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UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Jackson College of Graduate Studies

Functional Insect Protein Extracts for Food Applications

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN NUTRITION AND FOOD SCIENCE

By

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Edmond

May 2nd, 2019



Functional Insect Protein Extracts for Food Applications

A THESIS APPROVED FOR THE DEPARTMENT OF HUMAN ENVIRONMENTAL SCIENCES

May 2nd, 2019

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To my father, Dr. Amarender Vadivelu my mother S. Hemalapathi and my brother V.A. Nirmal Kumar

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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Kanika Bhargava for all the support, advice and guidance throughout my graduate program and research work. This journey would not have been possible without her support. I greatly appreciate the time she spent on giving me valuable inputs on my research. She has helped me realize my research potential and strengths.

I would like to thank the expert in Entomophagy studies, Dr. Aaron Dossey, CEO/founder Griopro®All things bugs LLC, for providing me with all the necessary information and suggestions. I would like to extend my special thanks and gratitude to my committee members, Dr. Tawni Holmes and Dr. Sanjeewa Gamagedara for all their support and advice throughout this study.

Words are not enough to thank my parents, Dr. Amarender Vadivelu and S. Hemalapathi, who have always encouraged me to pursue my higher studies and support me both financially and emotionally. Thanks to my fellow lab mates: Mojdeh Akbaran, Sindhu Bharathi and Roshaniben Chaudhari for all the team work and inspiration.

Abstract

Entomology, or the consumption of insects has been extensively accepted worldwide but is uncommon in the United States. There has been positive research on the sustainability, renewability, nutrition and safety of crickets as food. Due to the current trend of an increasing human population, food security has become a challenge worldwide, especially in developing countries. Studies on crickets as a protein source are still limited. Hence, a thorough investigation of crickets as an alternate insect protein source and incorporation of cricket proteins into low nutrient foods, such as breads and pasta, can help improve the nutritional content and food security. This study aimed to extract the proteins from defatted cricket powder, study its functional properties, incorporate the protein extract into pasta and carry out product analysis. In this research, proximate analysis of fresh cricket powder was studied and is proven to contain 63.43% protein, 20.86% fat, 4.65% ash, 7.56% carbohydrates, 3.50% moisture and 472Kcal/100g. Due to the high-fat content present in the powder, defatting was carried out using hexane and ethanol, reducing the fat content from 20.86% to 9.27% by rotary evaporation. Extraction of proteins was carried out using NaOH and ascorbic acid, ascorbic acid gave the highest yield of 87.75%. The defatted protein extract using ascorbic acid was a good source of essential amino acids having an overall protein content of 69.69%. Nutritional and functional properties of the protein extract were studied for further product formulation and analysis like color, texture, water activity, cooking quality, shelf life study and sensory evaluation. Results demonstrated that insect protein extracts helped improve the nutritional content of freeze-dried pasta thus offering an alternative protein source to the food industry.

Keywords: cricket, sustainable, food security, insect proteins, entomophagy

CHAPTER ONE: INTRODUCTION

1.1 Introduction and statement of the problem

Scientists anticipate that by 2050, the world's population will have reached nine billion people (van Huis et al., 2013) and agricultural land will be reduced. Protein is mainly derived from animal food from several sources such as poultry, fish, and meat; live stock requires a lot of land and water compared to insects. According to the Academy of Nutrition and Dietetics (2017), the required protein dietary allowance for adults is 0.36 grams per pound of body weight. Since the required protein allowance is not being met, there is a nutrient shortfall which can be resolved by utilizing natural resources. This study suggests crickets can be a potential source of protein for human consumption. Rearing crickets require little space and minimal water consumption compared to livestock. Studies on crickets as a protein source, however, is still limited. Hence, a thorough investigation of crickets as an alternate insect protein source and incorporation of cricket proteins into low nutrient foods, such as bread and pasta, can help improve the nutritional content and food security.

Protein is one of the three macronutrients that are vital for the human body. Amino acids are the building blocks of protein that ensure proper growth, maintenance, and development of the body. The essential amino acids must come from food. Protein sources can be obtained from several sources such as poultry, fish, meat, and legumes, which would require a lot of land and water when compared to insects. The study is based on how crickets can be considered a sustainable and healthy alternative to livestock. In the United States, insects as food are not widely popular. It is still required to help people understand that crickets are a very good source of protein in our diets. Encouraging them to accept and analyze the best and efficient method of protein extraction is still in progress. This study is based on the theories of food sustainability, food

security, and inadequate intake of protein in our diets. The purpose of this study is to discover if insects can be a sustainable source of protein for future generations.

Crickets contain many of the B vitamins including B12, thiamin, riboflavin, niacin and pantothenic acid. They also deliver a variety of minerals such as phosphorous, zinc, selenium and iodine. Advocates of cricket consumption point out that crickets offer nearly five times as much magnesium as beef.

The adverse effects of consuming animal proteins are having negative impacts on the environment. Amongst other sources of food, agriculture and meat production have a negative impact on human health and the ecosystem. Meat production, and particularly beef production, has a large carbon footprint, and since consuming meats has been increasing over the years in areas that are still developing, the meat industry is contributing to a wide range of climatic factors and thereby affecting the environment negatively (Premalatha, Abbasi, Abbasi, & Abbasi, 2011). Meat production utilizes a large area of land, animal feed, and energy. Studies suggest that insects can be an alternative source to meat and can contribute to a lower environmental impact (Belluco, Losasso & Maggioletti, 2013).

The insects are natural resources. Herbivorous insect species depend on plants, and thereby their collection depends on the season (van Huis et al., 2016). However, edible insects are available every season, which makes insects a sustainable source, as it provides a possibility for year-round harvesting. Edible insects often balance other protein sources which are not available during a certain period of the year (van Huis et al., 2016). Commonly, insects provide nutrients which are not available in staple food.

According to the World Health Organization (WHO): Edible insects overall meet the requirements for amino acids with high values for phenylalanine, tyrosine, tryptophan, lysine, and

threonine. Specifically, species from the order Orthoptera, like crickets, are a good source of proteins and signify a valuable protein alternative. Most edible insects provide adequate amounts of essential amino acids required for the human body (Yi et al., 2013).

Insect food sources are commonly used in tropical and subtropical countries but are less normalized in Western society. The study of insects demonstrates considerable nutritional, economical, and ecological benefits (De Foliart, 1999). The advantage of insects compared to livestock such as cattle is the lower emission of greenhouse gases and ammonia per kg mass gain (Oonincx, Itterbeeck, Heetkamp, Brand & van Loon, 2010). Most of the insects can be collected from natural resources that are always renewable and available. Commercial insect farming can advance the livelihood of communities through its future demand. Insects can be consumed in three ways: recognizable whole insects, non-recognizable processed ingredients in food and as an extract such as protein isolate. In this research, we mostly focus on crickets as a source of non-recognizable source of protein to enrich the protein content in low-nutrient foods such as bread, pasta, and extruded foods.

Hypothesis and Objective of the study

- **1.2.1 Hypothesis:** Fortification of cricket protein extract in low nutrient-dense food will serve as an alternative protein source and provide better nutritional value.
- **1.2.2** Objectives and specific aim: To analyze optimum extraction methodologies that provides us with the best yield and maximum extraction of proteins from crickets.
- 1. Study the functional properties of protein extract.
- 2. Fortification of cricket protein extracts in freeze-dried pasta and perform product analysis.

CHAPTER TWO: REVIEW OF THE LITERATURE

2.1 Facts on insect proteins

This review of the literature will further advance the use of natural resources like insects to help food sustainability, food security, and inadequate intake of protein in our diets. The benefits of consuming insect proteins are related to high nutritional content, especially being high in proteins and important micronutrients, low environmental impacts and improved food security. It is crucial to conduct studies that will help improve the knowledge on how to have a sustainable protein source for the future generations without any impact to our environment.

When a comparison study was made between livestock and insect sources the results showed that the conventional animal protein sources including beef, pork, and chicken meat may be insufficient to meet the demand of the world population (van Huis, 2016). Hence doubts arise on whether livestock can be a sustainable protein source in the future, subsequently opening a door to alternative sources. Edible insects demonstrate great potential as an environmentally sustainable nutrient source for the future as insects are renewable natural resources that are always available round the year. There are several positive benefits of utilizing insects as a sustainable food source including their high nutritional content. Besides fats and proteins, insects are also a predominant source of vitamins and minerals. Quantitative methods would be most appropriate to find an answer to analyze the nutritional content like the proteins, fat, vitamins and minerals present in insects. Insects have good food conversion efficiency and produce lower greenhouse gas emissions (GHGEs) while requiring a minimal amount of water and land when compared with their vertebrate counterparts in traditional animal husbandry. (Van Huis, 2016)

From this study, we can infer that consuming insects can contribute positively to the environment, food and nutritional security, and healthy life for present and future generations.

The following are the classifications where insects provide the most important benefits for a sustainable and secure food supply: 1) Efficiency 2) Biodiversity 3) Food security. Therefore, the remainder of this review of the literature explores what current empirical results reveal about these three categories of findings.

2.2 Efficiency

Efficiency is a very important factor to consider while deciding to prove that insect sources are the most sustainable form of proteins in order to obtain the desired result. Insects are very productive at transforming a wide range of organic matter into edible insect body mass, or they show a high feed conversion ratio (Gahukar, 2016). Cows consume eight kilograms of feed to gain one kilogram in weight, whereas insects require less than two. (Vogel, 2016). This is partly due to insects being poikilothermic, using less energy for body warmth (Premalatha, Abbasi, Abbasi & Abbasi, 2011). Insects require minimal water consumption when compared to livestock 1) By consuming less water and 2) By consuming less feed which requires irrigation. One of the examples stated in a study showed that a single female cricket can lay up to 1,500 eggs in its 5-6-week lifespan, and a female mealworm can lay over 500 eggs (3-6 months) (Siemianowska, et al., 2013), whereas cattle require four breeding animals for each animal marketed (Gahukar, 2011). Therefore, proving crickets can be an efficient source of proteins compared to livestock. Furthermore, many insects can eat plants, biomass or agricultural byproducts that humans cannot, thus not competing with human food supply like other livestock.

Insects also have greater feed conversion efficiency than cattle. For example, crickets only require two kilograms of feed to gain one kilogram body weight (van Huis et al., 2013). Insects also require less feed compared to the production of the equivalent amount of protein from conventional meat sources. To produce 1 kg of meat, 7.7 kg feed is required for beef, 6.3 kg for

sheep, 3.6 kg for pork, 2.2 kg for chicken, and 1.7 kg for crickets (van Huis, 2010). Due to the greater feed efficiency, insects show a greater potential as a sustainable protein source. This can be a great way to improve food security because insects have an equivalent amounts of proteins but consume less feed when compared to conventional meat sources.

2.3 Biodiversity

Biodiversity is often associated with the start of any species present in the ecosystem. The UN FAO (United Nations Food and Agricultural Organization) reports that 75% of our food comes from only 12 plant sources and five animal species. In the face of climate change, livestock diseases, droughts and increased demands from human population expansion, the development of more animal livestock diversity are critical to food security. However, there are one million species of insects and 4-30 million crickets estimated to exist on earth (Gahukar, 2016) indicating crickets are found in abundance and therefore can prove to be a good food alternative with a minimal impact to biodiversity.

Studies indicate that insects, could serve as a more sustainable and environmentally friendly alternative for the production of protein in regard to GHG and NH₃ emissions, land and water use, compared with beef (Akhtar & Isman, 2018). Most importantly, insects have the potential to contribute to food and feed security. Considering the economic, nutritional, and ecological advantages of insects as a traditional food source, studies on insect proteins can be considered a very important study. This promotion deserves more attention from national governments, assistance programs and recommendation by media and government official (Alemu, Olsen, Vedel, Pambo & Owino, 2017). In addition, behavioral adoption of entomophagy necessitates overcoming highly negative cognition and emotions of disgust and fear factors. If governments took the initiative to include insects as a food alternative, it can improve sustainability

and protect the environment. This change can be a huge benefit to the ecosystem and the food industry. After appropriate analysis showing insects can be a sustainable source of proteins, the next questions that followed up were the consumer acceptance and the need of proteins.

2.4 Food security

As the human population grows, it is important to reduce consumption and harvesting materials from the earth and its ecosphere (Dossey, Morales-Ramos & Rojas, 2016). One way to improve food security for the growing population is by using the vastly depleted natural resources. As demonstrated in one of the studies, utilizing natural resources like insects can be a boon to the world population (Steinfeld et al., 2006).

One important way to help the world population is by "Ensuring environmental sustainability" which is one of the United Nations' Millennium Development Goals. Climate change, reduced the productivity of agricultural lands, overfishing, dwindling freshwater resources, pollution from fertilizers and pesticides, and many other factors show that this population increase will place an inconsistent problem on Earth's ecosphere, with an urgency that remains largely ignored (Dossey, 2013).

In North America about 70% of protein is animal-derived (Dossey et al., 2016). Meat, fish, and poultry contribute 40% while dairy contributes 20%. Beef accounts for 53% and chickens account for 21% of meat purchased (Montanabeefcouncil.org, 2014) (Dossey et al., 2016). These animal-based products are increasingly scarce, and production is not sustainable. It is often overlooked and may be deemed unimportant. However, this aspect of studying insect proteins contributes to a lot of positive benefits like providing an optimum supply of nutrients without any impact to our environment.

2.5 History of entomophagy

The word entomophagy in greek is broken down into entomos, meaning insect, and phagein, meaning to eat. Insects have served as a food source for many years all over the planet. In the Middle East, as far back as the 8th century B.C., servants carried locusts arranged on sticks to royal banquets. In Greece, eating cicadas was considered a delicacy. In the 21st century, two billion people already started consuming a wide variety of insects on a regular basis, and in 2013, the United Nations Food and Agriculture Organization (FAO) reminded the world population that there are more than 1,900 edible insect species on Earth that contain high amount of protein, fiber, healthy fat and vitamins and minerals. According to the FAO, accepting entomophagy and incorporating it as a part of our diet is a perception of culture. Native Americans were very accustomed to eating grasshoppers, locusts and crickets. Today, insects are considered delicacies in many parts of the world, particularly in the tropics where a variety of edible insect species can be found all-round the year. As the domestication of animals and plants took place, eating insects became less popular and very limited. Now, considering all the environmental impacts, nutrition, and harvesting techniques insect seems to be a very good option for consideration to incorporate it as a part of our diet.

2.6 Nutrient facts and benefits of cricket insects

There are many species of cricket consumed globally, and various analysis have been carried out with regards to the different nutritional aspects of crickets such as the field cricket (Gryllus genus), and the house cricket (Acheta genus). Thailand's field cricket Gryllus bimaculatus (raw), contains 120 kcal/100g (van Huis et al., 2013), The nutritional composition of a single field cricket approximately contains 58% protein, and 10% fat in contrast, the composition of a single house cricket is around 65% protein, and 20% fat (Wang, Bai, Li, & Zhang, 2004).

Field crickets provide an adequate amount of essential amino acids that fulfill the requirements suggested by the World Health Organization (Wang et al., 2004). In another study, it was proven that the protein of the house cricket was superior to soy protein for amino acid intake when fed to rats (Finke et al., 1989).

Vitamin B12, which is abundantly found only in animal sources can also be found adequately in-house crickets, at 5.4 µg per 100 g in adults and 8.7 µg per 100 g in nymphs; the recommended dietary amount is 2.4 µg daily (van Huis et al., 2013 & Baik, 1999). According to Rumpold et al. (2013), the majority of insects exhibit high amounts of potassium, calcium, iron, magnesium, and selenium, as well as zinc. Acheta domesticus contains about 6-11mg of iron per 100g (Rumpold et al., 2013); in comparison to, ground beef that contains about 2.2mg iron per 100g. Insects, and specifically crickets, have long been overlooked as a source of essential nutrition in Western culture. Cricket flour is a complete protein, so it contains essential branched-chain amino acids that the body needs for muscle development. Proteins are considered long chains of amino acids, which are the important molecules that we get from our diet. Without enough diverse protein sources in the diet, there is a high risk in certain amino acid deficiencies, which can lead to trouble building muscle mass, low concentration and memory, unstable blood sugar levels and trouble maintaining or losing weight.

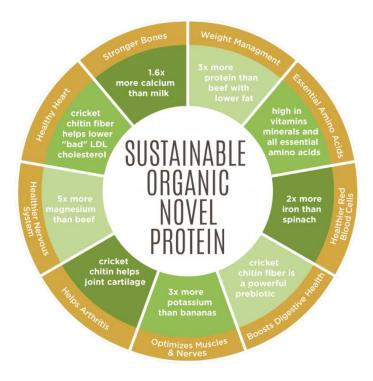


Figure.1 Nutritional benefits of Crickets (Picture Copyright © 2018 NMP Proteins)

TABLE 1.

Comparison of the Nutritional Value of Insects Between Other Common Sources of Proteins

200 calorie serving	Protein (g)	Fat (g)	Saturated fat (g)	Omega-3 (g)	Fiber (g)
Mealworms	16.2	14.8	4.9	3.3	2.5
Whole eggs	19.2	15.2	4.8	0.1	0
Salmon, Farmed	20.4	13.4	3.0	2.5	0
Beef (90% lean)	22.4	11.2	4.4	0.04	0
Tofu	24.6	12.6	2.7	0.5	2.7
Crickets	31.0	8.1	2.6	1.8	7.2

Borrowed from: Reinagel (2013).

From table 1 we can see a clear illustration that crickets rank superior in terms of nutrition compared to other common sources of proteins that are consumed on a daily basis.

Crickets are amongst the top five insects consumed in the world (Barennes, Phimmasane, & Rajaonarivo, 2015; Six Foods Chirps Chips, 2016). In fact, 13% of all insects consumed come from the Orthoptera, which includes crickets, grasshoppers, and locusts (van Huis et al., 2013). Cricket protein is used for product fortification for both their nutritional and functional properties. In one serving (20 g) of cricket protein powder, there are 90 calories, 4.5 g fat, 1.0 g fiber, and 13.0 g protein as outlined in Figure 2 (All things bug LLC, 2016).



Figure 2. Cricket powder nutrition label (Griopro®All things bugs LLC, 2016)

2.7 Production of cricket powder

Cricket powder is processed naturally: whole crickets are ground, pasteurized, and dried. Companies who produce cricket powder can either purchase live crickets from local cricket farmers, frozen crickets from cricket farms, or breed or raise their own crickets (Mermelstein, 2015). At cricket flour processing companies, once the crickets have been gathered, they are ground wet to a size of 100 microns. After the grinding process, the slurry of wet crickets is pasteurized at 80 °C for 10 min to kill spoilage microorganisms, then spray dried and packaged (Vandeweyer et al., 2017; Mermelstein, 2015).

2.8 Product development using insects proteins

These days, consumers focus is on a nutrient dense snack with protein as their main nutrient supply. Optimal protein intake can be helpful in preventing diseases, weight loss and an essential nutrient for maintaining muscle mass. Some populations, such as athletes and active individuals, require more protein and thus benefit from the availability of convenient sources to meet their daily protein requirements (Webb, 2014).

A protein fortified snack made with a complete protein source could be the answer to meeting consumer demands. Due to the unsustainability of conventional protein sources, insect protein has been proposed as an alternative high protein source (Oonincx, Itterbeeck, Heetkamp, Den Brand, van Loon, & van Huis 2010).

Studies have shown that whey protein supplements were unable to supply an overall protein source that has all the recommended EAA requirements (Almeida, Monteiro, da Costa-Lima, Alvares, & Conte-Junior, 2015). Likewise, even plant proteins do not contain a complete protein source that has all the essential amino acids, whereas cricket protein contains all essential amino

acids that aid in growth synthesis. Insect protein utilized in low nutrient dense foods like pasta can be a novel, convenient way to promote entomophagy and allow consumers to meet their daily nutrient requirements. Products like pasta contain only a good amount of fiber, manganese and selenium. Refined pasta is higher in calories, carbs, B vitamins and iron but lower in protein, fiber and most of the micronutrients. Thus, fortifying pasta with cricket powder can be a very nutrient rich snack alternative for future generations.

CHAPTER THREE: METHODOLOGY

3.1 Materials

The experiment was mostly conducted in the Department of Chemistry and Department of Human Environmental Sciences Laboratory at the University of Central Oklahoma, Edmond OK. Freshly ground Cricket powder was purchased from Griopro[®] All things bugs LLC and samples were analyzed at Eurofins Scientific Testing Laboratory, Iowa. Ethanol, Anhydrous (Histological) Cat No.: A405-20; A405F-1GAL; A405P-4 and Hexane (n-Hexane) S25352A were used for defatting. Sodium Hydroxide NC-0874, NC-2091, S1013, S1101, CF1131 and Ascorbic acid S25184 were used for protein extraction. Rotary evaporation (IKA HB 10) was used for removal of hexane and ethanol from the defatted sample and the samples were passed through the nitrogen gas to dry it to a powder form. Vortex (Vortex Gene scientific industries), Centrifuge (Jouan MR 1822) and home freeze dryer (Harvest right HRFD-SMBL) were used for carring out the protein extraction protocol. To carry out the functional properties of the defatted protein extract, water bath (WB10, polyscience), convention oven (MO144A-1, Thermo Fisher Scientific Inc.), magnetic stirrer (MS-H280-pro, Scilogex, LLC.), were used. Product analysis involved handling equipment's like the pasta maker (ISILER 9) Hunter colorimeter (MiniScan EZ 4500L Spectrophotometer), water activity meter (WA-160A), Genesis R&D software (SQL 2008 database) for nutritional labeling of the product and TVT 600 Perten instruments texture analyzer was used to carry out texture analysis.

3.2 Proximate analysis

Proximate analysis of freshly ground cricket powder, defatted cricket powder and protein extract were carried out. The various analysis included moisture by vacuum oven (AOAC 925.09), protein- combustion (AOAC 990.03, AOAC 992.15), Ash (AOAC 942.05), fat by acid hydrolysis (AOAC 954.02), carbohydrates (CFR 21-calc), and calories. Elemental analysis of sodium by ICP (AOAC 984.27, 927.02, 985.01, 965.17) was carried out to analyze the amount of salts present in the protein extract by using sodium hydroxide for extraction purpose.

3.3 Defatting procedure

The freshly ground spray-dried cricket powder was obtained from (Griopro[®]All things bugs LLC). Ethanol (99.5%) and Hexane (100%) was used for defatting cricket powder at a solvent to the material ratio of 5 mL/g. The solution was centrifuged at 4800 rpm for 10 minutes. The filtrate was then passed through nitrogen gas. The fat and the solvent were separated using a rotary evaporator and the fat percentage was calculated (L'hocine, Boye & Arcand, 2006).



Figure 3: Rotary evaporator (IKA HB 10)

3.4 Protein extraction

The ethanol defatted cricket powder was used for protein extraction by NaOH (Alkaline) and Ascorbic acid (Acidic nature). For this study protocol, 0.5M NaOH and Ascorbic acid were carried out separately in the ratio of 6:1 ml/g. The tubes were vortexed, centrifuged at 3500g at 4°C for 20minutes. The supernatant and gel layer were decanted and kept aside. The second extraction was carried out on the pellet, vortexed, centrifuged and the pellet was frozen overnight at -20°C and freeze-dried to obtain a final protein extract with moisture content less than 5% (Zhao, Gutiérrez, Johansson, Landberg & Langton, 2016). Further nutritional analysis like the Carbohydrates, Fat by Acid Hydrolysis, Moisture by Vacuum Oven, Calories, Ash, Protein Combustion, and elemental analysis like salts were carried out on the extract. Finally, the Extraction yield and extraction rate of protein were calculated as follows:

Extraction yield (%) =
$$100 * \frac{extract}{Sample}$$

Extraction rate of Protein (%) = $\frac{protein\ content\ in\ extract}{protein\ content\ in\ sample}*Extraction\ yield\ (\%)$



Figure 4: Centrifuge (JOUAN MR1822)

3.5 Functional properties

3.5.1 Water absorption capacity (WAC)

Two grams of cricket protein extract was weighed into a centrifuge tube and 50ml of distilled water was added. The sample was vortexed for 15 minutes. The mixture was then kept in a water bath (37°C) for 30minutes and centrifuged at 1300rpm for 15minutes. The pellet was weighed (M2) and then dried at 105°C to constant temperature (M1). The sampling procedure was done for original cricket powder, defatted cricket powder and the protein extract. A comparison study was done to see the difference in the functional properties. The WAC was then calculated as follows (Bernard, Edmond, Michel, Soumaila & Patrice, 2015).

$$WAC\% = \frac{M2-M1}{M2} * 100$$

3.5.2 Fat absorption capacity (FAC)

One gram of cricket protein extract sample was mixed with 10ml of rapeseed oil for 30minutes using a magnetic stirrer with a stirring speed of 1000/minute. The sample was then allowed to stand at room temperature for 30 minutes and centrifuged at 5000rpm for 30minutes. The volume of the supernatant was measured using a 10ml graduated cylinder. The density of oil had to be measured. The volume of the oil absorbed was multiplied by the density of the oil in order to determine the final weight of oil absorbed (Bernard et.al, 2015).

$$FAC\% = \frac{(V1-V2)*P}{W}*100$$

V1- Initial volume of oil used

V2 – **Volume remaining (not absorbed)**

P- Density of oil

W – Weight of sample

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3.5.3 Foaming capacity

Three grams of cricket protein extract was transferred into a 50ml graduated cylinder. The extract sample was leveled, and the volumes were noted. 30ml of distilled water was added to the sample, the cylinder was swirled and allowed to stand for 120 minutes while the change in volume was noted every 15 minutes (Coffman & Gracia, 1977).

$$FC\% = \frac{Vt - V0}{V0} * 100$$

V0 = Original volume of sample (ml)

Vt = Total volume after different times

3.5.4 Emulsifying activity

Two grams of cricket protein extract and 50ml of distilled water were mixed using a magnetic stirrer. After thorough mixing of the sample, Peanut oil was added continuously in small quantities from a burette. Mixing continued until the emulsion breakdown occurred, basically where a separation of two layers was observed (Beuchat, 1977).

EC (%) =
$$\frac{VE*100}{V*W}$$

W = Weight of sample

VE = **Volume** of emulsion layer **E**

V = Total volume of mixture



Figure 5: Addition of peanut oil using a burette

3.6 Statistical analysis

All experimental analysis carried out in this research study were done in triplicates. ANOVA (one-way) analysis was carried out to compare the mean values of the insect protein extract. A comparison of freshly ground cricket powder, defatted cricket powder and protein extract was studied as well.

3.7 Preparation of Pasta – product fortification

The pasta was prepared with the use of a pasta maker. The pasta was formulated with all-purpose flour, purchased from Walmart, while the protein powder was obtained by extraction using ascorbic acid and was used for product fortification. The purpose of fortifying cricket powder in pasta was to formulate a low nutrient-dense alternative snack. The pasta was formulated using 90% wheat flour and 10% Cricket powder and 3 eggs. It was blended together and left to stand at room temperature until it becomes soft for approximately 30 minutes and then rolled into sheets and shaped out. The product was then freeze-dried, and moisture content

was reduced to less than 5%. When freeze-dried, a hostile environment is created for microorganisms. Thus, inhibiting all the microorganisms and enhancing the shelf life of the product. The product is then packaged using Mylar bags (from Harvest right). Mylar bags blocks out light and air, another plus is that Mylar bags can be resealed once opened and they can be washed out and reused, like a canning jar. Pasta without Cricket Powder was used as a control for product comparison. Various analysis and cooking quality were carried out on the freeze-dried cricket pasta (Duda, Adamczak, Chełmińska, Juszkiewicz & Kowalczewski, 2019).



Figure 6: Pasta Maker (ISILER 9)



Figure 7: Freeze-Dryer (Harvest right home freeze dryer)

3.8 Color

The color of the cricket fortified pasta was measured using a Hunter colorimeter (MiniScan EZ 4500L Spectrophotometer). Differences in color were recorded in the L*a*b* scale in terms of lightness (L*) and color (a* – redness, b* – yellowness). Color measurement was done in triplicates. The color comparison was then studied for both, one with cricket fortification and another one without cricket powder. Color was also studied for cooked pasta. As well, the total color difference for the sample and control (ΔE) was calculated using the following formula:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

where ΔL^* , Δa^* and Δb^* are differences in the L^* , a^* , b^* values between the reference sample and the test sample respectively.



Figure 8: MiniScan EZ 4500L Spectrophotometer

3.9 Water activity

Water activity (a_w) is carried out using the water activity meter (WA-160A). In the field of food science, water activity plays a very important role in determining the shelf life of the product and in limiting the growth of the yeast, molds and fungi. The cricket fortified pasta used in this study is freeze-dried and thus reduces the water activity to a much lower extent and limits spoilage.

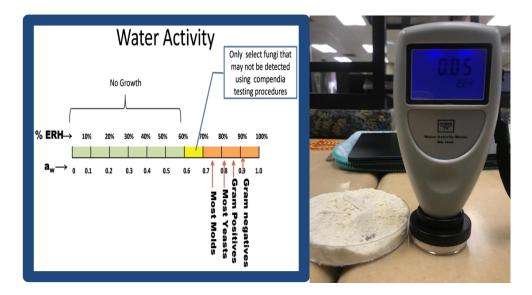


Figure 9: Water activity graph showing the range of growth at water activity (aw) and relative humidity (%) and Figure 10: Water activity meter (WA 160A)

3.10 Pasta Cooking Quality

3.10.1 Cooking time

Cooking indicates the quality of pasta. Freeze dried pasta was cooked in tap water that had a pH of 7.0 (1L/100g). Optimal cooking time (T) was indicated when the white core of pasta vanished when squeezed between two glass plates (approved method 66-50, AACC 2000).

3.10.2 Water uptake in Cooked Pasta and Cooking loss

Water uptake was evaluated on a 50 g dry pasta sample by measuring the weight (W) of pasta before and after cooking and calculated using the equation:

Water uptake (%, db) =
$$\left(\frac{W(Cooked\ Pasta)}{W(Dry\ Pasta)} - 1\right) x\ 100$$

Cooking loss was measured by evaporation of cooking water in a tarred beaker and is placed in an air oven at 105°C and evaporated to dryness. The residue was weighed and reported as a percentage of dry pasta (Larrosa, Lorenzo, Zaritzky & Califano, 2016).



Fig 11: freeze-dried All-purpose flour pasta before cooking (Control)

Fig 12: freeze-dried Cricket pasta before cooking

Fig 13: Control and fortified cricket powder pasta after cooking

3.13 Texture Analysis

The TVT 600 Perten instrument was used to carry out texture analysis of approximately 5-6 grams of cooked pasta from the control and Cricket fortified pasta. A 36 mm cylindrical probe was used (stable micro systems). From texture profile analysis curve, textural parameters of firmness and total work of shear were obtained. From the force-time curve, Pasta hardness was defined as the maximal peak force attained during the first compression. Cohesiveness was calculated as the ratio of the area under the second peak to the area under the first peak. Resilience was defined as the ratio of the area under the second half of the first peak to the area under the first half of the same peak (Petitot, Boyer, Minier & Micard, 2010).

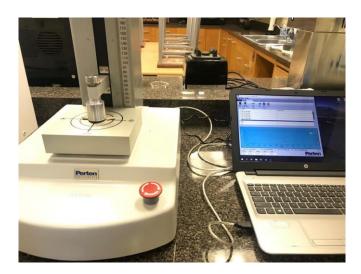


Fig 14: Texture analysis equipment (TVT 6700 Perten Instruments)

3.14 Statistical Analysis

All tests included in determining product analysis were conducted in triplicates unless indicated otherwise. An analysis of variance (ANOVA) was performed using general linear models' procedure to identify significant differences (p<0.05) among the samples.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Proximate analysis

Proximate analysis of freshly ground cricket powder, defatted cricket powder and protein extract was carried out. Ash, carbohydrates, moisture, calories, fat and protein content was determined on dry basis. Table 2 shows the proximate analysis of ground cricket powder. The results were analyzed as single measurements. High content of protein and fat was found in the samples. Protein content of 63.43% was observed and can be considered as an alternative to livestock and as seen from the table fat percentage was slightly on the higher side with a fat content of 20.86%, concluding that defatting procedure could help reduce the fat content in the cricket powder as indicated by Zhao et.al (2016).

Table 2: Proximate analysis of freshly ground cricket powder were carried out and the analysis are expressed as single measurements.

Freshly ground cricket powder	Ash (%)	carbohydrates (%)	moisture (%)	calories (Kcal/100g)	fat (%)	protein (%)
Dry matter basis	4.65%	7.56%	3.50%	472	20.86%	63.43%

Table 3: Proximate analysis of defatted cricket powder using Ethanol and defatted cricket powder using Hexane, the analysis was done in triplicates and expressed as Mean \pm standard deviation.

Sample	Ash (%)	carbohydrates (%)	moisture (%)	calories (Kcal/100g)	fat (%)	protein (%)
Defatted Cricket powder using Ethanol	5.09±0.04ª	7.21±1.08 ^a	4.7±0.86ª	407.33±9.07 ^a	9.27±1.14ª	73.72±0.76 ^a
Defatted Cricket powder using Hexane	5.44±0.08 ^b	6.88±1.41 ^b	3.6±0.2 ^b	424±5 ^b	11.98±1.13 ^b	72.08±0.40 ^b

Table 3 shows the comparison of proximate analysis between the ethanol and hexane defatted cricket powder, the analysis was done in triplicates and expressed as Mean \pm standard deviation. Statistical analysis was carried out, and for each analysis, the P<0.05, thus there is sufficient evidence at 5% level of significance to conclude that there is a difference in means of Ash content, carbohydrates, moisture, calories, fat and protein content among two methods of defatting.

Table 4: Proximate analysis of defatted protein extract using NaOH and Ascorbic acid.

Sample	Ash (%)	carbohydrates (%)	moisture (%)	calories (Kcal/100g)	fat (%)	protein (%)
Defatted Protein extract (NaOH)	23%	0.5%	4.8%	367	13.34%	61.75%
Defatted Protein extract (Ascorbic acid)	3.55%	7.54%	11.6%	378	7.62%	69.69%

Table 5: Proximate analysis of freshly ground cricket protein extract using NaOH and Ascorbic acid. Sodium by ICP AOAC 984.27, 927.02, 985.01, 965.17 was carried out and was found to be 5.24 % in the freshly ground cricket protein extract using NaOH.

Sample	Ash (%)	carbohydrates (%)	moisture (%)	calories (Kcal/100g)	fat (%)	protein (%)
Freshly ground Cricket Protein extract (NaOH)	16.71%	1.36%	3.1%	424	20.64%	58.19%
Freshly ground Cricket Protein extract (Ascorbic acid)	3.19%	8.33%	4.6%	476	21.44%	62.44%

Table 4 and Table 5 shows the proximate analysis of the protein extract carried out by NaOH and ascorbic acid. The analysis is done as single measurements. From the analysis we could conclude that ascorbic acid had a higher protein content of 69.69%, low fat of 7.62% and low ash. Whereas Sodium Hydroxide had a high percentage of Ash, and this could possibly be due to the addition of alkali which is required for pH adjustments (Liceaga-Gesualdo & Li-Chan, 1999). The moisture content ranged from 3.1 to 11.6% which is similar to the study conducted by Hall, Jones, O'Haire & Liceaga, 2017.

4.2 Defatting

Due to the high fat content in the original cricket powder, defatting was carried out. Original cricket powder had 20.86% of fat. Defatting was carried out using two solvents hexane and ethanol, using hexane we were able to extract out 8.88% fat and using ethanol we were able to extract out 11.59% fat, thus reducing the fat content to 11.98% and 9.27% respectively.

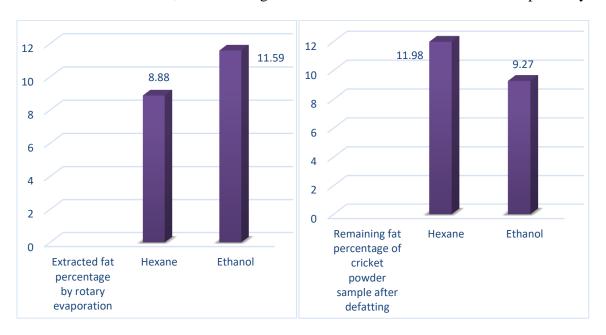


Table 5: Remaining fat percentage in the defatted cricket sample after fat extraction

Remaining fat percentage of cricket sample defatted using Hexane	11.98 ±1.13 ^a
Remaining fat percentage of cricket sample defatted using Ethanol	9.27 ± 1.14^{b}

Expressed as mean \pm standard deviation (n=3). Values marked with different letters showed significant differences (p<0.05).

P-value is 0.0439, it is less than 0.05, thus we reject the null hypothesis, and we say the result is statistically significant. Defatting of cricket powder using two solvents hexane and ethanol showed statistically significant defatting efficiency rate. Ethanol had a significantly higher extraction rate in comparison to Hexane. This is because ethanol is a polar solvent, it can extract

more than the non-polar solvent hexane (Li, Naghdi, Garg, Adarme-Vega, Thurecht, Ghafor, Tannock & Schenk 2014)

4.3 Protein Extraction

Protein extraction was carried out to isolate proteins from other substances present in the sample. Lipid removal was carried out to minimize error during the extraction process.

Protein extraction was carried out using the ethanol defatted cricket powder and extracted using ascorbic acid (acidic) and sodium hydroxide (alkaline) medium to determine extraction efficiency. Protein yield was calculated by the formula

Extraction yield (%) =
$$100 * \frac{extract}{Sample}$$

P value for protein yield is 0.0015. The analysis was done in triplicates and expressed as Mean ± standard deviation. Statistical analysis was carried out and the P<0.05, thus there is sufficient evidence at 5% level of significance to conclude that there is a difference between extraction yield obtained from ascorbic acid and NaOH. Ascorbic acid had a higher yield percentage than NaOH, this can be described by the solubility of the cricket proteins that was found to be highly dependent on the pH during the extraction process. Based on research findings from Bußler, Rumpold, Jander, Rawel, & Schlüter (2016) protein yields were higher in the acidic region at pH 2 and 3 this explains why ascorbic acid had a higher yield than sodium hydroxide. Extraction rate of protein was calculated using the formula:

Extraction rate of Protein (%) =
$$\frac{protein\ content\ in\ extract}{protein\ content\ in\ sample} * Extraction\ yield\ (\%)$$

Table 6: Extraction yield, true protein content and extraction rate of protein from ethanol defatted cricket powder.

4.4 Functional properties

Sample	Yield %	True Protein %	Extraction rate of Protein %
Defatted Protein extract (Ascorbic acid)	87.75±1.53 ^a	69.69%	82.95%
Defatted Protein extract (NaOH)	80.78±0.17 ^b	61.75%	67.66%

^{*}Means \pm standard deviation with different superscripts within columns indicate significant differences among samples (p<0.05).

Water/oil absorption capacity, emulsifying capacity, and foaming capacity were studied, the water absorption capacity of original cricket powder and protein extract was 71 %, respectively. While WAC of defatted powder varied and was around 55% and the P-value = 0.003, where P<0.05. Oil absorption capacity was determined, and it varied between the original, defatted and protein extract. However, was close to the range from another similar study of, which shows that water and oil absorption capacities of cricket were 238.47% and 202%, respectively, and thus we could suggest that the water absorption capacity could be a desirable functionality as a food ingredient (Adebowale, Adeyemi & Oshodi 2005). Emulsion activity index ranged from 6 to 9 m²/g which is in between the range as described in Hall, et al. (2017). While foamability ranged from 160 to 175%. However, foaming capacity was very less for protein extracts and this could be explained by Yi, et al. (2013) where the study was to investigate foamability, foam stability and gelation of soluble proteins from five insect species and found poor foaming capacities at pH 3, 5, 7, and 10, since ascorbic acid was used in protein extraction pH was around 2.5 to 3 and that could

be a reason why it showed poor foaming capacity. Thus, functional properties demonstrate a potential to develop cricket protein extracts as a functional alternative protein source in food ingredient formulations.

Table 7: Comparison of functional properties between Original, defatted and protein extract of cricket powder sample.

Functional properties	Original Cricket powder	Defatted Cricket Powder	Protein Extract	P- Value
Water absorption capacity	71.05±6.15 ^a	55.365±1.22 ^b	71.32±1.20°	0.003
Oil absorption capacity	197.37±0.17ª	226. 83±4.72 ^b	307.17±2.25°	0.00
Foaming capacity	173.89±6.73 ^a	152.66±2.51 ^b	11.667±7.63°	0.00
Emulsifying activity	6.641±2.85 ^a	7.573±0.65 ^b	$8.784 \pm 1.80^{\circ}$	0.4554

^{*} Means \pm standard deviation with different superscripts within rows indicate significant differences among samples (p<0.05).

4.5 Product analysis (Color)

Color plays a very important role in the assessment of food. Consumers assess food not only based on the nutritional value or potential pro-health effects but also on sensory analysis which directly affect consumer preferences, choice and requirements (Costell, Tárrega, Bayarri, 2010). Color is one of the most easily evaluated characteristics. The fortification of cricket powder in pasta showed significant differences in color. Pasta samples that were fortified with 10% cricket powder were clearly darker (Table 8). While the color was similar to commercially available whole wheat pasta, widely considered to be a healthier option in the market. The results of the color analysis showed that the value of L* parameter, related directly with the lightness of the pasta,

decreased by almost 30% in the case of cooked sample. Moreover, color balance was shifted towards red (positive a*) and yellow (positive b*) in all the samples before and after cooking. Total color differences (ΔE), which represent the magnitude of the color difference between the control sample and fortified pasta with cricket powder, were in the range from 16.38 to 31.77 (p < 0.05) which is similar to the study of Duda, et al. (2019). Bellary et al. (2016) pointed out that a color difference is perceivable to the naked eye when $\Delta E > 3.0$. In the case of all the analyzed pasta samples, ΔE was found to be higher than 25. In the case of raw freeze-dried pasta samples and cooked, (Table 8) the fortified cricket powder was significantly different from the control sample.

Table 8: Comparison of color results between two samples, freeze-dried all-purpose flour pasta (Control) and 10% fortified cricket pasta before and after cooking*

Raw Pasta:

Sample	L^*	<i>a</i> *	<i>b</i> *	ΔΕ
Control	86.83 ± 0.73^{a}	1.72 ± 0.13^{a}	16.68 ± 0.64^{a}	-
Fortified cricket Pasta	$70.60 \pm 0.53^{\text{b}}$	$3.72 \pm 0.08^{\text{b}}$	15.78± 0.15 ^b	16.38

^{*}Means \pm standard deviation with different superscripts within columns indicate significant differences among samples (p<0.05).

Cooked Pasta:

Sample	L^*	a^*	b^*	ΔΕ
Control	68.77 ± 0.93^{a}	2.79 ± 0.11^{a}	25.37 ± 0.29^{a}	-
Fortified cricket Pasta	40.59± 0.79 ^b	$3.28 \pm 0.15^{\text{b}}$	10.72±0.40 b	31.77

^{*}Means \pm standard deviation with different superscripts within columns indicate significant differences among samples (p<0.05).

4.6 Water activity of freeze-dried pasta

Water activity (a_w) determines stability and the safety of food with respect to microbial growth rate of deteriorative reactions and physical/chemical properties. Measurement of water activity is a key parameter in the quality control of moisture sensitive products or materials. If there is too much water in a product, there is a risk of microbial growth and water migration. Pasta is already a dry product; hence the water activity is under control. Water activity (a_w) is used for the preservation of food, stabilization of the food supply, and developing different types of shelf-stable foods. Freeze drying is a method used to reduce the water activity of foods. Dried or low-moisture foods do not contain more than 25% moisture (Erkmen & Bozoglu, 2016). In addition, no difference P-value is 0.0032 (p < 0.05), was found in a_w of control and fortified cricket pasta potentially due to the low water mobility in pasta products.

Table 9: Water activity of the product

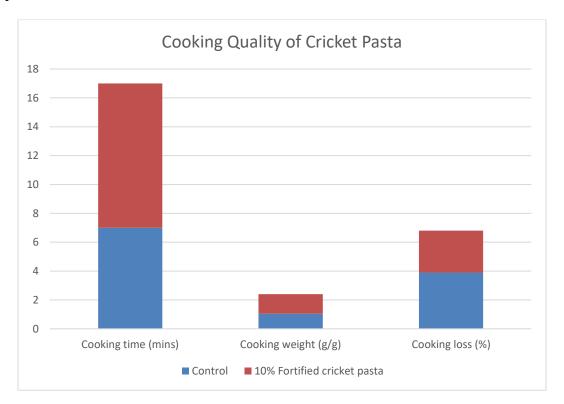
Sample	Water activity (aw)
Control	0.056±0.005
Fortified cricket pasta	0.086 ± 0.005

4.7 Cooking Quality

Cooking properties are important indicators of pasta quality. Addition of protein or fiber can significantly affect its properties (Khan, Yousif, Johnson, & Gamlath, 2013). Cooking properties of pasta fortified with CP are presented in Table 10. The addition of CP caused an increase of the optimal cooking time from 7min for control to 10 min for fortified cricket pasta which is similar

to the study by Duda et.al (2019). Generally, the use of protein additives of plant origin was found to increase cooking losses (Kaur, Sharma, Nagi, H & Ranote, 2013). On the contrary, the addition of CP resulted in reduced losses. This could be a good indicator for interpreting that the quality of the final product is of a high standard. The results of each cooking properties were statistically significant (P<0.05).

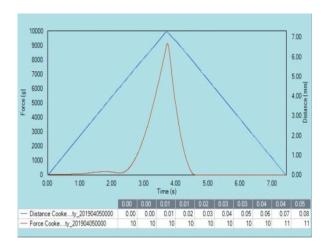
Table 10: Cooking properties of all-purpose flour pasta fortified with 10% cricket powder. CP-Cricket powder



4.8 Texture analysis

The texture properties of the pasta after cooking are extremely important macroscopic chemical-physical properties for assessing the quality of the pasta and consumer acceptance. Analysis of the texture of cooked pasta was carried out by examining two parameters: firmness and total work.

Cutting force is related to the firmness of cooked pasta. Maximum force on the graph measures firmness (Graph 1 & Graph 2). The firmness of pasta depends on a few factors like production, thickness of sample and time of cooking (Dziki, Biernacka, Laskowski, 2012). The results of texture analysis indicate that the firmness of fortified cricket pasta was higher than that of the control sample. Firmness was significantly different (P<0.05) among the control and fortified cricket pasta. Data in one of the literatures indicate that protein sources like the addition of egg increase the firmness, which is consistent with the obtained results in our study. A comparison between the various thickness of samples were carried out and results indicated that as the thickness reduced firmness reduced as well. In this particular study, the pasta sample was thick, and thus concluded the high value of firmness could be due to the thickness of the pasta sample. The force value obtained is in grams, the values were converted to Newton (1g =0.01 N)



12000 3.20 2.80 10000 2.40 8000 2.00 6000 1.60 1.20 4000 0.80 0.40 0.00 0.30 0.60 1.50 Force Cooke....ty_201904050002

Graph 1 - Control

Graph 2 – Fortified cricket pasta

Table 11: Texture analysis of Cooked Pasta

Parameter	С	CP 10
Firmness (N)	98.07 ± 0.33^{a}	117.68±0.52 ^b
Total work of shear (N/mm x s)	4.00 ± 0.03^a	20.43 ± 0.02^{b}

C, CP10, denote pasta with 0% cricket powder and 10% cricket powder, respectively. Mean values denoted by different letters (a, b) differ statistically. (P<0.05)

4.9 Nutritional Labelling

Table 12 and Table 13 discusses the nutritional content of all-purpose flour pasta and cricket fortified pasta. The nutritional profile of cricket pasta contains good nutritional qualities.

100 g pasta provides 330 kcal of energy, 2.5g fat, 45g carbohydrate, 28 g protein, 4 g dietary fiber, 135 mg sodium, 0 g sugar. The 10% fortified pasta has all the good properties in comparison to the control which is all-purpose flour pasta that is commercially available in the market. As we can see from the nutritional label, the protein content increases about 17%, total fat reduces from 2.5g to 1.5g, trans fat is 0g which is considered very healthy and the saturated fat is 13%. This type of fat comes mainly from animal sources of food, such as red meat, poultry and full-fat dairy products and thus is common to be present in insects as well. Since the saturated fat is slightly on the higher side, this research discusses defatting methods to help reduce the fat content. Overall, cricket proteins are considered to be a healthy and sustainable alternative that is required to support the maintenance of lean body mass, research indicates that cricket powder can influence gut health, increases 5 types of bacteria, including one closely related to Bifidobacterium animalis, a

commercial strain of which has been shown to improve bowel function, protect against diarrhea, and reduce some adverse effects of antibiotic treatment (Voelker, 2019).

Table 12: Control

Nutri Serving Size Servings Per	(100g)		cts
Amount Per Se	rving		
Calories 33	0 Cal	ories fron	n Fat 20
		% Da	aily Value*
Total Fat 2.5	5g		4%
Saturated	Fat 0.5g		3%
Trans Fat	0g		
Cholesterol	55mg		18%
Sodium 25n	ng		1%
Total Carbo	hydrate (35g	22%
Dietary Fi	ber 2g		8%
Sugars 0g]		
Protein 11g			
Vitamin A 2%	6 · '	Vitamin (0%
Calcium 2%	•	Iron 25%	
*Percent Daily Value diet. Your daily value depending on your	alues may be	e higher or l	000 calorie ower 2.500
Total Fat Saturated Fat Cholesterol Sodium Total Carbohydra Dietary Fiber Calories per grar Fat 9 • 0		65g 20g 300mg 2,400mg 300g 25g	80g 25g 300mg 2,400mg 375g 30g

Table 13: 10% cricket fortified pasta

		er	
Amount Per Se	rving		
Calories 33	0 Cal	ories fron	n Fat 15
		% Da	aily Value
Total Fat 1.5	5g		2%
Saturated	Fat 2.5g		13%
Trans Fat	0g		
Cholesterol	130mg		43%
Sodium 135	img		6%
Total Carbo	hydrate 4	45g	15%
Dietary Fi	ber 4g		16%
Sugars 0g	3		
Protein 28g			
Vitamin A 2%	6 ·	Vitamin (C 0%
Calcium 6%	•	Iron 25%	
*Percent Daily V diet. Your daily v depending on yo	alues may b	e higher or l	
Total Fat Saturated Fat Cholesterol Sodium Total Carbohydra Dietary Fiber Calories per graf	Less than Less than ate	65g 20g 300mg 2,400mg 300g 25g	80g 25g 300mg 2,400mg 375g 30g

CHAPTER FIVE: CONCLUSION AND FUTURE DIRECTIONS

Spray dried cricket powder was found to contain 20.86% fat and 63.43% protein. Due to the high fat content, ethanol and hexane were used for extraction of fat from cricket powder. Ethanol is an industrially friendly solvent and could extract more fat than the solvent hexane since ethanol is a polar solvent, and it can extract more than the non-polar solvent hexane. Proteins were extracted with 0.5M NaOH and ascorbic acid. Ascorbic acid (Vitamin C), which is safe to be used in an industrial scale shows very good extraction properties with a yield of 87.75%. The protein extract obtained was a good source of essential amino acid. While on the other hand alkaline extraction method provided an acceptable extraction yield of 80.78%. Future studies can be conducted on comparing plant-based proteins and insect-based proteins to study the amino acid profile. Proximate analysis of cricket powder reveals that, cricket is a very good source of protein, therefore can be extracted and incorporated in a low nutrient dense food.

Protein extracts made from ascorbic acid was analyzed for water/oil absorption capacity, emulsifying and foaming properties. Functional properties demonstrate a potential to develop cricket protein extracts as a functional alternative protein source in food ingredient formulations. Functional properties of proteins are physicochemical properties of proteins which affect their behavior in food systems during preparation, processing, storage, and consumption, and contribute to the quality and sensory attributes of food systems. Due to the high protein content of 69.69%, extracted cricket proteins can be used as an alternative source to meet the demands of the world population who is in need of a nutritional, sustainable and renewable source of protein. As consumptions of whole crickets are unacceptable to many, the use of powder obtained can be justified. This research concentrates on studying the properties of freeze-dried pasta fortified with 10% cricket powder. It was shown that fortification of cricket powder changed the characteristics

of the pasta significantly. It was found that the addition of CP increased the protein and mineral content in comparison to the control. At the same time, it was shown that fortified pasta had different cooking properties. The addition of CP resulted in a decrease in cooking loss, slightly higher cooking weight but caused an increase in the optimum cooking time from 7mins to 10mins. In the case of color analysis, the fortification of CP in pasta resulted in distinct differences in color. Pasta samples that were fortified with 10% cricket powder were clearly darker. While the color was similar to commercially available whole wheat pasta, widely considered to be a healthier option in the market and hence is acceptable. Since the product is freeze-dried, the moisture content is less than 5% which shows it is shelf stable and the water activity was also less than 0.5 thus all growth is inhibited. In terms of nutrients, reduced aw reduces losses of Vitamins C, E, B1. Fortified cricket pasta was also characterized by higher firmness than the control pasta and thus determines high-quality product. The results of this study will provide an alternative protein source for food applications. Thus, pasta with 10% cricket powder has the potential to be a good alternative to the food industry for the nutritional enrichment of traditional pasta. Future studies on evaluating more advanced protein extraction techniques, sensory evaluations, rheology, and cost analysis will be carried out to assess the consumer's acceptability of insect protein extracts.

References

- Academy of Nutrition and Dietetics. (2017). Dietary guidelines and my plate. Retrieved from https://www.eatright.org/
- Adebowale, Y. A., Adeyemi, I. A., & Oshodi, A. A. (2005). Functional and physicochemical properties of flours of six Mucuna species. *African Journal of Biotechnology*, *4*(12). Retrieved from: https://www.ajol.info/index.php/ajb/article/view/71409/60356
- Akhtar, Y., & Isman, M. B. (2018). Insects as an alternative protein source. In R. Y. Yada (Ed.), *Proteins in food processing* (pp. 263-288). Sawston, England: Woodhead Publishing. doi:10.1016/B978-0-08-100722-8.000115
- Alemu, M.H., Olsen, S.B., Vedel, S.E., Pambo, K.O., & Owino, V.O. (2017). Combining product attributes with recommendation and shopping location attributes to assess consumer preferences for insect-based food products. *Food Qual. Prefer* 55, 45–57. doi: 10.1016/j.foodqual.2016.08.009
- Almeida, C. C., Monteiro, M. L. G., da Costa-Lima, B. R. C., Alvares, T. S., & Conte-Junior, C.
 A. (2015). In vitro digestibility of commercial whey protein supplements. *LWT-Food Science and Technology*, 61(1), 7-11. doi:10.1016/j.lwt.2014.11.038
- Assielou, B., Due, E. A., Koffi, M. D., Dabonne, S., & Kouame, P. L. (2015). Oryctes Owariensis larvae as good alternative protein source: Nutritional and functional properties. *Annual Research & Review in Biology*, 8(3), 1.doi: 10.9734/ARRB/2015/19093
- Awoyinka, O. A., Ileola, A. O., Imeoria, C. N., Olumakinde, S. T., Oladele, F. C., & Asaolu, M. F. (2016). Functional properties and nutritional quality of some wild and edible bean in Nigeria. *Open Access Library Journal*, *3*(02), doi:10.4236/oalib.1102411

- Belluco S., Losasso, C., & Maggioletti M. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety 12*, 296-313. doi:10.1111/1541-4337.12014
- Beuchat, L. R. (1977). Functional and electrophoretic characteristics of succinylated peanut flour protein. *Journal of Agricultural and Food Chemistry*, 25(2), 258-261.doi:10.1021/jf60210a044
- Bellary, A.N., Indiramma, A.R., Prakash, M., Baskaran, R., & Rastogi, N.K. (2016) Anthocyanin infused watermelon rind and its stability during storage. *Innov. Food Sci. Emerg. Technol.* 33, 554–562. doi:10.1016/j.ifset.2015.10.010
- Bonomi, F., D'Egidio, M.G., Iametti S., Marengo M., Marti A., Pagani MA & Ragg EM (2012).

 Structure quality relationship in commercial pasta: A molecular glimpse. *Food Chem.*135(2): 348–355. doi:10.1016/j.foodchem.2012.05.026
- Bußler, S., Rumpold, B. A., Jander, E., Rawel, H. M., & Schlüter, O. K. (2016). Recovery and techno-functionality of flours and proteins from two edible insect species: Meal worm (Tenebrio Molitor) and black soldier fly (Hermetia Illucens) larvae. *Heliyon*, 2(12), doi: 10.1016/j.heliyon.2016.e00218
- Barennes, H., Phimmasane, M., & Rajaonarivo, C. (2015). Insect consumption to address undernutrition, a national survey on the prevalence of insect consumption among adults and vendors in Laos. *PloS one*, *10*(8), e0136458. doi:10.1371/journal.pone.0136458
- Coffmann, C. W., & Garciaj, V. V. (1977). Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, 12(5), 473-484.doi:10.1111/j.1365-2621.1977.tb00132.x

- Costell, E., Tárrega, A., & Bayarri, S. (2010). Food Acceptance: The role of consumer perception and attitudes. *Chem. Percept*, *3*, 42–50 doi: 10.1007/s12078-009-9057-1.
- De Foliart, G. R. (1999). Insects as food: Why the western attitude is important. *Annual Review of Entomology*, 44, (21). Retrieved from http://arjournals.annualreviews.org/loi/ento
- De Foliart, G. R. (2012). The human use of insects as a food resource: A bibliographic account in progress. Retrieved from http://www.food-insects.com/
- Dossey, A.T., (2010) Insects and their chemical weaponry: New potential for drug discovery. *Nat Prod Rep.* 27(12), 1737-57. doi: 10.1039/c005319h
- Dossey, A. T., Morales-Ramos, J. A., & Rojas, M. G. (Eds.). (2016). *Insects as sustainable food ingredients: production, processing and food applications*. Cambridge MA: Academic Press. doi: 10.1016/B978-0-12-802856-8.00012-0
- Duda, A., Adamczak, J., Chelmińska, P., Juszkiewicz, J., & Kowalczewski, P. (2019). Quality and Nutritional/Textural Properties of Durum Wheat Pasta Enriched with Cricket Powder. Foods, 8(2), 46. doi:10.3390/foods8020046
- Dziki, D., Biernacka, B., & Laskowski, J. (2012). Influence of cooking time and common wheat flour addition to semolina on spaghetti mechanical properties. *Acta Agroph.*, 19(2), 277-287. Retrieved from: http://www.acta-agrophysica.org/Influence-of-cooking-time-and-common-wheat-flour-addition-to-semolina-on-spaghetti,104776,0,2.html
- Erkmen, O., & Bozoglu, T. F. (2016). Food microbiology principles into practice: Vol. 2.

 Microorgniasms related to foods, foodborne diseases and food spoilage, Retrieved from:

 https://books.google.com/books?hl=en&lr=&id=pxDRCwAAQBAJ&oi=fnd&pg=PR15

 &dq=Osman,+T.+Faruk,+2016.+water+activity&ots=vChfV0V1nr&sig=xTe0wXsN-

- gbKUOAR6Ohl8oHCumc#v=onepage&q=Osman%2C%20T.%20Faruk%2C%202016. %20water%20activity&f=false
- Freye, H. B., Esch, R. E., Litwin, C. M., & Sorkin, L. (1996). Anaphylaxis to the ingestion and inhalation of Tenebrio Molitor (mealworm) and Zophobas Morio (superworm). *Allergy and Asthma Proceedings*, 17(4), 215-9. doi: 10.2500/108854196778996903
- Gahukar, R.T. (2016). Edible insects farming: Efficiency and impact on family livelihood, food security, and environment compared with livestock and crops. In A.T. Dossey, J. Morales Ramos, & M. G. Rojas (Eds.). *Insects as sustainable food ingredients* (pp.85-111). Cambridge, MA: Academic Press.
- Gahukar, R. T. (2011). Entomophagy and human food security. *International Journal of Tropical Insect Science*, 31(3), 129-144. doi:10.1017/S1742758411000257
- Hall, F. G., Jones, O. G., O'Haire, M. E., & Liceaga, A. M. (2017). Functional properties of tropical banded cricket (Gryllodes Sigillatus) protein hydrolysates. *Food chemistry*, 224, 414-422. doi:10.1016/j.foodchem.2016.11.138
- Kim, H. W., Setyabrata, D., Lee, Y., Jones, O. G., & Kim, Y. H. B. (2017). Effect of House Cricket (Acheta Domesticus) Flour Addition on Physicochemical and Textural Properties of Meat Emulsion Under Various Formulations. *Journal of Food Science*, 82(12), 2787-2793 doi: 10.1111/1750-3841.13960.
- Khan, I., Yousif, A., Johnson, S.K., & Gamlath, S. (2013) Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Res.Int.*, 54, 578–586. doi:10.1016/j.foodres.2013.07.059
- Kaur, G., Sharma, S., Nagi, H.P.S., & Ranote, P.S. (2013) Enrichment of pasta with different plant proteins. *J. Food Sci. Technol.50*, 1000–1005. doi:10.1007/s13197-011-0404-2

- Lensvelt, E.J. & Steenbekkers, L.P. (2014). Exploring consumer acceptance of Entomophagy: A survey and experiment in Australia and the Netherlands. *Ecology of Food and Nutrition*, 53(5), 543-561. doi:10.1080/03670244.2013.879865
- L'Hocine L., Boye J.I., & Arcand Y. (2006). Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. *J Food Sci.* 71(3):C137-45. doi.org/10.1111/j.1365-2621. 2006.tb15609.x
- Li, Y., Naghdi, F. G., Garg, S., Adarme-Vega, T. C., Thurecht, K. J., Ghafor, W. A., ... & Schenk,
 P. M. (2014). A comparative study: The impact of different lipid extraction methods on current microalgal lipid research. *Microbial Cell Factories*, 13(1) doi:10.1186/1475-2859-13-14
- Liceaga-Gesualdo, A. M., & Li-Chan, E. C. Y. (1999). Functional properties of fish protein hydrolysate from herring (Clupea Harengus). *Journal of Food Science*, 64(6), 1000-1004.doi:10.1111/j.1365-2621.1999.tb12268.x
- Larrosa, V., Lorenzo, G., Zaritzky, N., & Califano, A. (2016). Improvement of the texture and quality of cooked gluten-free pasta. *LWT-Food Science and Technology*, 70, 96-103 doi: doi.org/10.1016/j.lwt.2016.02.039
- Martins, N., Roriz, C.L., Morales, P., Barros, L., & Ferreira, I.C.F.R. (2016). Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends Food Sci. Technol*, *52*, 1–15. doi: 10.1016/j.tifs.2016.03.009
- Oonincx, D.G.A.B., Itterbeeck, J.V., Heetkamp, M.J.W., Brand, H.V.D., & van Loon, J.J.A. (2010). An exploration on greenhouse gas and ammonia production by insect species

- suitable for animal or human consumption. *PLoS ONE*, *5*(12), e14445. doi: 10.1371/journal.pone.0014445
- Premalatha, M., Abbasi, T., & Abbasi, S.A. (2011). Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects. *Renewable & Sustainable Energy Reviews*. 15(9).4357-4360. doi: 10.1016/j.rser.2011.07.115
- Pauter, P., Różańska, M., Wiza, P., Dworczak, S., Grobelna, N., Sarbak, P., & Kowalczewski, P. Ł. (2018). Effects of the replacement of wheat flour with cricket powder on the characteristics of muffins. *Acta Scientiarum Polonorum Technologia Alimentaria*, 17(3), 227-233. doi:10.17306/J.AFS.0570.
- Prabhasankar, P., Jyotsna, R., Indrani, D., & Rao, G.V. (2007) Influence of whey protein concentrate, additives, their combinations on the quality and microstructure of vermicelli made from Indian T Durum wheat variety. *J. Food Eng.*80, 1239–1245. doi:10.1016/j.jfoodeng.2006.09.013
- Petitot, M., Boyer, L., Minier, C., & Micard, V. (2010). Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. *Food Research International*, 43(2), 634-641.doi: 10.1016/j.foodres.2009.07.020
- Rumpold, B. & Schlüter, O. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science & Emerging Technologies 17*, 1–11. doi:10.1016/j.ifset.2012.11.005
- Shim, S.M, Seo, S.H, Lee, Y, Moon, G.I, Kim, M.S, Park, J.H. (2011) Consumers' knowledge and safety perceptions of food additives: Evaluation on the effectiveness of transmitting information on preservatives. *Food Control*, 22, 1054–1060. Retrieved from http://www.nal.usda.gov/

- Siemianowska, E., Kosewska, A., Aljewicz, M., Skibniewska, K.A., Polak-Juszczak, L., Jarocki, A., & Jedras, M. (2013). Larvae of mealworm (Tenebrio Molitor L.) as European novel food. *Agricultural Sciences*. *4*(6), 287-291. doi: 10.4236/as.2013.46041
- Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: Environmental issues and options*. Retrieved from http://www.europarl.europa.eu/climatechange/doc/FAO%20report%20executive%20sum mary.pdf
- Van Huis, A. (2016). Edible insects are the future? *The Proceedings of the Nutrition Society*, 75(3), 294-305. doi: 10.1017/S0029665116000069
- Van Huis, A., Itterbeeck, J. V., Klunder, H., Martens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: Future prospects for food and feed security*. Retrieved from https://www.fao.org/docrep/018/i3253e/i3253e.pdf
- Vogel, G. (2010). For more protein, filet of cricket. *Science*, 327(5967), 811 doi:10.1126/science.327.5967.811
- Voelker, R. (2019). Can insects compete with beef, poultry as nutritional powerhouses? *Jama*, 321(5), 439-441.doi:10.1001/jama.2018.20747
- Wang, D., Bai, Y. Y., Li, J. H., & Zhang, C. X. (2004). Nutritional value of the field cricket (Gryllus testaceus Walker). *Insect Science*, 11(4), 275-283 doi:10.1111/j.1744-7917.2004.tb00424.x
- Yi, L., Lakemond, C.M.M., Sagis, L.M.C., Eisner-Schadler, V., van Huis, A., & van Boekel, M.A.J.S. (2013). Extraction and characterization of protein fractions from five insect species. *Food Chemistry*, 141, 3341-3348. doi:10.1016/j.foodchem.2013.05.115 23993491

Zhao, X., Vázquez-Gutiérrez, J. L., Johansson, D. P., Landberg, R., & Langton, M. (2016). Yellow mealworm protein for food purposes - extraction and functional properties. *PLoS One*, 11(2). doi:10.1371/journal.pone.0147791