourishment (FAO, 2013).

Historically, entomophagy is as old as the Old Testament. In the books of Exodus and Leviticus consumption of bees, beetles, and locusts is mentioned. In the New Testament, John the Baptist survives in desert by nourishing on locusts (González & Contreras, 2009). Also in Islamic and Jewish literatures, there are examples of feeding on insects- mostly locusts (FAO, 2013).

The Greek historian Diodorus cites examples of locust eating in Ethiopia in the first century BC. (Bodenheimer, 1951). Bodenheimer (1951) quotes instances of Australian aborigines consuming insects. He also mentions urban population of China and Japan who:

> "improve their daily rice meals by the addition of small quantities of any kind of animal, from toads and mice to insects." (Bodenheimer, 1951: p. 24)

Bodenheimer (1951) further speaks of silkworm pupae as a highly appreciated food and mentions grasshoppers eaten in Japan. Regarding the Middle Eastern consumption of insects, he mentions locusts and wild honey believing to be appreciated as food even until today. There are also examples of North American and South American Indians consuming insects, especially at hard times of famine (Bodenheimer, 1951).

Today insects are abundant throughout African continent and are used as sources of food.

Between 150 and 200 species of edible insects are eaten in Southeast Asia. The indigenous people of Mexico have a deep knowledge of plant and animal species while insects form a part of their diet (FAO, 2013; Ramos- Elorduy, 1997).

Although in Western culture consuming edible insects is considered a primitive habit and is normally looked upon with disgust, Bodenheimer (1951) believes that this horror and repulsion is solely based on customs and prejudice. He explains:

> "it is rather doubtful whether primitive man ever felt an instinctive aversion against the eating of insects. Scores of writers have explained at great length how most of the vegetarian insects in themselves, by their environment and by their food habits, belong to the cleanest of animals, actually being much cleaner than most other animals which are served at our tables." (Bodenheimer, 1951: p. 8)

It is still a long way until insects become accepted by Western population as a legitimate food source. Education and better information, in addition to proper marketing, will lead to better approval of entomophagy as the knowledge tends to lessen the prejudices, fear and negative view of the general public towards edible insects (Sogari, et al., 2017).

1.2 Consumed Species

According to FAO, the most common insects consumed are (Figure 1.1):

- Beetles;
- Caterpillars;
- Bees, wasps and ants;
- Grasshoppers, locusts and crickets;
- Cicadas, leafhoppers, planthoppers, scale insects and true bugs;
- Termites;
- Dragonflies;
- Flies;
- Others.

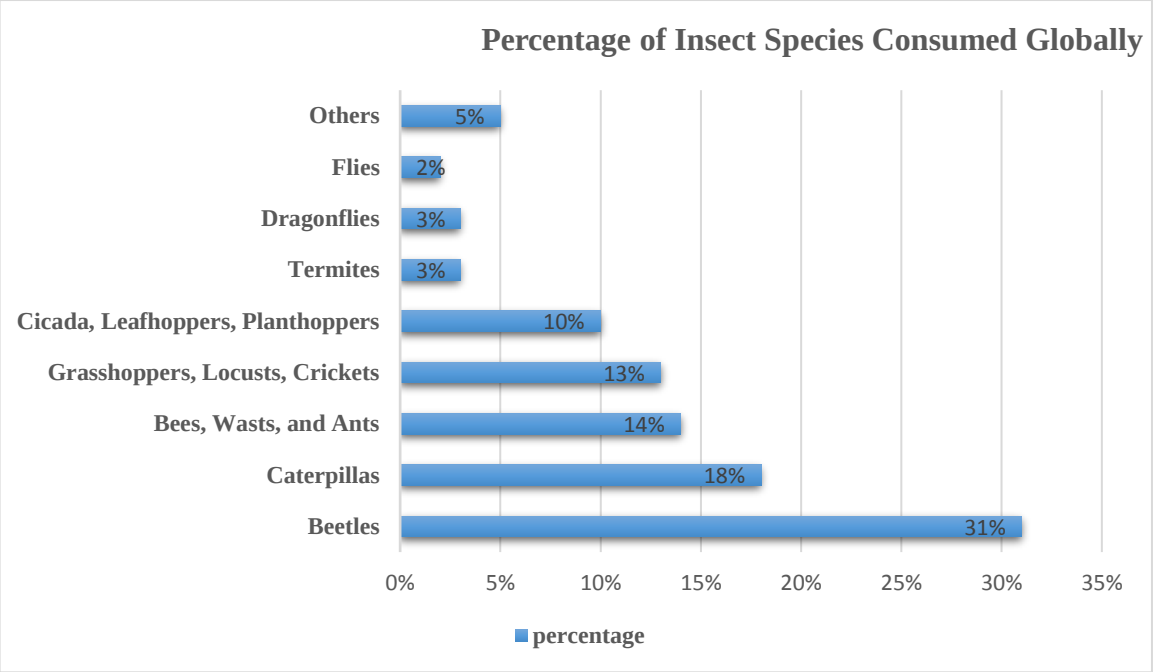

Figure 1.1: Percentage of insect species consumed globally (FAO, 2013)

In addition to the insects cited in Figure 1.1, a comprehensive list of all edible insects is given by Wageningen University in the Netherlands (Jongema, 2017).

1.3 Nutritional Value

From a nutritional perspective, it's an accepted fact that animal-based protein is superior to protein derived from plants. Insects are a considerable alternative opportunity to provde animal-sourced nutrients. Nutritional analyses have mostly studied insects for their protein, fat, ash, moisture and fiber content and have proven insects to be a good source of protein and fat, comparable to the protein and fat content of milk and meat. Edible insects can provide us with enough energy and protein. They are good sources of amino acid, monounsaturated and polyunsaturated fatty acids. Insects are also rich in several micronutrients such as zinc, copper, iron, magnesium, calcium, manganese, phosphorous, selenium (FAO, 2013; Rumpold & Schlüter, 2013; Shockley & Dossey, 2014). The main components of insects are determined to be protein, fat, fiber, nitrogen-free extract (NFE) and ash (Figure 1.2).

Protein is the main component of the nutrient composition of insects followed in second by fat. Considering the average protein content, Orthoptera (grasshoppers, locusts and crickets) provide the most protein, as high as 61.32%, followed by Blattodea (cockroaches) standing at around 57.3%. The highest fat content belongs to Coleoptera (beetles) with 33.4%, followed by Isoptera (termites) standing at 32.74%. (Rumpold & Schlüter, 2013).

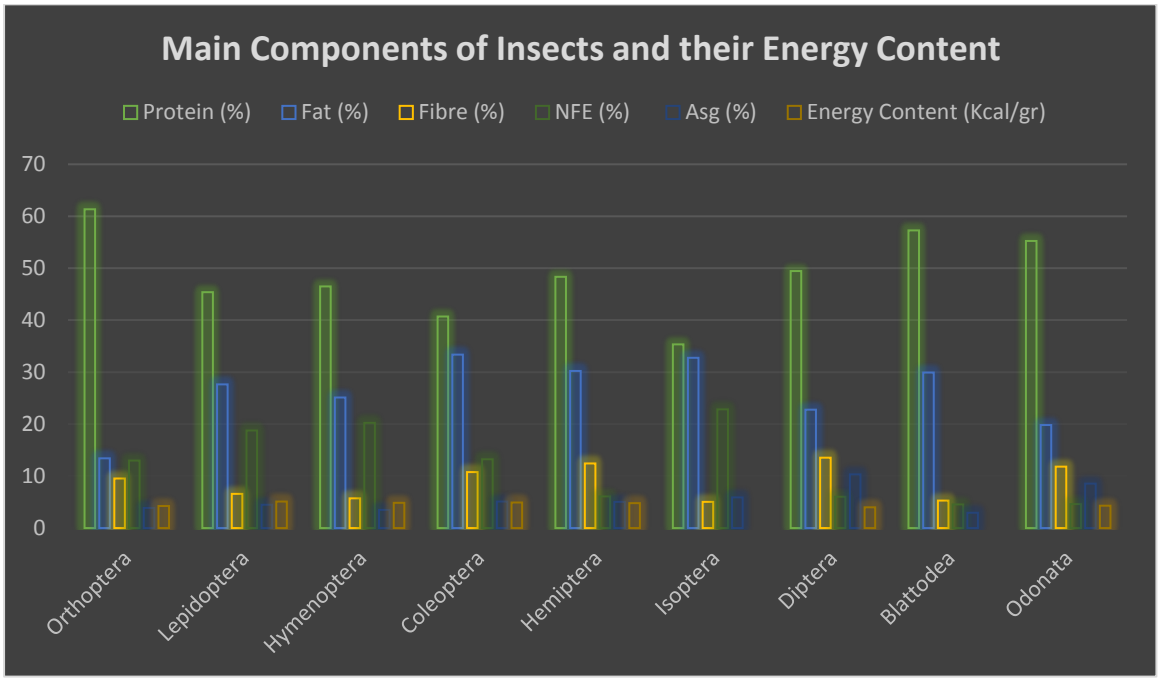

Figure 1.2: Nutritional content of insect families (FAO, 2013)

The protein content of insects is satisfying in comparison to casein and soy. It is highly digestible (between 77% and 98%) as well (Rumpold & Schlüter, 2013). In addition, removing the chitin and some other food processes can even improve the protein content (Belluco, et al., 2013).

A particular study of entomophagy among the Luo of Kenya found that consumption of insects can provide man with minerals such as iron, zinc and calcium which can be seen in Table 1.1 (Christensen, et al., 2006)

Table 1.1: Content of iron, zinc, calcium and ash in some insect genera

Insect	Genera	Iron (mg/100 g dry matter)	Zinc (mg/100 g dry matter)	Calcium (mg/100 g dry matter)	Ash (% of dry matter)	Dry Matter (g)
Onyoso mammon	**Ant**	17.7	11.1	32.6	1.7	1.19
Oyala	**Termite**	332	11.9	84.7	6.8	5.96
Ogawo	**Termite**	93.9	8.1	83	2.4	1.11
Agoro	**Termite**	161	14.3	132	6.8	9.68
Onjiri mammon	**Cricket**	1562	25.1	341	7.8	5.54

Table 1.2: Recommended nutritional intake (mg/day) for males and females, respectively, based on high bioavailability (Christensen, et al., 2006)

Recommended daily intake (mg/day)	Iron	Zinc	Calcium
Children	5.9	3.3	700
Adolescent (male/female)	12.5/20.7	5.1/4.3	1000
Adults (male/female)	9.1/19.6	4.2/3	750

In developing countries, where other sources of animal are not accessible or affordable, deficiency of minerals such as iron and zinc can be answered through promotion of entomophagy. Consuming insects can also help calcium deficiency (although to a lesser degree) in developing countries where other animal sources are either not accessible or unaffordable (Christensen, et al., 2006).

In addition to minerals, insects are a sufficient source of vitamins. For example, Angolan caterpillar, *Usta terpsichore* (Saturnidae) is rich not only in iron, copper and zinc, but also in thiamine (vitamin B1), and riboflavin (B2) (Belluco, et al., 2013).

1.4 Benefits of Entomophagy

Feed-conversion ratio is the amount of weight gained per gram of food consumed. Insects provide us with a greater conversion ratio compared to animal protein. Feed-conversion ratios ranges from 1.85:1 to 33.33:1 with the average of about 2.5-5:1 for the majority of studied insects. Compare that to the feed-conversion ratio for chicken which is 2.6:1 and for that of sheep which is 9:1, ranging from 7.47-10.35:1, while standing at 10:1 for the livestock. Insects' bodies adapt to the temperature of the environment they are inhabiting so that they don't need to use a large part of their food to maintain their body temperatures, this simple fact is the reason for their higher feed-conversion ratio. Another factor for consideration is the energy efficiency of insects from an edible weight perspective. We can consume a great amount of insect bodies compared to that of the cattle, birds or fish where there are wasted parts like bones, claws, skins and shell (Ramos-Elorduy, 2008; Ramos-Elorduy, 1997).

Insects reproduce faster and grow faster. An individual insect can reproduce up to thousands of offspring while livestock reproduce only few and it takes months (and sometimes years) for them to reach adulthood. All that contributes to achievement of a greater rate of protein production out of insects compared to livestock (Abbasi, et al., 2016).

FAO has estimated that up to 70% of all agricultural land is applied to livestock production. An increased demand in meat consumption mean an increase in land use. In comparison, insects need much smaller spaces. People are already rearing their own insects in the corners of their kitchens. Industrial application of insect production demands less complicated machinery and user-friendly control systems. It has been estimated that for each hectare of land used in mealworm protein production, 2.5 hectares is needed for milk, 2-3.5 hectares are needed for pork and chicken production and 10 hectares is needed for beef protein (Abbasi et al., 2016; FAO, 2013).

Most insect species feed on organic matter in nature. They easily exploit organics sources

such as plants and animals. Insects raised on organic waste proved to be more efficient in producing biomass and weight. We can use insects as recyclers of organic matter, producing nutritious insect biomass in return. From an environmental point of view, insects are more beneficial as they can be reared on organic waste adding more value to them. Insects can also be used in feeding of poultry and fishes producing equal or better results than soya or fish flour (FAO, 2013; Ramos- Elorduy, 1997).

Another difference between insect production and livestock production is water. Less water is needed for producing insects compared to the estimated numbers for animal protein. 2,300 liters of water is needed for producing 1 kg chicken. That number arises to 3,500 liters for 1 kg of pork and even more, the necessary water stands at 22,000 liters for producing 1 kg of beef while that number has been estimated up to 43,000 liters (FAO, 2013).

Livestock production is to blame for around 35-40% of worldwide methane and 9% of CO_2 emissions, speeding the impact of global warming. Add to that the effects of deforestation in order to produce more land for livestock pasture and their nurture. Compare that with the low levels of greenhouse gases and NH_3 emission by insects (Table 1.3) (Abbasi, et al., 2016).

Table 1.3: Resource use and global warming potential (GWP) of mealworm production in comparison with traditional protein sources (Abbasi et al., 2016)

Protein Source	Energy (MJ/kg edible protein)	Land (m^2/kg edible protein)	Water (L/kg live weight)	GWP (kg CO_2 eq/kg edible protein)
Mealworms reared with mixed grains and carrot	173	18	–	14
Mealworms reared without energy inputs and on organic waste	0.29	0.04	2.5	0.06
Beef	177–273	142–254	9700	77–175
Pork	95–237	46–63	2800	21–54
Chicken	80–152	41–51	1500	19–37
Milk	36–144	33–58	800	25–39

It can be said that entomophagy is more favorable in comparison with animal protein consumption. We need less land and water resources for insect production with lesser greenhouse gases emission compared to livestock production (FAO, 2013).

Insects have been cited as being clean as they mostly feed on plants (Ramos- Elorduy, 1997). Add to that the fact that edible insects are numerous and diverse in comparison with animal protein (FAO, 2013).

1.5 Food Safety

Insects are a novel protein source with food safety aspects that are not yet fully determined. Potential hazards include contaminants like mycotoxins, pathogens, pesticide residues and heavy metals (van der Spiegel & van der Fels-Klerx, 2013).

Experimentally, insects consumed in tropical countries do not harbor any citable threat or

health problems. Generally, insect pathogens that can harm invertebrates, do not cause health issues for vertebrates (van Huis 2015). Safety records of more than 2000 insect species consumed globally is equal or better than records of more widely consumed foods or other nutrition resources (Abbasi, et al., 2016; van der Spiegel & van der Fels-Klerx, 2013).

There are few studies on the microbiological safety of insects as a food source. Insects can be a source of different kinds of food pathogen bacteria. However, it has been suggested that a well-managed insect farm is able to remain free from pathogens. In addition, strict microbiological regulations in animal farming, and the food derived from them, are enough to justify insect farming and allow them entry to the food market. Insects, no different from other foods, may cause allergic symptoms. According to Food and Drug Administration (FDA), involuntary ingestion of insects or their parts are considered common food contaminants (Belluco, et al., 2013; van der Spiegel & van der Fels-Klerx, 2013).

Heating can be a sufficient step for inactivation of Enterobacteriaceae. However, spore-forming bacteria survives this treatment. Other preservation techniques, such as drying and acidifying, are practical and promising (van Huis, 2012).

In Europe, new food derived from novel sources must comply with European legislation in addition to national legislation. However, no legislation has yet been passed in EU regarding insects as food. But insects as feed have been allowed for fish in aquaculture (van der Spiegel & van der Fels-Klerx, 2013; van Huis, 2015).

1.6 Farming Insects

Traditionally, insects are collected manually where people learn (from their elders) how to find and collect them (Ramos- Elorduy, 1997). In tropical areas insects can be found in huge numbers and big sizes (FAO, 2013). However, it must be considered that intense harvesting of insects from nature will lead to destruction of their habitat and degradation of biodiversity. Therefore, we can rely on farming as a sustainable source of edible insects (Abbasi, et al.,

2016). Insects can be raised and controlled in a designated space, i.e. a farm (FAO, 2013).

Silkworms and bees have been domesticated since ancient times. Larvae of bees and silkworm pupae are consumed as byproducts. Cochineal (*Dactylopius coccus*) is another domesticated insect that gives carminic acid used in red dye in human food; it is also used in pharmaceutical and cosmetic industries (FAO, 2013; van Huis, 2012).

In tropical countries such as Thailand, crickets are farmed for human consumption. It is noteworthy to mention that these crickets are reared in sheds in a farmer's backyard. Palm weevil (*Rhynchophorus ferrugineus*), and giant water bug (*Lethocerus indicus*) are also commercially farmed in Thailand. In temperate zones, farming occurs mainly by family-run enterprises dedicated to rearing insects such as mealworms, crickets and grasshoppers. These insects are commercially sold, mostly as pet food (FAO, 2013; van Huis, 2012).

Semi-cultivation of insects is another measure relying on particular insect species' biology and ecology. It has benefits such as availability and predictability of edible insects. Through semi-cultivation, food security and conservation of the insect habitat can be ensured (FAO, 2013).

1.7 Commercialization

Currently, in many countries, insects are more expensive than meat (Ramos- Elorduy, 1997). In Cyprus there are only one or two enterprises dedicated to rearing, mass producing and selling of insects as animal feed. Shcokley 2014, has stated that in the United States there are a few large and many small farms that rear insects such as crickets (*A. domesticus*) mealworms (larvae of *Tenebrio molitor*), and waxworm (larvae of *Galleria mellonella*) for pet feed. In many other countries commercial farming and selling of insects exist (Ramos-Elorduy, 1997). There are challenges in the way of new enterprises; challenges such as legislation and the regulation of the edible insect sector, which need involvement of governments and international community (FAO, 2013; Shockley & Dossey, 2014).

There are two main technological questions in the way of use of insects as a major human food source:

1- How to turn insects into healthy and safe food products?
2- How to produce enough insects that are cheap and sufficient to meet market demand?

The ultimate answer to the above questions is farming and mass production of insects. We cannot depend on harvest of insects from the wild, as it involves risks such as ecological damage and also consumption of insects affected by pesticides and environmental contaminants. Insects in the wild might be exposed to parasites, pathogens and diseases, induced by other agents that exist in the wild. All these dangers can be eliminated in farming, where insects are reared in safe and controlled spaces (Shockley & Dossey, 2014).

The aim of this thesis is to evaluate some of the nutritional quality of three commercially sold edible insects, in addition to their microbial aspects, as a new and reliable source of human food and animal feed.

CHAPTER 2
THEORETICAL FRAMEWORK

2.1 Nutritional Quality of Insects

Food composition is the amount of energy, protein, fat, carbohydrates, fiber, minerals, and vitamins in a selected food. Other factors such as contaminants, additives or bioactive compounds can also be included. Food composition data are important as they lay the ground for almost all work in nutrition. These data are a bridge between agriculture and nutrition, ensuring that food production is nutrition-sensitive and has to meet people's nutritional and health demands (FAO, 2017).

The most important factors that influence the nutrient content of foods are environment, rearing, storage, processing and genetics (FAO, 2017). The nutritional values of edible insects are variable, depending on the metamorphic stage of the insect, their habitat and diet (FAO, 2013). In addition, from more than 2000 known species of edible insects, only little data is available regarding their nutrient composition. Most of the published data are presented on a dry matter basis, which cannot be used directly for the assessment of human nutrition and for food composition databases, as, in general, foods are consumed fresh and we need a fresh weight basis in our Food Composition Databases (FCDBs) (Nowak, et al., 2016).

Food composition, and thus, the nutritional value of insects has been repeatedly mentioned in scientific literature and is comparable to everyday sources of animal protein. Insects are rich in protein, providing us with all the essential amino acids in their recommended ratios. Fatty acid composition in insects is within the accepted limits and suitable for health. In addition, the amount of fiber content makes insects a nutritionally balanced food (FAO, 2013; Belluco, et al., 2013).

2.1.1 Analytical Methods for Protein Content

Proteins are composed of different elements including nitrogen, oxygen, sulfur, carbon and hydrocarbon. The building blocks of proteins are only twenty α-amino acids bound throw peptide bonds. However, nitrogen is the prominent element present in proteins (Nielsen, 2010a; Nielsen, 2010b).

Universally, the protein content of foods has been found on the basis of total nitrogen content, using the Kjeldahl or similar methods. The protein content is determined by multiplying the nitrogen content by a factor. To obtain the nitrogen content two assumptions are made (FAO, 2003):

1. Dietary carbohydrates and fats do not contain nitrogen,
2. All of the nitrogen exists as amino acids in proteins.

The nitrogen content of proteins varies according to the molecular weight of amino acids (the number of nitrogen atoms it contains) and the amount of non-protein nitrogen (NPN). The variation is something around 13 to 19 percent which yields the conversion factor in a range of 5.26 (1/0.19) to 7.67 (1/0.13) (FAO, 2003).

2.1.1.1 Kjeldahl Method

In the Kjeldahl method, sulfuric acid digests proteins and other food components in the presence of catalysts. Through the mentioned procedure, the total organic nitrogen turns into ammonium sulfate. Then, an alkali neutralizes ammonium sulfate and distills it into boric acid. The borate anions are titrated with standardized acid and converted to nitrogen. In this analysis, the result gives the crude protein content of the sample. It’s to be noted that nitrogen also comes from non-protein content (Nielsen, 2010a).

2.1.1.2 Dumas Method

The Dumas Method is an automated method that in recent years has replaced the traditional

Kjeldahl method. The Dumas Method measures the nitrogen content of samples through combustion at high temperatures and then, with a conversion factor, it calculates the protein content (Nielsen, 2010b; Nielsen, 2010a).

In Dumas method, samples are burned at high temperatures (700-1000° C) in the presence of a flow of pure oxygen. During this combustion, all carbon turns into carbon dioxide while nitrogen is converted to N_2 and nitrogen oxides. The nitrogen oxides are reduced to nitrogen in a copper reduction column at high temperatures (600° C). Pure helium carries the total nitrogen released and a thermal conductivity detector measures the nitrogen through gas chromatography. The protein is then calculated by multiplying the nitrogen content by a protein conversion factor (Nielsen, 2010a).

The Dumas Method has obtained popularity because it is easy to use and gives the results faster than the Kjeldahl method (Müller, 2014).

The Kjeldahl method determines only organic nitrogen and ammonia while the Dumas method calculates the inorganic fraction of nitrogen (like nitrite and nitrate) as well. This difference in calculating the non-protein-nitrogen content of the sample will lead to different results between the two methods. As an example, in a lettuce sample with a nitrate content of 33,000 mg/kg dry matter, this difference in calculation will amount to 0.75% Nitrogen or 4.7% crude protein with the conversion factor of 6.25 (Müller, 2014)

It has been determined that about 2% of "Dumas Protein" was not determined by the Kjeldahl methods and the following relationship between Dumas and Kjeldahl protein values was established (Formula 2.1) (Müller, 2014):

$$\text{Kjeldahl Protein} = 0.959 \times \text{Dumas Protein} + 0.285 \qquad 2.1$$

2.1.1.3 AOAC Official Method 990.03

In principle, nitrogen freed by combustion at high temperature in pure oxygen is measured by thermal conductivity detection and converted to equivalent protein by appropriate

numerical factor (AOAC, 2002).

Any device or instrument that is capable of measuring nitrogen by combustion may be used. The device must be equipped with the following instruments (AOAC 2002):

- Furnace, in order to maintain high combustion temperatures (950° C) for pyrolysis of samples in pure oxygen;
- Isolation system, capable of isolating liberated nitrogen gas from other combustion products for subsequent measurement through thermal conductivity detector (a device for converting NO_x products to N_2 or measuring N as NO_2 may be required and designed);
- Detection system, to interpret detector response as percent nitrogen, w/w.

Crude protein is calculated by Formula 2.2 (AOAC, 2002):

$$\text{Crude protein, \% (w/w)} = \%\ N \times 6.25 \qquad 2.2a$$

Or,

$$\%N \times 5.70 \qquad 2.2b$$

In case of wheat grains.

2.1.2 Analytical Methods for Fat Content

Lipids rarely solve in water while soluble in some organic solvents such as ethyl ether, petroleum ether, acetone, ethanol, methanol and benzene. Most fat content, however, is in the form of triglyceride. While there are non-glyceride components such as sterols, like cholesterol, these non-glyceride fats are not considered important sources of energy (FAO, 2003; Nielsen, 2010b; Nielsen, 2010a).

There are gravimetric methods accepted by AOAC for calculation of crude fat, which includes phospholipids and esters in addition to small amounts of non-fatty material. However, total fat can be expressed as triglyceride equivalents (FAO, 2003).

The lipid content of a certain food is depended on the solvent used. Fat content is calculated by solvent extraction methods but it can also be determined via non-solvent wet extraction methods. There are also instrumental methods that are based on the physical and chemical properties of lipids. The method of choice depends on different factors (Nielsen, 2010b). Since lipids are soluble in organic solvents while remain insoluble in water, water insolubility is the essential analytical property for separation of lipids from proteins, carbohydrates, and water in foods (Nielsen, 2010a).

2.1.2.1 Solvent Extraction Methods

For total lipid (fat) content measurement of foods, organic solvent extraction methods are commonly used. In some other cases, hydrolysis (alkaline or acid) through Mojonnier extraction is prefered. Acid hydrolysis is the preferable method for multi-component foods. The accuracy of direct solvent extraction methods, depends on the solubility of the lipids in the solvent used. In addition, the ability to separate the lipids from complexes with other macromolecules needs to be considered. Because of the differences in solvent polarity, the lipid content of a food may be different considering the solvent we use for extraction (Nielsen, 2010a).

2.1.2.2 Hydrolytic Methods

Association of Analytical Communities (AOAC) Official Method 996.06, extracts fat and fatty acids by hydrolytic methods (acidic hydrolysis for most foods, alkaline hydrolysis for dairy products, and combination of acidic and alkaline hydrolysis for cheese). Pyrogallic acid is used to minimize oxidative degradation of fatty acids during analysis. Triglyceride, triundecanoin is added as internal standard. Fat is extracted into ether and then methylated to Fatty Acid Methyl Esters (FAMEs) using BF_3 in methanol. FAMEs are quantitatively measured by capillary gas chromatography against internal standard e.g. triglyceride, triundecanoin. Total fat is determined as sum of individual fatty acids expressed as triglyceride equivalents. Saturated and monosaturated fats are determined as sum of

respective fatty acids (AOAC, 2002).

2.1.2.3 Soxtec/Hydrotec™Total Fat Solution

Foss total fat solution gives the total fat analysis in a single integrated process. It consists of an extraction unit, a hydrolysis unit and a single filter that is common to both units. This method carries out the hydrolysis process in a faster manner. In addition, the Soxtec™ 8000 can do total or crude fat analysis depending on our requirements (Foss, 2017).

Fat is semi-continuously extracted with an organic solvent. By volatilization and condensation, the heated solvent is poured above the food samples. Solvent is then allowed to drop onto the sample and soaks it in order to extract the fat. With 15-20 minutes' intervals, the solvent is siphoned back to the heating flask and the process restarts. In the end, the fat content is measured by weight loss of sample or weight of fat removed (Foss, 2017; Nielsen, 2010b).

2.1.2.4 AOAC Official Method 991.36

In principle, soluble material is extracted from dried test samples of meat and meat food products through a two-step treatment with petroleum ether as solvent. Solvent is recovered by condensation, while the extracted soluble material is left. Fat (crude) content is measured by weight after drying (AOAC, 2002).

The instrument or device must be composed of the following parts (AOAC, 2002):

- Extraction system, capable of extracting six test samples. Extraction unit for solvent addition to cups, two-stage extraction process, and solvent recovery cycle. Service unit to supply hot oil through insulated tubing to extraction unit and to pump air for evaporation of last traces of solvent from cups;
- Thimbles and stand- 26 × 60 mm, cellulose thimbles, and stand to hold six thimbles;
- Extraction cups- Al, 44 id, 60 mm height;
- Glass beads- 3-4 mm diameter;

- Mechanical convection oven with the ability to maintain temperatures of 125 ± 1° C.

Reagents are as follows:

- Petroleum ether,
- Sand,
- Cotton.

Formula 2.3 calculates the percent fat in the sample (AOAC, 2002):

$$\text{Fat content, \%} = \frac{(B\text{-}C) \times 100}{A} \qquad 2.3$$

Where:

A = g test sample weight,

B = g weight of extraction cup after drying,

C = g weight of extraction cup prior to extraction.

2.1.3 Analytical Methods for Dietary Fiber Content

Most dietary fiber is plant cell-wall composed of polysaccharides- such as cellulose, hemicellulose and linin- that are not easily digested in the small intestine. These fibers need to be fermented by bacteria in the colon, producing different quantities of short-chain fatty acids and gases like carbon dioxide, methane and hydrogen. The produced short-chain fatty acids enter into metabolism and work as a direct source of energy for the colonic mucosa (Nielsen, 2010a; FAO, 2003).

Another form of dietary fiber is chitin. The body of insects is mostly made of insoluble chitin which is considered as a component of the defense mechanism of the insects. In commercial insects, chitin ranges from 2.7 to 49.8 mg per kg of fresh weight (Kouřimská & Adámková, 2016).

Because food labeling requires the expression of dietary fiber, (an) official analytical

method(s) for its determination must exist, which in turn, demands a universal definition of dietary fiber. The American Association of Cereal Chemists (Now AACC international) has defined dietary fiber as:

> "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promotes beneficial physiological effects, such as laxation, and/or blood cholesterol attenuation, and/or glucose attenuation." (AACC, 2001: p. 112)

Dietary fiber is determined gravimetrically so that carbohydrates, lipids and proteins are either solubilized by chemicals or removed through enzyme-catalyzed hydrolysis. The insoluble or indigestible material is collected by filtration. The residue gets recovered, dried and weighed (Nielsen, 2010a).

Among the polysaccharides, solely starch is digestible in the small intestine. Therefore, all polysaccharides, except for non-resistant starch, is considered dietary fiber. Starch usually causes the most problem in dietary fiber analysis. In methods for the determination of fiber, all digestible starch has to be removed. An incomplete separation of starch will increase the weight of the residue, providing a higher estimation for fiber (Nielsen, 2010a).

Three methods have been developed and accepted by bodies such as AOAC International, BCR (Bureau Communataire de Reference) and EC (European Community):

1- AOAC (2000) enzymatic, gravimetric method – Prosky (1985.29)
2- The enzymatic, chemical method of Englyst and Cummings (1988)
3- The enzymatic, chemical method of Theander and Aman (1982)

It is recommended that the AOAC (2000) analysis – Prosky (985.29) or another similar method be used for determination of dietary fiber (FAO, 2003).

In all fiber analysis methods, a heating step (95-100 °C) for 35 minutes is needed. This heating process gelatinizes starch granules and makes them susceptible to hydrolysis. The starch molecules that are resistant in this process and remain unhydrolized, are measured as dietary fiber (Nielsen, 2010a). However, all digestible materials have to be removed from the sample. Lipids, for example, are removed easily with organic solvents posing no threat to the analysis. Protein and minerals that remain, need to be corrected by nitrogen and ash analysis (Nielsen, 2010a).

2.1.3.1 Fibertec™ 8000

The Fibertec™ 8000 is a fully automated system that determines the crude fiber and detergent fiber content according to Weende, van Soest and other recognized methods. Each sample is calculated separately, according to the official procedures. The single-step or sequential extractions include boiling, rinsing and filtration performed under controlled and reproducible conditions (Foss, 2017).

2.1.3.2 AOAC Official Method 978.10

In principle, crude fiber is loss amount on ignition of dried residue remaining after digestion of sample with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solutions under specific conditions. This method is applicable to materials from which the fat can be, and is extracted, to obtain a workable residue, including grains, meals, flours, feeds, fibrous materials, and pet foods. Sample solution is exposed to minimum vaccum needed to regulate filtration, and heating of sample solution prevents gelling or precipitation of possible saturated solutions (AOAC, 2002).

The instrument should be composed of the following units (AOAC, 2002):

- Digestion apparatus, with condenser to fit 600 mL beaker, and hot plate adjustable to temperatures that will bring 200 mL H_2O at 25° C to rolling boil in 15±2 min;
- Desiccator, with efficient desiccant;

- Filtering device, with stainless steel screen;
- Liquid preheater;
- Filtration apparatus, to permit application of minimum vacuum necessary for filtration and washing of each sample within 3-5 min;
- Crucible;
- Cleaning solution (whether acid or base);
- Filtering device.

The required reagents are as follows (AOAC, 2002):

- Sulfuric acid solution;
- Sodium hydroxide solution;
- Bumping chips or granules.

Formula 2.4 determines the values of crude fiber (AOAC, 2002):

$$\text{Crude fiber, \%} = \text{loss in weight on ignition} \times 100/\text{weight test portion, g} \qquad 2.4$$

2.1.4 Analytical Methods for Ash Content

Ash is the inorganic residue that remains after either ignition or complete oxidation of organic matter in a foodstuff. Two important types of ashing is used: dry ashing, and wet ashing (oxidation). Dry ashing is often used for immediate composition and for some specific kinds of mineral analyses while as a preparation for the analysis of certain minerals, wet ashing is used (Nielsen, 2010a).

Dry ashing refers to the use of a muffle furnace with temperatures of 500-600° C. Water and volatile compounds vaporize while organic elements are burned and produce CO_2 and N_2. Most minerals, however, are converted to oxides, sulfates, phosphates, chlorides, and silicates. Wet ashing oxidizes organic elements by using acids and oxidizing agents, or combination of both. In this procedure, minerals become solubilized without volatilization

(Nielsen, 2010a).

Ash content specified the complete mineral content in foods. Ashing, therefore, is the first step in preparing a food sample for specific elemental analysis. It gets even important when there are certain foods high in particular minerals (Nielsen, 2010a).

2.1.4.1 AOAC Official Method 923.03

In principle, 3-5 g well-mixed test portion must be weighted into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after reaching room temperature. Ignite in furnace at 550° C (dull red) until light gray ash constant weight results. The, cool it in desiccator and weigh the sample soon after reaching room temperature. Reignited CaO is a satisfactory drying agent for desiccator (AOAC, 2002).

2.1.5 Analytical Methods for Carbohydrates Content

Carbohydrates form more than 70% of the caloric values of human diet. Ingested carbohydrates are exclusively of plant origin with only lactose from milk that is of non-plant origin. Only monosaccharides can be absorbed from the small intestine while at least 90% of the carbohydrate in nature is in the form of polysaccharides that need to be hydrolyzed to monosaccharides in order to become ingested (Nielsen, 2010b).

2.1.5.1 Phenol-Sulfuric Acid Method for Total Hydrocarbon

The phenol-sulfuric acid method is a simple and rapid calorimetric method for determination of total carbohydrates including mono-, di-, tri-, oligo-, and polysaccharides. As the absorptivity of different carbohydrates is not the same, the results of this method must be expressed in an arbitrary form related to one carbohydrate (Nielsen, 2010b).

Since carbohydrates are destroyed by strong acids and/or heating, a series of complex reactions break down any polysaccharides, oligosaccharides and disaccharides into monosaccharides. Further, pentoses are dehydrated to furfural and hexoses to hydroxymethyl furfural, which react with phenol to produce a gold-like color. This color is stable and the

results are reproducible (Nielsen, 2010b; Nielsen, 2010a).

2.1.5.2 Total Carbohydrate by Difference

Total carbohydrate by difference as the standard for calculating the carbohydrate content of foods. This methods works through calculation of other food constituents such as protein, fat, water, alcohol, and ash and then entering the amounts into a Formula 2.5 in order to obtain total carbohydrate (FAO, 2003):

Total carbohydrate = 100 – (weight in g [protein + fat + water + ash + alcohol] in 100 g of food 2.5

Available carbohydrate signifies the amount of carbohydrate that human enzymes can digest and quickly enters the metabolism a concept that is beneficial in energy evaluation. There are two ways to calculate available carbohydrate content. One is by difference and the other is direct analysis. To calculate the amount of available carbohydrates by difference, we need to exclude the dietary fiber and enter the other components in Formula 2.6 (FAO, 2003):

Available carbohydrate = 100 – (weight in g [protein + fat + water + ash + alcohol + dietary fiber] in 100 g of food 2.6

Direct calculation sums the analyzed weights of individual available carbohydrates or as monosaccharide equivalents; See Table 2.1.

Table 2.1: Methods for calculating carbohydrate in foot samples

Total Carbohydrate:
By difference:
=100 - (weight in grams [protein + fat + water + ash + alcohol] in 100 g of food)
By direct analysis:
=weight in grams (mono- + disaccharides + oligsaccharides + polysaccharides, including fiber)
Available Carbohydrate:
By difference:
=100 - (weight in grams [protein + fat + water + ash + alcohol + fiber] in 100 g of food)
By direct analysis:
=weight in grams (mono- + disaccharides + oligosaccharides + polysaccharides, excluding fiber)

It has been recommended that available carbohydrates be determined by difference, yielding to acceptable results for energy evaluation of almost all foods. But for novel foods or foods or foods with a reduced energy content, a standardized, direct analysis should be made (FAO, 2003).

2.2 Microbiological Evaluation of Edible Insects

Food cannot be consumed before having met the safety and quality standards. Chemical and biological hazards resulting from contamination, adulteration or mishandling of foods must be avoided. Each national food control system is equipped with analytical laboratory services with both chemical and microbiological analytical capabilities (Andrews W. , 1997)

Insects are a rich source of nutrient and moisture that provides a suitable environment for microbial growth. Insects have proven to be a safe source of nourishment from a microbiological perspective but there is a lack of sufficient data in the literature. More studies

are required regarding microbiota associated with edible insects (Amadi EN, 2016; Garofalo & Osimani, 2017).

There are only a few studies that have concentrated on microbiological aspects of entomophagy (Megido, et al., 2017). The Standard ISO food microbiology methods have been suggested and used for microbiological analyses (Grabowski & Klein, 2017).

Full microbiological analysis demands the individual microorganisms to multiply in a liquid medium enriched with agar. A wider range of media with their formulation is available. The formulation depends on the type of organisms and the purpose of study (Adams & Moss, 2008).

2.2.1 Food Sampling, Sample Handling and Storage

Interpretations about the quality of whole food are based on a small sample of it, hence, the adequacy and condition of the sample is important and established procedures has to be followed. Set procedures require us to (FAO, 2009):

- Segregate the material that is evidently harmful for human consumption,
- From hygienic point of view, we need to disregard the rejected material,
- Protect food and food ingredients from contamination throughout sampling, handling, transport and storage.

Samples need to the transferred to laboratory under aseptic conditions. Containers in which the samples are held must be clean, dry, leak-proof, wide-mouthed, and sterile with a suitable size for samples. Samples should be delivered to laboratory with their original storage conditions kept as closely as possible (Andrews & Hammack, 2003).

Samples must be held frozen at all times. Refrigerated samples should not be analyzed more than 36 hours after collection, unless necessary and thus, specified (Andrews & Hammack, 2003).

Aseptic technique has to be used for handling product. Before handling or analysis, work environment has to be cleaned. Frozen samples cannot be thawed before analysis. When necessity demands, we can obtain an analytical portion by thawing the frozen sample in the container in which it was delivered to laboratory. A sample can be thawed within 18 hours, at a temperature of 2-5° C. If rapid thawing is necessary or desired, the samples have to be thawed at a temperature less than 45° C for a time of not more than 15 minutes (Andrews & Hammack, 2003).

Any food sample contains various degrees of non-uniform distribution of microorganisms. In order to reach an even distribution, liquid samples should be shaken and dried samples need to be mixed with sterile spoons before the analytical unit is extracted from a sample of 100 g or greater (Andrews & Hammack, 2003).

Soaking foods has been suggested prior to mixing and diluting so that resuscitation of sublethally damaged cells occurs. In addition, it facilitates better release of cells which exist within tissues. Thirdly, in working with hard or sharp food samples such as cereals and nuts, softening helps prevent damages to stomacher bags used for preparing primary dilutions. When homogenization is done, samples cannot be allowed to stand for more than a few minutes before separating a portion for dilution and plating, the reason being that fungal propagules may sink to the bottom of the container, which in its turn, may lead to under- or overestimation of population (Andrews & Hammack, 2003).

2.2.2 Culture Media

The ideal enumeration medium has been described as having the following attributes: It needs to thoroughly suppress bacterial growth without affecting growth of food fungi. In addition, it has to be nutritionally adequate and allow the growth of fastidious fungi. Mold colonies has to be constrained while spore germination should not be restrained. No one particular medium is satisfactory for determination or enumeration of all yeasts and molds

(Corry, et al., 2003).

2.2.2.1 Brain Heart Infusion (BHI) Agar

BD Brain Heart Infusion (BHI) Agar, is a medium with general purposes used for cultivation of a wide range of microorganisms such as bacteria, yeasts and filamentous fungi. Many types of pathogens have been cultivated through this agar. It is widely recommended as a universal medium for aerobic bacteriology and for primary recovery of fungi (BD, 2017).

BHI is recommended in standard methods for water testing and in antimicrobial susceptibility tests. Nitrogen, vitamins, minerals and amino acids that allow the growth of microorganisms is provided by beef heart, calf brain infusions and peptone mixture. Disodium phosphate acts as a buffer and dextrose acts as the fermentable carbohydrate providing carbon and energy (Condalab, 2017).

2.2.2.2 Alkaline Peptone Water (APW) and TCBS Agar

It is the recommended agar as an enrichment medium while Thiosulfate Citrate Bile Salts Sucrose (TCBS) is the selective agar medium for isolating *Vibrio* species. *Vibrio spp*. grows very fast in APW, and it takes 6 to 8 hours for it so be present at greater number compared to non-*vibrio* organisms. Universally, TCBS is the medium of choice for the isolation of *Vibrio*. It is easy to prepare and is highly selective and differential (CDC, 2017).

In TCBS agar, meat and casein are the sources of nitrogen, vitamins, minerals and amino acids necessary for growth and sucrose the provider of energy (Condalab, 2017).

2.2.2.3 SDA

Sabouraud Dextrose Agar, is used for cultivating yeasts, molds and aciduric microorganisms. It is suitable for cultivation of pathogenic fungi. It is also used for determining the mycological evaluation of food. The fermentable carbohydrate is dextrose which provides carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids

that are required for the growth. The high concentration of dextrose and the acidic pH make this agar very selective for fungi (Condalab, 2017).

2.2.2.4 EMB

Levin Agar (EMB), is the preferred medium for the investigation and differentiation of lactose-fermenting and lactose non-fermenting Enterobacteria in foods and dairy products, for the presence of coliforms. Gelatin peptone is the source of nitrogen, vitamins, minerals and amino acids necessary for growth while lactose is the fermentable carbohydrate that provides carbon and energy (Condalab, 2017).

2.2.2.5 Blood Agar Base

Azide Blood Agar Base, is made of sodium azide that has a bacteriostatic effect on Gram-negative bacteria thus the medium of choice for the isolation of streptococci and staphylococci in clinical, water and foods analysis. In this medium, peptone mixture and beef extract provide nitrogen, vitamins, minerals and amino acids that are required for growth. Sodium chloride provides the essential electrolytes for transport and osmotic balance. Sodium azide inhibits Gram-negative organisms. The agar can be further supplemented with 5% sheep blood allowing the investigation of hemolytic reactions of fastidious pathogens (Condalab, 2017).

2.2.2.6 Salmonella Shigella Agar (SS)

SS agar, is the medium of choice widely used for selecting, differentiating and isolating *Salmonella* and *Shigella* from feces, urine, and fresh and canned foods. It is a strong inhibitory agar in which beef extract and peptone mixture provide nitrogen, vitamins, minerals and amino acids required for growth. Lactose is the fermentable carbohydrate that provides carbon and energy (Condalab, 2017).

2.2.2.7 Campylobacter Agar Base

Campylobacter Agar Base (Preston)m is a medium designed for the isolation of *Campylobacter* in human, animal, bird and environmental samples. It is highly selective for *Campylobacter jejuni* and *C. coli.*. *Campylobacter spp.* is widely recognized in human and animal disease and is one of the main causes of acute diarrhea in man. Casein peptone and soy peptone are the providers of nitrogen, vitamins, minerals and amino acid. Sodium chloride supplies essential electrolytes for transport and osmotic balance (Condalab, 2017).

2.2.2.8 Yersinia Agar

Yersinia Selective Agar Base, is a selective and differential medium when used with supplement. It is based on CIN (Cefsulodin-Irgasan-Novobiocine) agar of Schiemann, which is recommended by ISO 10273 to isolate *Yersinia enterocolitica* in clinical and food samples. D-Mannitol is the fermentable carbohydrate while enzymatic digest of gelatin, casein, and animal tissues provide nitrogen, vitamins, minerals and amino acids required for growth (Condalab, 2017).

2.2.3 Blending and Diluting of Samples for Microbial Enumeration

Dilution plating techniques are used to determine the population of microorganisms per unit weight or volume of food samples. Peptone (0.1%) water commonly plays the role of a diluent for samples so that they become homogenized or blended (Corry, Curtis, & Baird, 2003).

Other diluents may be used as well which depends on the type of food. For high-sugar or high-salt foods, sufficient amount of solute must be used to minimize osmotic shock to fungal cells. For this purpose, buffered diluents consisting of up to 30% glycerol, 40% glucose, or 60% sucrose are suggested (Corry, Curtis, & Baird, 2003).

2.2.4 Plate Counts

In standard laboratories microorganisms in a food sample are allowed to multiply themselves

to form a visible colony. The Aerobic Plate Count (APC) indicates the level of microorganism in a product (Andrews W. , 1997).

2.2.5 Confirmation of Suspected Colonies by BD Phoenix™ System

The BD Phoenix is an automated microbiology system that performs identification and susceptibility of clinically relevant bacteria (BD, 2017).

Automated microbiology system like BD Phoenix™, uses smart software to detect microbes without addition of reagents (Figure 2.1). BD Phoenix allows simultaneous identification, flexible data entry, reduced waste disposal, single or batch inoculation, and gives rapid and accurate results.

Thus, any modification or improvement in conventional culture method that may reduce labor and time of analysis can be regarded as rapid method (Mandal, 2011).

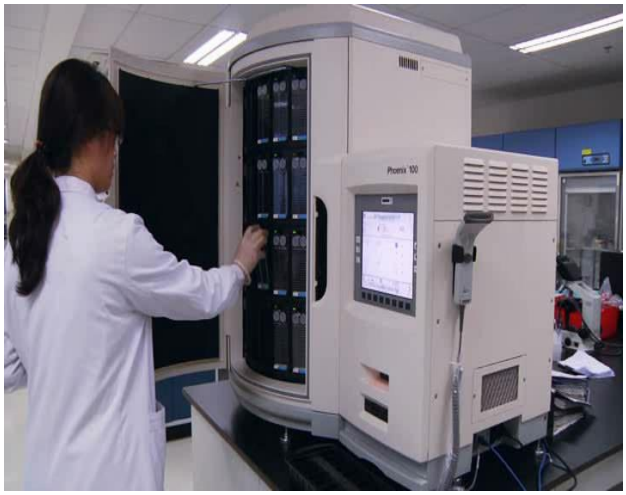

Figure 2.1: Automated BD Phoenix Instrument

CHAPTER 3
RELATED RESEARCH

With the publication of FAO's Edible Insects (Future prospects for food and feed security) in 2013, more emphasis has been placed upon insects as a source of human food and animal feed. Meanwhile, a good amount of studies and researches has been done on the topic of entomophagy.

One particular study listed the nutrient composition of about 236 edible insects from various insect families. Based on the presented data, the protein content of edible insects falls in a range of 35.34% for Isoptera (termites) and 61.32% for Orthopetra (crickets, grasshoppers, locusts). Further, the study concluded that the protein quality of the insects has to be studied in feeding trials. In conclusion, the authors found edible insects as a potential food and protein source. However, they recommend us that more research needs to be done in order to assess the value of insect protein in comparison to that of plant protein and ordinary livestock protein (Rumpold & Schlüter, 2013).

A study has listed the eight essential amino acid composition of common insects in addition to their nutritional values. The study states either tryptophan or lysine as the first limiting amino acid in the majority of food insects, while in some cases, these two amino acids are represented well. For examples, in their study some caterpillars, the palm weevil larva and aquatic insect flour are cited with an amino acid score of over 100. The range of (limiting) amino acid scores for edible insects is from 0 to 102, although, the study was unable to find any similarity in amino acid pattern among the insects listed and concluded that the limiting amino acid varies widely considering the type of insect (Bukkens, 1997).

Ramos-Elorduy (1997) discusses the viability of insects as a reliable source of food. She lists 34 important characteristics of insects- such as biodiversity; their short life cycle; their high

population and biomass; and good nutritive values- as the reason why they could be a sustainable food alternative. She concludes that insects are an attractive food source that can be used in current food technologies. However, according to the study, that can happen only when we are able to select suitable species that can be raised easily in small spaces (with the promise of an economic and nutritional use for insect species) in order to avoid exploitation and extinction (Ramos- Elorduy, 1997).

Another study determined the mineral value of five insects used among the Luo of Kenya. The study alludes to the high iron and zinc content (especially in crickets and also in termites) as their most noticeable finding. According to the study, insects are a potential source of iron and zinc which can combat iron and zinc deficiency in many developing countries. It was also found out that calcium content was much lower, contrary to the iron and zinc content (Christensen, et al., 2006).

Kourimska and Adamkova (2016) reviewed some of the literature and presented the energy value of selected insect species, falling into a range from 293 to 762 kcal per 100 g of dry matter. They found insects as a good source of minerals such as iron, zinc, potassium, sodium, calcium, phosphorus, magnesium, manganese and copper. In addition, insects contain a variety of water soluble or lipophilic vitamins. Interesting in their study, is the taste and flavor of selected insect species based on data by Ramos-Elorduy, presented in Table 3.1 (Kouřimská & Adámková, 2016).

Table 3.1: Sensory evaluation of some of the insect families

Edible Insect	**Taste and Flavor**
Ants, termites	Sweet, almost nutty
Larvae of darkling beetles	Wholemeal bread
Larvae of wood-destroying beetles	Fatty brisket with skin
Dragonfly larvae and other aquatic insects	Fish
Cockroaches	Mushrooms
Striped shield bugs	Apples
Wasps	Pine seeds
Caterpillars of smoky wainscots	Raw corn
Mealybugs	Fried potatoes

Eggs of water boatman	Caviar
Caterpillars of erebid moths	Herring

According to the authors, sensory properties is an important factor in entomophagy. Flavor is affected by pheromones which occur at the surface of insects. The environment where insects live, and their feed, also influence it. Feed selection is an important matter through which we may control the way insects taste. In addition, for the migratory locusts, it was shown that their chemical composition (nutritional composition) could be manipulated by their feed (diet) (Oonincx & van der Poel, 2011). Scalded insects are particularly tasteless because the pheromones are washed away by rinsing. When cooked, insects take the flavor of added spices and other ingredients (Kouřimská & Adámková, 2016).

In an interesting study, microbiological analysis of processed marketed edible insects was carried out, where, the authors observed great variety in the microbiota among the insects. Although they found relatively low microbial counts in insect samples, through pyrosequencing they observed several gut-associated bacteria, some of which may be considered opportunistic pathogens for humans. In addition, food spoilage bacterial and *Spiroplasma spp.* in mealworm larvae were found which are related to neurodegenerative diseases in animals and humans. The authors state:

> "although viable pathogens such as *Salmonella spp*., and *L. monocytogenes* were not detected, *Staphylococcus spp*., *Clostridium spp*., and *Bacillus spp*. (with low abundance) was also found. These numbers must be considered within the context of the low number of edible insect sample analyzed." (Garofalo & Osimani, 2017: p. 15)

In another study, Klunder et al. (2012) studied the microbiological content of fresh, processed and stored edible insects with an emphasis on farmed mealworm larvae and house crickets. They noticed that a short heating step eliminated Enterobacteriaceae, with some spore-forming bacteria surviving the boiling and cooking processes. They concluded that insects, which are a rich environment for growth of unwanted microorganisms, need to be processed and stored with care (Klunder, et al., 2012).

Abbasi et al. (2016) reviewed entomophagy in the light of environmental impact of livestock

production. They concluded that raising livestock for food consumption is a considerable cause of global warming and environmental damages which, within the context of human population growth and their demand for more food, will lead to difficulties in the future. They consider entomophagy as a viable option, their reason being insects' greater diversity, energy efficiency, reproductive thrust, and cleaner production in addition to their potential as human nutrition. They believe that by a considerable shift from conventional livestock to entomophagy we will be able to obtain higher quantities of animal protein (through mini-livestock) with a lesser carbon footprint and environmental impacts and costs. However, challenges and uncertainties remain to be addressed, which are not impossible to overcome (Abbasi, et al., 2016).

A study done by one of the pioneers of entomophagy, Arnold van Huis, thoroughly discusses potential of insects as food and animal feed. He believes that in future, diets based on meat will become too expensive and grain-livestock systems will prove to be environmentally unsustainable. Therefore, switching to edible insects as an alternative to the conventional meat production, whether for human consumption or as animal feedstock, is a serious option. The author states that the challenges such as food safety (pesticides, contamination, heavy metals, pathogens, allergenicity) and processing procedures (for transforming insects into protein meal) still remain to be addressed as regulatory standards are missing. He demands the collaboration of government, industry, and academia in order to develop a new economic sector related to insects as food and feed (van Huis, 2012).

Hartman et al. (2015) did a cross-cultural comparison on willingness of people to eat insects among the Chinese and the Germans. The Chinese were more in favor of insect-based food with regard to their taste, nutritional value, familiarity and social acceptance in comparison with the Germans. In most Western societies, an internalized aversions and negative attitude towards insects exists which is an obstacle to establish insects as a source of nourishment. The authors mention familiarity enhancement and taste education as preconditions to establishing entomophagy, citing the socio-culturally defined public bias towards insects as

the biggest challenge (Hartmann, et al., 2015).

Gerea et al. (2017) carried out a web-based survey regarding entomophagy and alternative protein sources in Hungary. Their result suggests that consuming insects is still thought to be exotic in Hungary. In addition, there was a direct link between those who were familiar with entomophagy and neophobia. Their survey revealed that less than 11% of the participants did not know about insects, soy, algae and whey protein as the alternative protein source. About 60% had heard about entomophagy but they didn't know what it actually meant. Food neophobia, in Hungary, is a barrier for consumption of insects, a fact that is true about most of Western countries (Gerea, et al., 2017).

In an interesting study, Varelas and Langton (2017) have discussed the potential of forest biomass waste as a source for rearing edible insects. In their study, they have summarized the chemical composition of various forest organic waste, as they contain various chemical groups and elements, depending on forest by-product (foliage, branches and tops, stem wood, bark, wasted round wood, stump and saw dust) origin. The organic waste stream contains significant amounts of cellulose, hemicellulose, linin, pectin, starch, protein, fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, rosin, waxes, polyphenols, suberin, oils, phytosterols, tannins, flavonoids and phlobaphenes. These by-products being used either raw or with the appropriate biotechnological pre-treatment, could become essential nutrient components in artificial diets for edible forest insects. In the end, the authors concluded that:

> "forest waste substrates could offer new perspectives on forest sustainable management, environmental protection and future global food security (Varelas & Langton, 2017: p. 203).

CHAPTER 4
MATERIALS AND METHODS

4.1 Materials

4.1.1 Providing the Samples

For the purpose of current study, 500 gr Migratory Locusts (*Locusta migratoria*), Morioworms (*Zophobas morio*), and Mealworms (*Tenebrio molitor*) were used as edible insect varieties (Figure 4.1) and sampling was done according to FAO's Food Composition Data (2003).

Figure 4.1: Frozen insect samples ready for homogenization
(a: morioworms; b: locusts; c: mealworms)

All insects were fasted for more than 48 hours, so that their gastrointestinal tract become free of any feed leftovers, and then put to freezing temperatures (Zielińska, et al., 2015). It is to be noted that throughout the analyses, all the samples were kept at freezing temperature

(Figure 4.1).

Figure 4.2: Insect farm in Antalya, Turkey from where the insects were obtained

4.1.2 Sample Preparation and Processing

Half of the thawed samples were blended and homogenized using a mechanical blender and then used for nutritional analyses (Figure 4.3). The other half of the samples were divided into two groups. One group was directly used for microbiological evaluation and the other group was washed with water, boiled for 5 minutes and then homogenized for yet another set of microbiological evaluation (Figures 4.4 and 4.5).

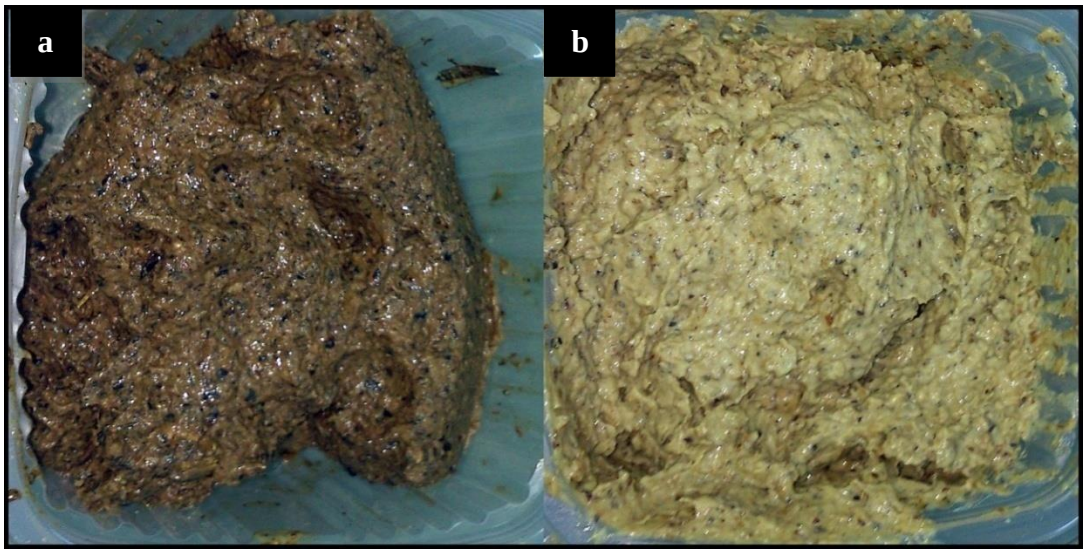

Figure 4.3: Homogenized samples used for nutritional and microbial analysis (a: locusts; b: morioworms)

Figure 4.4: Insects are being boiled (a: locusts; b: mealworms; c: morioworms)

Figure 4.5: Homogenized insects after boiling
(a: locusts; b: mealworms; c: morioworms)

4.1.3 Other Equipment

- Mechanical blender,
- Sealed sterile vials,
- Balance,
- Sterile beakers,
- Sterile graduated pipets,
- Sterile knives, forks, spatulas, forceps, scissors, and tablespoons for sample handling (FDA 2003).
- For other materials related to nutritional analysis, you may refer to Chapter 2 as well.

4.2 Methods

4.2.1 Nutritional Analysis

4.2.1.1 Protein Analysis

Protein content of the insects was obtained through Dumas Method using LECO FP-528 instrument, which is automated and delivers the results directly (Figure 4.6). Leco FP-528 works according with AOAC, official method 990.03 (LECO, 2017).

Figure 4.6: LECO FP-528 used for calculation of protein value

a) 1.0 gram of samples was weighted and put into ceramic boats;
b) The samples were dried in a 101° C furnace. After drying the samples, they were placed in desiccator to cool;
c) The device was set up by running five blanks until values were reproducible and lower than 0.375% protein. Then four EDTA standards were run so that three consecutive values agreed within <0.15% protein of each other;
d) Samples were then placed inside the autoloader and analyzed;

e) The instrument automatically gave the results.

4.2.1.2 Fat Analysis

Fat content was calculated using the FOSS Soxtec™ 8000 instrument (Figure 4.7). This automatic device corresponds with AOAC official method 991.36 (Foss, 2017).

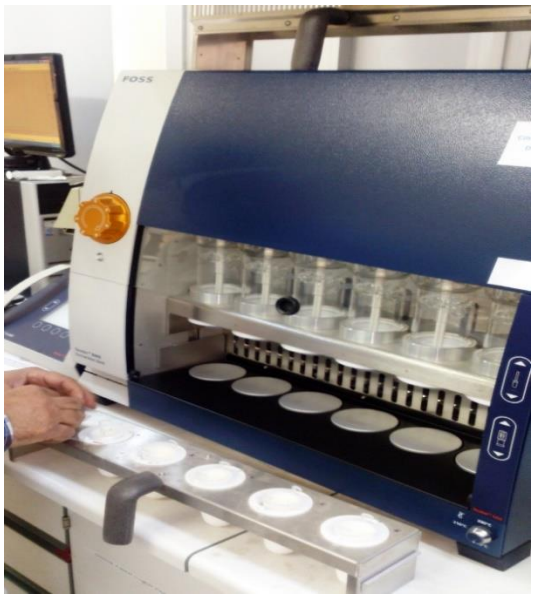

Figure 4.7: FOSS Soxtec™ 8000 used for determination of fat content

a) 3.0 gram of samples were weighted accurately and put into thimbles;
b) Sand was added to the samples and mixed using glass rod;
c) The thimble was placed into its stand and the samples were dried for one hour in 125° C oven. After drying, the samples were allowed to cool;
d) Test samples/sand mixture was loosened using the glass rod;
e) Glass rod was wiped with small amount of cotton and cotton was placed in top of thimbles and the thimbles were transferred to extraction unit;
f) Extraction cups were weighted and the thimbles were extracted using 40 mL petroleum ether while boiling for 25 min and rinsed for 30 min. The condensation rate was adjusted

to more than 5 drops per second;

g) After the extraction was completed, condenser valves were closed and ether was recovered;

h) The cups were dried in 125° C oven and then cooled and weighted.

4.2.1.3 Ash Analysis

Ash content was determined according to the AOAC 923.03 method, using a Protherm Furnace (Figure 4.8).

Figure 4.8: Ash furnace

a) 5 gram of samples were weighted and placed into ceramic boats;

b) The samples were allowed to be burned at 550° C (Figure 4.8);

c) The samples were burned enough until a light gray ash resulted;

d) The samples were allowed to cool in a desiccator and weighted at the moment they reached the room temperature.

4.2.1.4 Crude Fiber Analysis

Finally, Crude Fiber content was calculated using FossFibertec™ 8000 instrument (Figure 4.9). This device corresponds with AOAC official method 978.10 (Foss, 2017)

Figure 4.9: Foss Fibertec™ 8000

a) 2.0 gram of samples was extracted using petroleum ether;
b) The samples were then transferred to 600 mL reflux beaker;
c) 0.5 gram bumping granules were added and then 200 mL near-boiling temperature 1.25% H_2SO_4 was added in order to wet the sample;
d) Two blank runs were done;
e) Beakers were then placed on digestion apparatus at 5 min intervals and boiled for exactly 30 min;
f) California plastic Buchner's were sealed for a vacuum pressure of 735 mm and were placed;
g) By the end of the refluxing, near-boiling water was streamed through funnel to warm it;
h) The residue from funnel was washed into reflux beakers using near-boiling 1.25% NaOH;

i) Beakers were placed on reflux apparatus at 5 min intervals and refluxed for 30 min;
j) Near end of refluxing, filtration apparatus was turned on and the crucibles were placed;
k) The crucibles with residue were dried for two hours at 130° C and then cooled inside a desiccator and weighted;
l) Ash was burned for two hours at 550° C and then cooled in a desiccator and weighted.

4.2.2 Microbiological Analysis

The isolation and identification of microorganisms by conventional culture techniques was performed according to the procedures set by Food and Drug Administration (Andrews & Hammack, 2003).

25 grams of each of the raw and boiled samples was weighted and homogenized in 200 ml solution of Brain Heat Infusion (BHI) agar for detection and isolation of *E. coli, Staphylococcus spp., Camyplobacter, Yirsinia, Candida spp.,* and *Shigella;* and for *Vibrio spp.,* alkaline peptone water (APW) buffer was used.

From the homogenized solutions, 10 ml solutions (1 ml of sample + 9 ml of agar solution) were taken aseptically and then streaked onto petri dishes containing prepared agar mediums designed for the detection of each microorganism (Table 4.1). Subsequently, all microorganisms, except for *Campylobacter,* were aerobically allowed to incubate at 37° C for 24 hours while *Campylobacter* was incubated in microaerophillic conditions at 37° C for 24 hours. Figure 4.10 summarizes some of the steps taken for the microbiological data. After confirmation, BD Phoenix™ Automated Microbiology System gave the number of observed colonies.

Table 4.1: Agar solutions used for the detection, incubation and enumeration of each specified microorganism

Microorganism	**Agar**
Candida spp.	SDA
E. coli	EMB
Staphylococcus spp	Blood Agar Base
Vibrio spp.	TCBS
Shigella	SS
Camyplobacter	Campylobacter Agar
Yersinia	Yersinia Agar

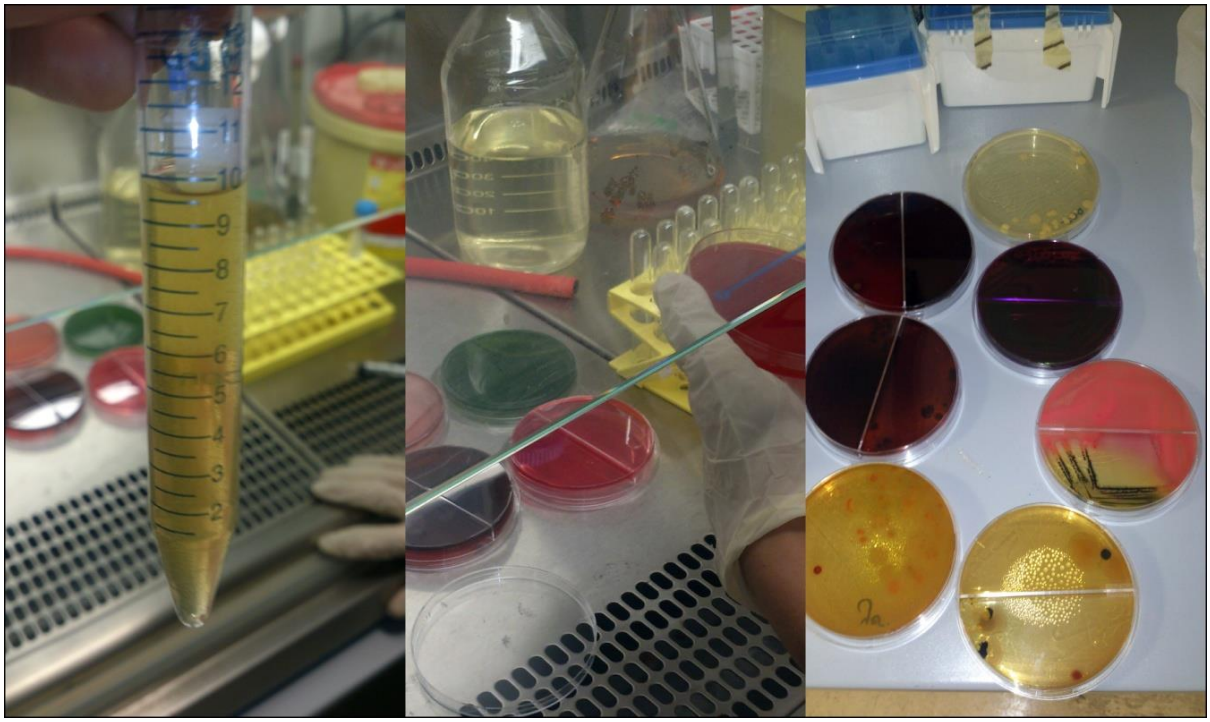

Figure 4.10: Some of the steps taken in microbial analysis

CHAPTER 5
RESULTS AND DISCUSSION

5.1 Nutritional Results and Discussion

Table 5.1 summarizes the protein, fat, crude fiber, and ash content of each of the studied insects.

Table 5.1: Nutritional values obtained from the studied insects

Analyzed Insects	**Nutritional Parameters (per 100 gr)**			
	Protein	**Fat**	**Crude Fiber**	**Ash**
Adult Migratory Locust	20.0	2.9	3.3	1.2
Mealworms	18.0	8.2	2.5	1.3
Morioworms	18.0	11.0	3.0	1.2

In addition, data from some other food sources like beef, chicken, fish, spaghetti and soybeans is added for the purpose of comparison. The data for conventional foodstuff has been taken from the United States Department of Agriculture's (USDA) food composition databases. The characteristics of the foodstuff cited is mentioned below (USDA, 2015):

- **Beef:** Round, top round, separable lean only, trimmed to 0" fat, choice, cooked, braised;
- **Chicken:** Broilers or fryers, breast, meat only, cooked, fried;
- **Fish:** Tuna, light, canned in oil, drained solids;
- **Spaghetti:** Protein-fortified, dry, enriched;
- **Soybeans:** Mature seeds, sprouted, raw.

Protein content of locusts is compared with the data provided by FAO- edible insects (2013) in Table 5.2.

Table 5.2: Comparison of protein content of adult migratory locust

Migratory Locust (*Locusta migratoria*)	**Protein (per 100 gr)**
Current Study	20.0
FAO Edible Insects (2013)*	13.0-28.0
Beef	36.1
Chicken	33.4
Fish	29.2
Spaghetti	21.8
Soybeans	13.1

*(FAO, 2013: p. 69)

Fat and ash content is compared with that of another study based on a diet of grass for adult locusts (Oonincx & van der Poel, 2011). For the crude fiber content, data from Mohamed's study, done on commercially sold migratory locusts in Sudan, has been taken (Mohamed, 2015). The data is presented in Table 5.3.

It is to be noted that the relatively smaller amounts (for fat, ash and fiber) found in the current study is most probably related to the locusts' diet (Oonincx & van der Poel, 2011). However, if we compare the data from current study with the data for the oriental migratory locusts (*Locusta migratoria manilensis)* from Phillipins given by Bukkens (1997), the resutls can be concluded as satisfactory, considering the small size of locusts in the current study (Table 5.3).

Table 5.3: Comparison of fat, ash, and fiber content of adult migratory locusts

Adult Migratory Locusts (*Locusta migratoria*)	**Fat (per 100 gr)**	**Ash (per 100 gr)**	**Fiber (per 100 gr)**
Current Study	2.9	1.2	3.3
Other Studies	18.6±8.07*	4.0±0.74*	14.2-17.1†
Bukkens (1997)	4.3‡	2.3‡	N/A‡
Beef	5.8	-	-
Chicken	4.71	-	-
Fish	8.21	-	-
Spaghetti	2.23	1.12	13.2
Soybeans	6.7	1.59	1.1

* (Oonincx & van der Poel, 2011: p. 13); † (Mohamed, 2015: p. 145); ‡ (Bukkens, 1997: p. 294)

For mealworms and morioworms, the obtained nutritional data has been compared with data obtained by (Finke, 2002) (Table 5.4 and Table 5.5).

Table 5.4: Comparison of protein, fat, ash, and fiber content of mealworms

Mealworms (*Tenebrio molitor*)	**Protein (per 100 gr)**	**Fat (per 100 gr)**	**Ash (per 100 gr)**	**Fiber (per 100 gr)**
Current Study	18	8.2	1.3	2.5
Finke (2002)*	18.7	13.4	9.0	5.7
Beef	36.12	5.8	-	-
Chicken	33.44	4.71	-	-
Fish	29.15	8.21	-	-
Spaghetti	21.78	2.23	1.1	13.2
Soybeans	13.09	6.7	1.6	1.1

*(Finke, 2002: p. 272-273)

Table 5.5: Comparison of protein, fat, ash, and fiber content of morioworms

Morioworms (*Zophobas morio*)	**Protein (per 100 gr)**	**Fat (per 100 gr)**	**Ash (per 100 gr)**	**Fiber (per 100 gr)**
Current Study	18	11	1.2	3.0
Finke (2002)*	19.7	17.7	10	3.9
Beef	36.1	5.6	-	-
Chicken	33.4	4.7	-	-
Fish	29.1	8.2	-	-
Spaghetti	21.7	2.2	1.1	13.2
Soybeans	13.1	6.7	1.6	1.1

*(Finke, 2002: p. 272-273)

5.2 Microbiological Results and Discussion

Microbiological results were obtained automatically through BD Phoenix™ and then multiplied by 10 (tenfold solutions) and presented in the form of colony forming units per milligram (cfu/mg).

It is to be noted that the number of colony forming units per milligram found in raw samples, is interpreted for ready-to-eat (RTE) foods according to Compendium of Microbiological Criteria, Food Standards Australia New Zealand, 2016 (FSANZ, 2016).

The microbial data related to mealworms is presented in Table 5.6.

Table 5.6: Microbiological results for mealworms

Studied Microorganisms	**Raw Samples (cfu/ml)**	**Boiled Samples (cfu/ml)**	**Raw Sample Interpretation**
E. Coli	1.0×10^2	-	Unsatisfactory
Staphylococcus spp.	1.4×10^2	-	Marginal
Camyplobacter	-	-	Satisfactory
Candida spp.	2.0×10^2	-	N/A*
Yersinia	-	-	Satisfactory
Virbio spp.	-	-	Satisfactory
Shigella	-	-	Satisfactory

*No Interpretation Available

Table 5.7 represents the microbial data for locusts and Table 5.8 shows the number of colony forming units per milligram of morioworms samples.

Table 5.7: Microbiological results for adult migratory locusts

Studied Microorganisms	**Raw Samples (cfu/ml)**	**Boiled Samples (cfu/ml)**	**Raw Sample Interpretation**
E. Coli	1.5×10^{2}	-	Unsatisfactory
Staphylococcus spp.	1.0×10^{2}	-	Marginal
Camyplobacter	-	-	Satisfactory
Candida spp.	1.5×10^{2}	-	N/A
Yersinia	-	-	Satisfactory
Virbio spp.	-	-	Satisfactory
Shigella	-	-	Satisfactory

Table 5.8: Microbiological results for morioworms

Studied Microorganisms	**Raw Samples (cfu/ml)**	**Boiled Samples (cfu/ml)**	**Raw Sample Interpretation**
E. Coli	0.3×10^{2}	-	Marginal
Staphylococcus spp.	-	-	Satisfactory
Camyplobacter	-	-	Satisfactory
Candida spp.	1.3×10^{2}	-	N/A
Yersinia	-	-	Satisfactory
Virbio spp.	-	-	Satisfactory
Shigella	-	-	Satisfactory

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Although our obtained data are a little lower compared to some of the other studies, two factors should be considered:

- Our commercial insects were bought from a small farm in Turkey, which rears insects mainly for pet consumption. The conditions, under which these insects are kept, and their feed is of outmost importance when it comes to commercializing insects.
- The nutritional and microbial data, presented in this thesis, provide enough of a stepping-stone to consider insects as a great source of protein and fat- whether for human consumption or animal feed.

Boiling for 5 minutes improved the odor of the insects significantly and resulted in an acceptable color enhancement for morioworms and mealworms. However, migratory locusts maintained their color with boiling. In addition, boiling resulted in detection of no trace of the studied microorganisms. These simple facts help us conclude that boiling the insects will provide us with better odor and color and is a satisfactory step resulting in the destruction of most microorganisms (FAO, 2013; Rumpold & Schlüter, 2013).

6.2 Recommendations

Further research is needed to confirm the vast benefits of insects as a source of human food and animal feed. More studies on other nutritional aspects of insects such as minerals, vitamins, amino acids, and fatty acids need to be done. It is highly recommended that researches in Turkish Republic of Northern Cyprus take entomophagy more seriously. In addition, governments and international organizations need to cooperate and regulate the laws in order to facilitate practices related to farming insects, and subsequently processing and commercializing them as a source of human food and animal feed.

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