PHYSICAL, CHEMICAL AND FUNCTIONAL CHARACTERIZATION OF EDIBLE CRICKET (*ACHETA DOMESTICUS*) PROTEIN CONCENTRATE

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Physical, chemical and functional characterization of edible cricket (*Acheta domesticus*) protein concentrate

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DECLARATION

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

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DEDICATION

I dedicate this work to my fiancée, family, relatives and friends.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the people who made major contributions to the completion of this work. First, I thank God who gave me the strength and wisdom and good health to undertake my studies and project. I wish to thank my supervisors Dr. John Kinyuru, Dr. Paul Njihia and Prof Glaston Kenji for their dedication and selfless support in providing guidance as I carried out my project. May God bless your good work and reward your kind acts. My sincere gratitude goes to my parents, sibling, colleagues and friends who were always there for me. Thank you so much. Lastly I thank JKUAT RPE innovations grant for funding this project and the Department of Food Science and Technology of JKUAT for providing facilities and technical support.

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LIST OF ABBREVIATIONS

FAO: Food and Agricultural Organization

UN: United Nations

WHO: World Health Organization

ABSTRACT

Edible insects have been shown to be good sources of proteins and a potential solution to food and nutrition insecurity. Therefore the aim of this study was to investigate physico- chemical and functional characteristics of cricket powder defatted using two different methods for future utilization as a food ingredient in processed food products. Adult crickets were obtained from JKUAT farm in Kenya, dried under controlled conditions and ground to powder. The proteins were concentrated using two solvent namely; hexane which resulted to a filtrate (HE) and water which resulted to a filtrate (AE-Pellet) and a residue (AE-Residue). Yield was determined gravimetrically and colour by colorimetric method. The crude protein, crude fibre, crude fat, crude ash and available carbohydrates were determined using standard analytical methods. Protein digestibility was determined using enzymatic digestion method while protein fractions were extracted and quantified gravimetrically. The water holding capacity was determined using standard AACC procedure. Emulsion capacity, emulsion stability, foaming capacity and foam stability was investigated. The effect of sodium chloride (NaCl) and pH on the functional properties was also determined. HE recorded high values for yield, lightness, hue angle and protein content ($P < 0.0001$, $P = 0.0003$). Hexane extraction was significantly efficient in extracting fat in relation to aqueous extraction ($P < 0.0001$). Crude fiber and protein digestibility were not significantly affected by concentration method ($p= 0.0476, 0.0822$). Aqueous extraction recorded significantly higher values for Globulin (19.42%), prolamin (6.26%) and Glutelin (10.10%). AE Pellet had higher emulsion capacity (41.70%) and emulsion stability (33.61%), foaming capacity (11.11%) and foam stability (10.15%). At pH 12 the protein solubility, emulsion capacity and water holding capacity of the protein concentrates was optimum. Increase in NaCl concentration resulted to a significant decline in emulsion capacity and stability of the protein concentrates ($P < 0.0001$). At Ph 4 and 4% NaCl foaming capacity was optimum with AE Pellet recording the highest values (21.73%).The least gelation concentration was 30% with only AE Pellet showing gelation potential. The extraction method, pH and NaCl significantly influenced the physico-chemical and functional properties of the protein concentrates.

CHAPTER ONE

INTRODUCTION

1.1 Background information

About 1900 species of insects are consumed as part of diet and a projected 2 billion people consume insects on a daily basis. In some regions insect consumption contributes largely as a dietary source of proteins (DeFoliart, 1989; Van Huis *et al.,* 2013). Edible insects are a source of food to many communities in the world usually as a delicacy or a source of food in times of calamities such as drought, war and floods (Sutton, 1998; DeFoliart, 1991, Adedire and Aiyesanmi, 1999). Insects are consumed at the egg, larvae, pupae and adult stage of life. Among the key species consumed include Lepidoptera, Coleoptera, Hymenoptera, Orthoptera, Hemiptera, Isoptera and Diptera (Yi et al., 2013).

In Kenya edible insects are consumed mainly by communities from the lake region for instance Lake flies are consumed by the Luo community living around Lake Victoria region (Ayieko & Oriaro, 2008). In addition termites are consumed by the Luo community (Kinyuru *et al.,* 2009). Apart from consumption African communities have used insects for medicinal purposes and witchcraft (Ayieko & Oriaro, 2008). In most developed countries the consumption of edible insects is infrequent and culturally unacceptable (Van Huis *et al.,* 2013). There is a global challenge of meeting the demand for protein which is amplified by constrained agricultural land and global warming that has resulted to adverse weather conditions. Edible insects seems to be a suitable solution to this dilemma (Van Huis *et al.,* 2013).

It has been shown that insects can be processed into products with long shelf life, crackers, muffins, sausages and meat loafs have been made by incorporating insects (Ayieko *et al.,* 2010). Additionally wheat burns containing termite have been made (Kinyuru *et al.,* 2009). There are people who do not consume insects since they perceive them as dirty and scary, therefore it has been proposed for the food industry to develop products that would include insect powder, insect alcohols and nutritious fluids from insects (Chen *et al.,* 2009). Chitin obtained from crabs and crayfish has

been approved in Japan to be incorporated in cereal products to provide fibre and calcium (Belluco *et al.,* 2013). There is potential for postharvest losses of insects due to fat auto oxidation and proteolysis of proteins therefore a necessity for processing (Kinyuru *et al.,* 2009). Therefore there is a necessity of providing proper preservation and also to diversify the utilization of edible insects.

Plant sources of proteins have been widely studied. For instance Soy proteins is one of the most refined isolate from soy bean which has been incorporated in meat products, nutritional beverages, infant formulas and as dairy product replacer based on an understanding of their functional properties (Chove *et al.,* 2007). Recent research has shown more interest towards production and processing of edible insects. This motivation was as a result of the proven safety of edible insects (Konyole *et al.,* 2012).

The utilization of edible insects in the food industry is highly dependent on understanding the chemical and functional properties of proteins and fats which occur in considerable high quantity in insects. Studies showing that soybean extracts have anticancer benefits has sparked new innovations of using soy extracts in the food industry (Chove *et al.,* 2007). Therefore there is a lot of focus on soybean by processors and researchers. However there is still limited information on the characteristics and functionality of protein isolates from edible insects (Yi *et al.,* 2013), despite the high protein content of insects. This study is aimed at investigating the characteristics of protein extracts from cricket (*Acheta domesticus*) powder for future utilization as a food ingredient in processed food products.

1.2 Problem statement

Very little is known of the characteristic and functionality of protein extract from insects. The ability of protein extracts from insect particularly to form gels is promising. However, more studies need to be done to come up with better concentration and purification methods and also compare the efficiency of the various protein concentration methods. Moreover the functional properties need more attention (Yi *et al.,* 2013). Among ingredients used in the food industry to create stable forms is egg white, however the food industry is looking for other

ingredients with good foaming capacity and stability to replace egg white since it is very expensive (Sciences *et al.,* 2010). Most of studies on the fatty acid profile of insect lipids have focussed on wild edible insects (Bukkens, 1997; Rumpold & Schluter, 2013), however some studies have also focussed on reared insects utilized as feed (Finke, 2002). As a result of limited information to support industrial utilization of insect proteins, edible insects are consumed alone and rarely as a part of a meal in most places (Kinyuru *et al.,* 2010). This shows that there is limitation in terms of diversification in utilization of the edible insects. The major value addition done on edible insects is sun drying, roasting and boiling (Babiker *et al.,* 2007; Ghaly 2009). Grinding and incorporation of edible insects meal in food products has been partially exploited. Communities that harvest these edible insects therefore do not reap the full economic benefits. The physical and chemical properties of proteins influences how they behave during processing and storage, this influences their application in the food industry (Messens *et al.,* 1997). Emulsifying and foaming properties are most critical in food formulations (Sciences *et al.,* 2010). The incorporation of extract from insect will promote consumption of insects by most people other than consuming a whole insect.

1.3 Justification

Many initiatives are on-going aimed at popularising edible insects. Kenya is leading in Africa in this venture with farming techniques being evaluated for mass production as a mini-livestock. Therefore, promotion and diversification of utilization of edible insects will be of great help to communities who harvest edible insects. This will make edible insect production become a sustainable source of livelihood. To enhance better utilization of edible insects particularly industrial processing of edible insects it is important to understand the efficiency of the protein concentration procedures that are available. Additionally, it is important to understand the functionality of the edible insect proteins. Ayieko *et al.,* (2010) and Kinyuru *et al.*, (2009), have demonstrated the potential of utilization of edible insects in food processing. In view of the studies this far, the aim of this study was to investigate the concentration potential of edible insect proteins and the functionality of the concentrated proteins.

1.4 Objectives

1.4.1 Main objective

i. To investigate physico- chemical and functional characteristics of cricket meal concentrated using two different methods for future utilization as a food ingredient in processed food products.

1.4.2 Specific objectives

i. To determine influence of hexane and aqueous extraction on physico-chemical characteristics of the protein concentrates.
ii. To determine influence of hexane and aqueous extraction on emulsion capacity, emulsion stability, water holding capacity, fat adsorption capacity, foam capacity and foam stability of the protein concentrates.
iii. To determine influence of pH and NaCl on protein solubility, emulsion capacity, emulsion stability, water holding capacity, foam capacity, foam stability and least gelation concentration of the protein concentrates.

1.5 Research hypothesis

i. Protein concentration methods have no significant influence on physical and chemical characteristics of the protein concentrates.
ii. Protein concentration methods have no significant influence on functional properties of the protein concentrates.
iii. There is no significant difference in the functional properties of proteins concentrates upon treatment with different concentrations of NaCl and pH regimes.

1.6 Scope

The study was conducted to determine yield, colour, proximate composition, amino acid profile, protein digestibility, protein fractions and functional properties of *Acheta domesticus* concentrates. However the determination of fatty acids, minerals, vitamins, anti- nutrients and allergens was not within the scope of the study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of insect consumption

Insects are a delicacy among some communities in the world and also a source of food in times of calamities such as drought, war and floods (Sutton, 1988; DeFoliart, 1992; Adedire & Aiyesanmi, 1999). The insect's acts as a good source of proteins, vitamins, minerals and energy and are more affordable to rural communities compared to animal proteins. The consumption of insects can help in reducing cases of malnutrition (Obopile & Seeletso, 2013). There are over 400 documented species of edible insects (Allotey & Mpuchane, 2003). Some of these species have been consumed over time by Africans, for instance consumption of termites is a part of the food culture among the Luo community living around Lake Victoria region (Kinyuru *et al.,* 2009). Insects have also been used for cultural practices such as medicine and witchcraft (Ayieko & Oriaro, 2008). However western communities view the consumption of insects as means for survival by Africans (Van Huis, 2003).

2.2 Production methods of edible insects

Edible insects have been for a long time harvested in the wild habitats such as vegetation, roots, branches and trunks of trees or soils (FAO, 2014). Women and children have been highly involved in harvesting and also in selling of the edible insects (Van Huis, 2015). The availability of the insects have been highly affected by seasons. For instance edible insects such as grasshoppers tend to swam at the beginning of the rainy season. In addition winged termites emerge from tunnels during the rainy season and are harvested using light traps (Kinyuru *et al*., 2009). The seasonality of edible insects shows the need to farm the insects to ensure constant productivity and supply. Farming of insects will also help reduce the pressure on wild harvesting.

2.3 House cricket *(Acheta domesticus)*

Cricket has for a long time been used in the feeding of animals (Finke, 2002). The consumption of insects is on an increasing trend. The rearing of insects for food consumption is advantageous, the feed conversion efficiency for *Acheta domesticus* is higher compared to conventional livestock, additionally rearing of insects to provide proteins has less environmental impact as compared to ranching (Van Huis, 2013).

The order Orthoptera comprises of Caelifera and Ensifera sub orders with approximately 15000 species. Cricket is under sub order Ensifera (Legendre *et al.,* 2010). The house cricket (*Acheta domesticus*) origin is Southwestern Asia. Cricket takes around 2-3 months to complete its lifecycle when raised at 80-90^{o}F. Cricket is 16-21mm long, the colour is yellowish brown and have wings covering the abdomen (Walker, 1999). There is a need of processing these insects in less recognizable forms so as to enhance consumption (Yi *et al.,* 2013).

2.4 Field cricket *(Gryllus bimaculutus)*

The black *G. bimaculutus* is the most widespread of the gryllus species, it is found in Europe, South Africa and Thailand (Otte & Cade, 1984). The insect has short hind femur, with short or long hind wings and a shiny protonum, the insect is currently being sold as a food for pets in the United States of America (Weissman *et al.,* 2012). Based on a study it was shown that *G. Bimaculutus* provided about 120kcal/100g of fresh insects (Van Huis, 2013). The protein content of adult cricket on fresh weight basis was found to be 8-25g/100g (Van Huis, 2013).

2.5 Nutrient content of insects

Edible insects have shown to have good protein content and thus a potential substitute, furthermore they provide good amounts of energy. They are able to meet the requirements in terms of amino acid profile in humans. Edible insects have high amounts of mono and poly unsaturated fats and also significant amounts of

micronutrients such as Iron, Zinc, Copper, Phosphorus, Riboflavin, Biotin and to some level Folic acid (Rumpold & Schlüter, 2013).

The protein content of edible insects is in the range of 35.34% for Isoptera and 61.32% for Orthoptera (Rumpold & Schlüter, 2013). 100 grams of caterpillars either moth or a butterfly was found to give about 76% of daily requirement of proteins and almost 100% of recommended intake of vitamins by humans (Agibdye *et al.,* 2009). The energy provided by edible insects is similar to energy provided by meat (Durst *et al.,* 2010). 100 grams of *Acheta domesticus* reared in the USA, was found to be providing 455.19 kcal of energy, 22.08% fibre, 22.08% fat and 66.56% protein (Blásquez, 2012).

In Kenya studies have reported significant amounts of protein, fat, minerals and vitamins in grasshoppers (*Ruspolia differens*) (Kinyuru *et al.,* 2011). Similarly high amounts of energy 556 Kcal/100g, protein 39.3% and fat 44.8% have been reported in edible termites (Kinyuru *et al.,* 2013).

2.5.1 Protein

Proteins varies in the number and distribution of amino acids, furthermore proteins differ on the basis of molecular weight, polarity, size and charge (Sikorski, 2007).The edible insects particularly of the order Orthoptera such as cricket have good protein content and therefore potential substitute for protein sources (Rumpold & Schlüter, 2013). The protein content of most insects is in the range of 9-25% on fresh weight basis, with that of *Acheta domesticus* being 19% (Finke & Winn, 2004). The crude protein of five insects studied was in the range of 19-22% with chitin nitrogen being included, crude protein content of *Acheta domesticus* was found to be 19.3% and that of *Zophobas morio* to be 20.6%. *Tenebrio molitor, Zophobas morio* and *Alphitobius diaperinus* showed higher protein quality compared to *Acheta domesticus* and *Blaptica dubia* with casein and soy protein being the reference (Yi *et al.,* 2013). In a study done in Benue state in Nigeria, edible insect collected from the forest recorded 74% crude protein content on dry matter basis. Based on a study the digestibility of edible insect proteins was 64% (DeFoliart, 2002). The quality of proteins is determined by the amino acid content and especially the essential amino

acids. The essential amino acid content of the larvae of Lesser mealworm (*Alphitobius diaperinus*), yellow worm (*Tenebrio molitor*) and superworm (*Zophobas morio*) are close to the human requirements (FAO/WHO/UNU, 2007). Additionally, based on a study on 100 species of edible insects it was found that they are able to provide essential amino acids at a rate of 10-30% which is the range recommended by the World Health Organization and the Food and Agricultural Organization (Chen *et al.,* 2009).

The sum of essential amino acids for *Alphitobius diaperinus* and *Zophobas morio* were 459 and 545 respectively compared to that of Soy bean which was 439 and the recommendation by FAO/WHO/UNU, (2007) which is 277 (Yi *et al.,* 2013). The essential amino acid index for *Tenebrio molitor* and *Zophobas morio* was found to be higher in comparison to soy proteins but lower compared to casein (Yi *et al.,* 2013), this shows the good quality of insect proteins.

2.5.2 Chitin

Chitin is the most abundant carbohydrate polymer in nature, it is mainly found as part of the exoskeleton of invertebrate, protozoa, fungi and algae. The chitin content however varies among the insect species, due to the chitin insects are good sources of fibre contributing about 10% of the dry insect (Belluco *et al.,* 2013). The chitin results to high energy values, the chitin for termite is estimated to be 761kcal/ 100g on dry weight basis, caterpillars studied in Zaire yielded 457kcal/100g on dry weight basis (Malaisse & Parent, 1980). However this energy is not available to humans. Chitin obtained from crabs and crayfish has been approved in Japan to be incorporated in cereal products to provide fibre and calcium (Belluco *et al.,* 2013). Chitin has been found to have beneficial effects to the human immunity (Sun *et al.,* 2007). About 90% of the insect skin is composed of chitin (Chen *et al.,* 2009). Edible insects have different amounts of chitin, *Bombyx mori* defatted pupae was found to have a chitin content of 5.55% and *Dendrolimus houi* adult had a chitin content of 17.83% (He *et al.,* 1999; Guo *et al.,* 2008). Chitin contains non protein nitrogen and this explains high crude protein content, *Acheta domesticus* has been found to have

considerable high chitin content compared to *Tenebrio molitor, Zophobas morio* and *Alphitobius diaperinus* (Yi et al., 2013).

2.5.3 Lipids

Lipids are grouped as non-polar and polar lipids. The polar lipids are widely distributed in plants, animals and bacteria (Sikorski, 2007). Insect lipid content ranges from below 10% to above 30% with high concentration of unsaturated fatty acids which include oleic, linoleic and linolenic acids (De Foliart, 1991). The lipid content and the composition of the lipid profile is dependent on the species and life stage of the insect (Tzompa-Sosa *et al.,* 2014).The lipid content of Caterpillars (Lepidoptera) is in the range of 8.6-15.2g/100g fresh insect, Grasshoppers (Orthoptera) has a fat content in the range of 3.8-5.3g/100g fresh insects (Bukkens, 1997). About 80% of the lipid content is in the form of triacylglycerol (Gilby, 1965). Phospholipids are the second most important class of lipids in insect crude fat and the concentration is below 20% (Ekpo *et al.,* 2009; Gilby, 1965). The total lipid content of four insects studied was in the range of 6.0-12.7%, with *Tenebrio molitor* recording the highest lipid content of 12.7g/100g fresh insect (Tzompa-Sosa *et al.,* 2014).The lipid content of *Acheta domesticus* was in agreement with the range reported in a number of studies which is 6-7g/100g insect (Barker *et al.,* 1998; Finke, 2002). The most abundant unsaturated fatty acid in *Tenebrio molitor,Alphitobius diaperinus, Acheta domesticus and Blaptica dubia* was Oleic and Linoleic acid and the most abundant saturated fatty acid was palmitic acid. These three fatty acids accounted for 84.7-89.8g/100g of the total fatty acid in the lipid extract (Tzompa-Sosa *et al.,* 2014).

2.6 Insect based food products

The consumption of insects is not very new, the new dimension in the food science field is how the insects are served. The lack of appropriate storage and preservation facilities particularly in the rural area where there is no electricity is among the factors contributing to food shortage (Ayieko *et al.,* 2010). Insects are processed by roasting, sun drying, grinding and mixing with other ingredients to make other products. Crackers and muffins have been made from termites and mayfly, meat loaf

and sausages from mayfly (Ayieko *et al.,* 2010). There is reduced consumption of meat products due to health reasons (Salvini *et al*., 1989; Rosner & Gore, 2001), as a result the consumption of insects is becoming more common among the health conscious people (Ayieko *et al.,* 2010). Based on the processing technique the products may have a longer shelf life which is important when it comes to food security. In Japan the art of preparing insects have been modified where by soysauce is introduced as an additive (Pemberton & Yamasaki, 1995). This has basically promoted acceptance of insects. Therefore there is potential for cricket to be used in processing of food products.

2.7 Status on industrial use of insect proteins

Edible insects have been utilized in processing food products due to their good nutrient content particularly high protein content. The utilization of edible insects has been pressed by studies that have demonstrated the safety of edible insects (Konyole *et al.,* 2012). Red colorant carmine extracted from cochineal insect widely used in industrial production of yoghurt (Yi *et al.*, 2013). Termites and mayfly have been shown to have great potential in local production of crackers and biscuits (Ayieko *et al.,* 2010). Furthermore termite meal has been used as a wheat supplement in the production of buns (Kinyuru *et al.,* 2009). In addition winged termites meal has been included in the formulation of extruded complimentary flour (Konyole *et al.,* 2012). This shows the potential of utilization of edible insects and also extracts from insects such as proteins. To meet the great need of industrial processing of edible insects there is a great need to focus on technologies that will aid in farming of edible insects.

2.8 Protein concentration methods

A number of methods exist for protein concentration from plant sources and insect sources. For plant protein sources such as soya bean, proteins are isolated from defatted soy flour by dissolving soy flour in water, altering the pH using 1M NaOH and 1M HCl, followed by centrifugation. The isoelectric point is then used to precipitate proteins from crude proteins solution, this is also called acid precipitation

(Chove *et al.,* 2007). Additionally, ammonium sulphate and alcohol precipitation are also used (Alsohaimy *et al.,* 2007).

For the case of insect proteins insect flour is washed severally with hexane at different ratios to extract fat, this is followed by drying the filtrate and passing it through screens (Babiker *et al.,* 2007). Frozen insect under Nitrogen is dissolved in deionized water, ascorbic acid is added and then mixing is done. The obtained suspension is then passed through screens to obtain the proteins (Yi *et al.,* 2013).

2.9 Influence of concentration method on physico- chemical and functional characteristics of protein concentrates

There are various extraction methods used in obtaining protein concentrates. The methods used have varying effects on the physico- chemical and functional properties of the protein concentrates. For instance hexane extraction procedure has been observed to yield concentrates with lower fat content and high protein content compared to ethanol and methanol (L'hocine et al., 2006). The differences observed in the physico-chemical and functionality of the protein concentrates is usually associated with reagents and solvents used in the defatting procedure which potentially induce change through denaturation, aggregation and dissociation. Defatting has been a common practice in concentrating proteins. Hexane has been the commonly used solvent in defatting of plant materials such as soy bean (L'hocine et al., 2006). However defatting by use of hexane has become unpopular due to effects on functionality and safety issues (Gandhi et al., 2003). Other than hexane, ethanol and methanol are other organic solvents that can be utilized in deffating and consequently protein concentration. Ethanol has shown comparable defatting efficiency as hexane however methanol has proven to be a poor choice (L'hocine et al., 2006). Aqueous concentration has been recommended as an alternative method. This method has been shown to improve some functional properties, for instance foaming and gelling properties of soy bean isolate (Hua et al., 2005). In addition in a study it was concluded that protein extracted by isoelectric point irrespective of the source showed good emulsion ability and stability (Alsohaimy et al., 2007).

2.10 Influence of ph and Nacl on functional properties of protein concentrates

The functional properties of protein concentrates are highly influenced by the proteins. However there are other components such as fat that have an influence on functional properties. pH has been recorded to have significant influence on the functional properties of edible insects extracts. This is due to the enhanced ionization for instance at high pH (Alsohaimy *et al.,* 2007). The high ionization ends up influencing functional properties such as solubility, gelation and emulsion capacity. For instance it was observed that the solubility of protein extracts from Grasshoppers was high at high pH (Olaofe *et al.,* 1998). Similarly the gelation of edible insect concentrates has been recorded at pH range of 7-10 (Yi *et al.,* 2013). In addition in a study it was shown that the emulsion forming ability by protein extracts was high at the isoelectric point (Chove *et al.,* 2002).

Salts have also been recorded to having diverse effects on the functional properties of edible insect concentrates. For instance it has been observed that high salt concentration reduces the solubility of proteins while low salt concentration enhances the solubility of proteins (Narayana & Rao 1984; Akintayo *et al.,* 1999). This is due to the salting out effect that occurs at high salt concentration. However the addition of salt have been shown to have positive effects on some functional properties. In most plant protein concentrates the addition of salt have resulted to enhanced gelation of the protein concentrates (Akintayo *et al.,* 1999; Adebowale *et al.,* 2005). In addition NaCl was reported to positively influence water holding capacity of protein concentrates compared to other salts (Aremu *et al*., 2008). This therefore implies that the choice of salt is also a factor to always consider.

CHAPTER THREE

METHODOLOGY

3.1 Sample preparation

The crickets were farmed at Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were fed on a diet consisting of 21% protein content feed for the first 14 days after eggs hatching followed by a 14% protein diet afterwards. Four weeks after hatching, the diet was supplemented with leafy vegetables such as pumpkins, cassava and morning glory depending on their seasonal availability.

On the 10^{th} week, 10kgs of crickets were harvested by hand picking from trays and put in white buckets that were periodically opened and closed. Crickets were then cleaned using clean tap water, freeze dried in polythene bags to a moisture content of 5.3%. Whole crickets were ground using a stainless steel blender to obtain a cricket powder and stored at 4°C in one polythene bag.

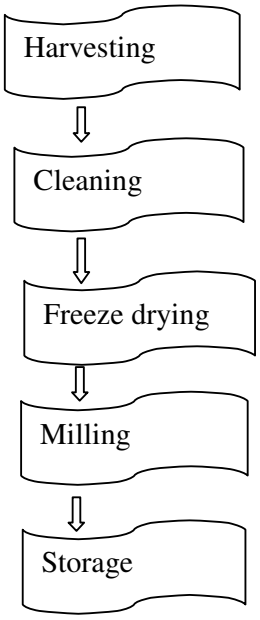

Figure 3.1: Process flow diagram

3.2 Study and experimental design

The experiment employed unbalanced randomized complete block design. The treatments were two extraction procedures i.e. hexane and aqueous extraction. Hexane extraction method yielded one protein concentrate (HE). Aqueous extraction yielded two protein concentrates one which was a filtrate (AE Pellet) and the other a residue (AE Residue).

3.3 Concentration of cricket meal proteins

Concentration of cricket proteins was done by extraction of fat from the cricket meal using the following methods:-

3.3.1 Hexane extraction

The concentration was based on a modified method used by (Babiker *et al.*, 2007). Dried cricket powder was mixed with hexane in the ratio of 1:5. The mixture was stirred using a mechanical shaker (KS 250 basic) for 16 hours and let to settle for 5 min, hexane and the extracted fat was then decanted. The residue was washed with hexane to remove any traces of oil. The defatted powder was dried in open air at room temperatures. The dried powder was sieved through 500µm screen and then stored at 4^{o}C after determination of yield. The sample codes for the resulting concentrates are as shown in (Table 3.1).

3.3.2 Aqueous extraction

The extraction was based on the method by Yi *et al.,* (2013). About 400 g of cricket meal was blended using a blender (Signature SG-201) at speed 2 with 1200 ml of demineralized water mixed with 2 g ascorbic acid beforehand, for 1 min. The obtained insect suspension was sieved through a stainless steel filter sieve with a pore size of 500µm resulting to a filtrate and residue, the residue was then freeze dried. Centrifugation of the filtrate was then done at 10,000 rpm for 30 min at 4^{o}C, resulting to a supernatant which was decanted and a residue (pellet) which was freeze dried.

Table 3.1: Sample codes and description of the protein concentrates

Sample codes	Description
HE	Hexane defatted cricket meal
AE Pellet	Aqueous defatted cricket meal (Filtrate)
AE Residue	Aqueous defatted cricket meal (Residue)

3.4 Determination of yield

The amount of protein concentrate was determined gravimetrically and the weight reported in percentages on dry matter basis.

3.5 Determination of colour by colorimetric method

The colour of the samples was determined using a hunter lab colour difference meter (Minolta, Tokyo, Japan) according to Almenar *et al.,* (2008). The colour was measured on the surface of the samples. Reflected colour *L**, a* and b* values were determined directly. Results were tabulated and the *L*,* chroma and hue angle values were used to determine the colour components of the samples.

Chroma value= $(a^2 + b^2)^{0.5}$ Hue angle= $\tan^{-1}(b^*/a^*)$

*L** = value (0=black=high value, 100=white)

3.6 Determination of proximate composition

The moisture content, crude ash, crude fat, crude protein and crude fiber of the protein concentrates was determined according to the standard method of AOAC, (2005). The moisture content was determined by oven drying method at 105°C where 2 g of the sample were used until a constant weight was obtained. Crude ash content was analysed by incineration where 5 g of the sample were dry ashed in an electric muffle furnace (Shimadzu KL- 420, Japan) at 550 °C for 16 hours to a constant weight. Crude fat was extracted from 5 g of the concentrated cricket proteins using Soxhlet apparatus with petroleum ether as the solvent and quantified gravimetrically. Crude protein content of the concentrated cricket proteins was

determined according to the Kjeldahl method and conversion factor of 6.25 was used to calculate the crude protein content from the nitrogen content. Crude fibre was determined by sequential digestion of samples with 1.25% H_2SO_4 and 1.25% NaOH using a fibreglass container drying in an oven for 5 hours at 105 °C and ashing in the muffle furnace at 550 °C for 16 hours. The fibre content was then determined gravimetrically. Available carbohydrate was calculated based on weight difference using crude protein, crude fat, crude fibre, and crude ash data as follows.

Available carbohydrates (%) = 100 - (crude fibre + crude protein + crude ash + crude fat)

3.7 Determination of amino acid profile

1 g of the cricket meal was weighed into a 250 mls Bombelroll tube, hydrochloric acid was added to 50 mls of volume. The contents of the Bombelroll tube were shaken gently and the tube closed tightly with a stopper. The tube was heated at 110 – 115 °C in an autoclave for 7 hours. The tube was then cooled to room temperature. The contents of the tube were evaporated to dryness by rotary vacuum evaporator. The residue was dissolved in 50 mls of sodium citrate pH 2.2 and filtered through Whatman no. 42 paper. An aliquot of the solution was taken, filtered through 0.45 µm membrane filter and loaded to the amino acid analyzer.

Compute mg/ g sample using the formula

$$\text{mg / g sample} = \frac{10 \times v}{aw}$$

Where

x = Amount of amino acid (ng/volume sample injected)

v = Total volume of sample

a = Volume sample injected

w = Sample weight (g)

3.8 Determination of *in-vitro* protein digestibility

Digestibility of protein in the insects was determined by the method outlined by Mertz *et al.,* (1984) and adapted by Kinyuru *et al.,* (2010). Initial protein content of the samples was determined using micro-Kjeldahl nitrogen determination method AOAC, (2005). The second stage involved pepsin digestion, where 0.2 g of the sample was weighed into centrifuge tubes. Then 20 mls buffered pepsin was added and mixed. A blank was prepared in the same way but without a sample. The tubes were placed in a water bath at 37ºC for 2 hours with gentle shaking after every 20 min. The tubes were then placed in an ice bath for 30 min to attain a temperature of 4ºC followed by centrifugation at 6000 rpm for 15 min. The supernatant was discarded and 10 mls of buffer solution added, then shaking and centrifugation was done. The supernatant was discarded and the residue filtered using a filter paper. The filter paper was rolled and inserted into a Kjeldahl flask and dried for 15 min in the oven. 10 mls of Concentrated Sulphuric acid, 1 g Potassium Sulphate and 1 ml of 10% Copper Sulphate solution were added to the Kjeldahl flask containing the dried filter paper and sample. Then digestion, distillation and titration were done according to the micro-Kjeldahl nitrogen determination.

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

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

B = % protein in the sample after pepsin digestion

3.9 Determination of protein fractions

The protein fractions were extracted based on a method by Agboola *et al.,* (2005)**.** Albumin was determined by mixing 50 g of protein extract with 250 mls water, followed by stirring for 4 hours, centrifuging at 3000 rpm for 30 min freeze drying and weighing. Globulin was extracted by mixing the residue after extracting albumin in 250 mls of 5% NaCl and stirred for 4 hours. The supernatant was transferred to another flask, freeze dried and weighed. Glutelin was extracted by dissolving the residue after extraction of globulin in 250 mls of 0.1M NaOH with continuous

stirring for 1 hour. The supernatant was freeze dried and then weighed. Prolamin was extracted by dissolving the residue after extraction of glutelin in 250 mls of 70% ethanol, followed by stirring continuously for 1 hour. The supernatant was freeze dried and then weighed.

3.10 Determination of functional properties of the protein extracts

3.10.1 Protein solubility

1 g of the protein extract were dissolved in 10 mls of water. The solution was poured into centrifuge tubes, shaken thoroughly and centrifuged at 4000 rpm for 10 min. Protein content in the original sample and the supernatant were determined by microkjedal method as suggested by Wu *et al.,* (1998). Protein solubility was calculated using the equation suggested by Were *et al.*, (1997).

Protein solubility= (protein content of supernatant/ total protein content in the sample) ×100

3.10.2 Emulsion capacity and stability

Emulsion capacity and emulsion stability were determined based on a method by Naczk *et al.,* (1986). Emulsion capacity was determined by mixing 1 g of protein concentrate with 100 mls of distilled water. The suspension was homogenized for 10 min. At the 5^{th} min, corn oil was added continuously and stirred. The emulsion was centrifuged at 3000 rpm for 10 min. Volume of the emulsified layer was then recorded.

Emulsion capacity (EC) %= (volume of emulsified layer/ volume of the suspension) × 100.

Emulsion stability was determined by mixing 1 g of protein concentrate with 100 mls of distilled water. The suspension was homogenized for 10 min. At the 5th min corn oil was added continuously and stirred. The emulsion was heated at 85 ^{o}C for 30 min then cooled back to room temperature. The emulsion was centrifuged at 3000 rpm for 10 min and the volume of the emulsified layer recorded.

Emulsion Stability (ES) % = (volume of emulsion layer/ volume of suspension) × 100.

3.10.3 Water holding capacity

Water holding capacity (WHC) was determined by the AACC (2000). About 1 g of the sample was put in a centrifuge tube followed by 3 mls of water. Centrifugation was then done at 2060 rpm for 10 min (Beckman CS-6 centrifuge). After centrifugation the supernatant was decanted and its volume determined.

Water holding capacity= (volume of water added – volume of the supernatant)/weight of sample.

3.10.4 Fat absorption capacity

Fat-absorption capacity (FAC) was determined using the procedure of Lin *et al.,* (1974). A protein concentrate (0.3 g) was mixed with corn oil (3 ml) in a pre-weighed 50 mls centrifuged tube for 1 min. After centrifugation at 2060 rpm for 30 min (Beckman CS-6 centrifuge), the supernatant was discarded and the tubes were re-weighed. The FAC was expressed using the following equation:

FAC (%) = 100 × (weight of sample + oil/ weight of sample).

3.10.5 Foam capacity and stability

The method described by Coffmann & Garcia, (1977) was used. 10 g of protein concentrates were mixed with 100 mls of distilled water, the suspension was blended for 2 min using a blender (Signature SG-201) at speed 2. The initial solution volume (V_1) and final volume after mixing (V2) were recorded. Foaming Capacity (FC) was calculated as

$FC = ((V2-V1)/V_1) \times 100.$

Foaming Stability (FS) was determined as the foam volume (V_3) that persisted after 5 min.

$FS = ((V3-V1)/V_1) \times 100.$

3.10.6 Least Gelation concentration

Least gelation concentration was determined using a method described by Yi *et al*., (2013). Protein concentrate suspensions at 30 % w/v was made in test tubes and heated in a water bath for 30 min. The test tubes were then removed from the water bath cooled using running water and then put in a cold room (4°C) for 2 hours. Gel formation was determined visually after inverting the test tubes.

3.11 Effect of NaCl on the functional properties of protein extracts

NaCl concentrations of 2%, 4%, 6%, 8%, and 12% were prepared in a volumetric flask. The salt solution were then independently used in place of distilled water in the determination of water holding capacity, emulsion capacity and emulsion stability, foaming capacity and foam stability and least gelation concentration of the protein concentrates.

3.12 Effect of pH on the functional properties of protein extracts

Solutions of pH 2, 4, 6, 8 and 12 were prepared using 0.1 M NaOH and 0.1 M HCL. The solutions with varying pH were then independently used in place of distilled water to determine protein solubility, water holding capacity, emulsion capacity and stability, foaming capacity and foam stability and least gelation concentration of the protein concentrates.

3.13 Ethical considerations of handling insects

Edible crickets were harvested for the purpose of research under the supervision of a trained expert. Distress to the crickets was minimized by immediate freeze drying in plastic bags.

3.14 Data analysis

Data was reported in means and standard deviations. To determine the effect of the extraction methods on the physico-chemical and functional characteristics, data was analysed using one way analysis of variance (ANOVA). To determine influence of pH and NaCl on the functional properties data was analysed using two way analysis of variance. Mean separation was done using Bonferroni's method at $p \leq 0.05$. Data analysis was performed using Stata, version 12.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Yield and Colour of protein concentrates

The yield of hexane and aqueous extraction methods and colour characteristics of the protein concentrates is shown in (Table 4.1). Aqueous extraction gave two protein concentrates i.e. AE Pellet and AE residue. Hexane extraction resulted to a higher yield with HE recording the highest amount (66.35%) and AE Pellet the least.

Table 4.1: Yield and colour characteristics of *Acheta domesticus* protein concentrates

Protein concentrates	Yield (%)	*Lightness (L*)*	Hue angle	Chroma
HE	66.35 ± 0.87^{c}	65.50 ± 0.52^{b}	81.08 ± 0.31^{b}	12.69 ± 0.51^{a}
AE Pellet	32.72 ± 1.34^{a}	58.53 ± 0.46^{a}	77.84 ± 0.08^{a}	13.61 ± 0.18^{ab}
AE Residue	48.32 ± 0.92^{b}	59.07 ± 0.85^{a}	78.30 ± 1.32^{a}	14.13 ± 0.20^{b}

Means with different superscript letters in each column are significantly different at $p<0.05$

(n=3).

The yield from hexane extraction i.e HE was higher than 48.64% obtained from defatted mealworm larvae while yield by aqueous extraction i.e AE Pellet and AE residue was close to values obtained from defatted mealworm larvae (35.84%) and silkworm pupae flour (48.64%) respectively (Kim *et al.,* 2016). Hexane and aqueous extraction were aimed at removing fat and consequently increase the protein content. The higher yield in hexane extraction could be attributed to the fact that losses tend to occur during aqueous extraction (Yi *et al.,* 2013)**.**

The hexane extraction method showed considerably higher values for lightness and hue angle compared to aqueous extraction ($P < 0.0001$) with HE recording the highest

value for lightness (L^*) and hue angle while AE Pellet and AE residue the least. The brown colour of the protein concentrates is attributed to melanin (Wittkopp & Beldade, 2009) therefore the high L^* recorded in HE could be due to higher removal of melanin during the extraction process (Kim *et al.,* 2016). Based on the hue angle all the protein concentrates were within the orange region (Stancil & Jordan, 1985). There was significance difference in the chroma values however with slight numerical difference, the low values indicated lower saturation. Potentially positive visual appeal could result to a higher probability to taste and liking of a food product (Yeomans *et al.,* 2008). Colour is a major attribute that influences the acceptance or rejection of edible insects (Tan *et al.,* 2015). Evidently the protein concentration methods significantly affected the yield and colour characteristics of the protein concentrates.

4.2 Proximate composition

Crude protein was the highest component while crude ash was the least. Hexane extraction yielded a protein concentrate with high protein content compared to aqueous extraction (P = 0.003) (Table 4.2). The protein content of the protein concentrates was similar to 64.38% reported by (Rumpold & Schlüter, 2013). The protein content of AE Pellet and residue was within 65 -75% and 58 – 69% reported in pellet and residue from five different insects including *Acheta domesticus* (Yi *et al.*, 2013).

Table 4.2: The proximate composition (% dry weight basis) of *Acheta domesticus* protein concentrates

Protein concentrate	Crude protein	Crude fat	Ash	Fibre	Available CHO
Cricket meal	62.72± 1.64[a]	18.87 ± 0.1[d]	4.19 ± 0.08[b]	7.50 ± 1.60[a]	6.71 ± 2.16[a]
HE	72.63± 0.83[c]	6.76± 0.133[a]	4.50 ± 0.06[b]	7.51 ± 0.29[a]	8.61 ± 0.79[a]
AE Pellet	66.66± 0.82[b]	13.91± 0.24[c]	2.93 ± 0.24[a]	6.29 ± 0.54[a]	10.21 ± 0.86[ab]
AE Residue	65.79± 1.3[ab]	11.49± 0.13[b]	3.13 ± 0.19[a]	6.22 ± 0.72[a]	13.38 ± 1.33[b]
P value	0.003	<0.001	0.001	0.476	0.0034

Means with different superscript letters in each column are significantly different at $p<0.05$

(n =3).

Hexane extraction proves to be a suitable method for concentrating proteins to be used in the food industry. Soy proteins are highly utilized as non- meat ingredients in the meat industry. The protein content of concentrated soy protein is 62-69% (Kim *et al.,* 2016), this shows that the protein concentrates obtained in this study matches well with soy protein concentrates and have the potential of being used as a supplement for soy protein in the food industry.

Hexane extraction proved to be a more efficient compared to aqueous extraction with the HE recording the least crude fat and cricket meal the highest ($P < 0.001$). Similarly high fat extraction yield was realized through solvent extraction compared to aqueous extraction (Tzompa-Sosa *et al.,* 2014; Yi *et al.,* 2013). The crude fat content of the protein concentrates was lower than 18.55% - 22.80% reported in crickets (Finke, 2002; Rumpold & Schlüter, 2013). Previously hexane extraction has been used in preparation of sweet lupin pea protein and soy proteins (L'Hocine *et al.,* 2006; Sciences *et al.,* 2010).

The ash content of cricket meal and HE was the highest while the least was recorded in AE Pellet and AE Residue. Hexane extraction resulted to a significantly higher ash content compared to aqueous extraction ($P < 0.001$). The values obtained in this study were within 3.55% - 5.10% reported in adult *Acheta domesticus* (Rumpold & Schlüter, 2013). Studies have revealed that drying methods influences ash content of foods hence the importance of optimizing drying methods to ensure consistency in quality of products (Özcan *et al.,* 2005).

Hexane and aqueous extraction had no significant influence on the crude fibre content of the protein concentrates ($P = 0.467$). The crude fiber content of the protein concentrates was close to 7.8% as reported by (Videan *et al.,* 2007). Studies have concluded that crude fibre in insects represents chitin since chitin has a linear structure to that of cellulose (Finke, 2002). Chitin-chitosan has been extracted from insects for use as a supplement, this is due to it's potential in reducing blood cholesterol and in the management of obesity (Koide, 1998). Crude fibre represents the indigestible components from the edible insects, however studies have shown that chitinase found in humans could potentially help in the digestion of chitin (Boot *et al.,* 2005). This therefore implies that there should be no fear when it comes to the consumption of edible insects.

AE residue had the highest available carbohydrate content while there was no significance difference in the other protein concentrates. The available carbohydrate was close to 3.55-9.10% reported in *Acheta domesticus* by (Rumpold & Schluter, 2013). The variation observed in nutrient content could be attributed to differences in diets, environment and age of insect (Banjo *et al.,* 2006).

4.3 Amino acid profile of cricket meal

The amino acid profile of the cricket meal showed Arginine as the dominant amino acid while Methionine and Tyrosine were the least (Table 4.3).

Table 4.3: Amino acid profile of cricket meal

	Amino acids (mg/g)													
	Histidine	Arginine	Lysine	Glutamine	Serine	Glutamic acid	Proline	Valine	Methionine	Tyrosine	Isoleucine	Leucine	Hydroxyprline	Phenylalanine
Cricketmeal	0^{a}	101 ± 0.01^{g}	0^{a}	0^{a}	0^{a}	0^{a}	15.88 ± 0.18^{e}	12.49 ± 0.21^{c}	11.21 ± 0.56^{b}	11.22 ± 0.38^{b}	0^{a}	17.28 ± 0.06^{f}	12.44 ± 0.24^{c}	13.68 ± 0.26^{d}

Means with different superscript letters are significantly different at $p<0.05$ *(n =3).*

Histidine, Lysine, Glutamine, Serine, Glutamic acid and Isoleucine were not reported. Wang *et al.,* (2005) recorded Glutamic acid (90.7) as the highest amino acid in Field cricket while Histidine (19.4) was the least. Similarly Glutamic acid (21) was obtained in higher amounts in adult crickets as reported by Finke, (2002). Edible insects are able to provide some of the essential amino acids such as phenylanine, methionine, proline, leucine among others and thus important to human nutrition. However cricket proteins do not provide all the essential amino acids for instance Histidine (0 mg/g) therefore they should to be consumed as part of a diet or used as an ingredient in formulation of processed products (Table 4.3). There was a considerable high amount of hydrophobic amino acids i.e Methionine, Phenylanine, Valine and Proline and zero amounts of polar amino acids Glutamine, Histidine and Serine. This could potentially result to low solubility of the cricket meal.

4.3 Protein digestibility and protein fractions

The protein digestibility and protein fractions of the protein concentrates are shown in Table 4.4. Hexane and aqueous extraction methods had no influence on the protein digestibility of the protein concentrates (P = 0.0822) however studies have shown that heat processing of edible insects may either decrease or increase the protein digestibility of edible insects (Kinyuru *et al.,* 2010).

Table 4.4: Protein digestibility and protein fractions (%) of *Acheta domesticus* protein concentrates

Protein concentrates	Protein digestibility (%)	Albumin (%)	Globulin (%)	Glutelin (%)	Prolamin (%)
HE	84.23 ± 0.74[a]	7.05 ± 0.02[b]	14.49 ± 0.03[a]	8.37 ± 0.02[a]	4.27 ± 0.02[b]
AE Pellet	85.28 ± 0.20[a]	6.29 ± 0.11[a]	19.42 ± 0.59[c]	8.25 ± 0.05[a]	6.26 ± 0.01[c]
AE Residue	84.70 ± 0.23[a]	6.09 ± 0.08[a]	17.86 ± 0.07[b]	10.1 ± 0.06[b]	4.05 ± 0.47[b]
P value	0.0822	<0.001	<0.001	<0.001	<0.001

Means with different superscript letters in each column are significantly different at p<0.05

(n =3).

The protein digestibility of the protein concentrates was within 76% -98% reported by Ramos-Elorduy *et al.,* (1997) in edible insects from Mexico and Kinyuru *et al.,* (2010) in winged termites and grasshoppers. Additionally the protein digestibility of the protein concentrates was close to 85% for salmon and 89% for beef (Bodwell *et al.,* 1980). Protein digestibility is a key parameter that shows the quality of cricket proteins (Belluco *et al.,* 2013). The good protein digestibility of the cricket proteins

proves the suitability of application of cricket protein concentrates in formulation of food products. The protein digestibility of proteins is influenced by factors such as heat for instance denaturation of proteins may promote the unfolding of the polypeptide chain rendering it susceptible to the digestive enzymes (Opstvedt *et al.,* 2003).

There was a general trend in protein fraction distribution with globulin being the highest component, followed by glutelin and albumin and prolamin being the least component. There was significance difference in protein fraction distribution, AE Pellet recorded the highest amount of globulin while HE recorded the least. AE Pellet had the highest amount of prolamin while HE and AE residue had the least. Globulin and albumin have been found in considerable amounts in *inago* (Mitsuhashi, 1997). According to (Akpossan *et al.,* 2015) glutelin was the major component in defatted caterpillar flour, followed by albumin, prolamin and globulin was the least. Additionally, glutelin has been recorded as the major protein component in edible insects (Aguilar-Miranda *et al.,* 2002). The variation in distribution of protein fraction with other studies could be due to sex (Mitsuhashi, 1997) species difference and method used in determination.

4.4 Functional properties

The extraction procedures had influence on the functional properties of the protein concentrates (Table 4.5). The aqueous extraction method showed high values for emulsion capacity and emulsion stability with the AE Pellet recording the highest value (P = 0.0053). However, there was no significance difference in emulsion and emulsion stability in HE and AE Residue.

Table 4.5: Functional properties of *Acheta domesticus* protein concentrates (n =3).

Protein concentrates	Emulsion capacity (%)	Emulsion stability (%)	Water holding capacity (ml/g)	Fat adsorption capacity (%)	Foaming capacity (%)	Foaming stability (%)
HE	26.83 ± 3.85[a]	21.86 ± 2.10[a]	2.03 ± 0.32[a]	337.24 ± 33.20[a]	1.42 ± 0.47[a]	1.26 ± 0.28[a]
AE Pellet	41.70 ± 2.64[b]	33.61 ± 3.76[b]	2.73 ± 0.29[a]	352.75 ± 20.03[a]	11.11 ± 0.46[c]	10.15 ± 1.01[c]
AE Residue	35.53 ± 3.69[ab]	26.31 ± 3.54[ab]	2.74 ± 0.24[a]	348.40 ± 35.21[a]	6.39 ± 1.07[b]	4.15 ± 0.53[b]
P value	0.0053	0.0118	0.074	0.8166	<0.001	<0.001

Means with different superscript letters in each column are significantly different at $p<0.05$

(n =3).

The emulsion capacities obtained in this study were lower than 46.8% while the emulsion stability were greater than 8.5% obtained in large African cricket (*Gryllidae* sp) (Yemisi *et al.,* 2005). Similarly the emulsion capacity of the protein concentrates was higher than 14.93% and the emulsion stability was lower than 74.22% obtained in defatted *Imbrasia oyemensis* Larvae flour (Akpossan *et al.,* 2015). The observed deviation in emulsion capacity and stability could be attributed to partial denaturation of proteins and change in the distribution of molecular charge that exposes hydrophobic amino acids (Bußler *et al.,* 2016). The denaturation of the protein concentrates could have been influenced by the different protein concentration methods. The ability of the protein concentrates to form emulsions is of important specifically in communited meats, salad dressing and in cake production (Yemisi *et al.,* 2005). The ability of a protein to form stable emulsions shows that it can be used

in the food industry as an emulsifier. Emulsion formation by proteins is affected by pH and even salt concentration. In a study it was shown that emulsion forming capacity of soy proteins was highly dependent on pH (L'Hocine *et al.,* 2006). Similarly in a study it was shown that the emulsion forming ability by protein extracts was high at the isoelectric point (Chove *et al.,* 2002).

There was no significance difference in water holding capacity of the protein concentrates ($P = 0.074$). The water holding capacity of the protein concentrates was close to 2.38 mls/g reported in *Gryllidae* sp (Yemisi *et al.,* 2005). However the protein concentrates exhibited higher water holding capacity than 1.87 mls/g obtained in Yellow Mealworm protein extract (Zhao *et al.,* 2016). The considerably high water holding capacity of the protein concentrates is desirable in processes where water retention is preferred such as in the meat and baking industry. The deviation observed in water holding capacity of the protein concentrates could be attributed to differences in amino acid profile, conformation, hydrophilicity, protein concentration, charge characteristic and conformation of the protein concentrates (Naik *et al.,* 2012). The ability of protein to hold water is a desirable characteristic in the food industry. The water retention by proteins influences the mouth feel and texture characteristics of meat and baked products (Aremu *et al.,* 2008).

Hexane and aqueous extraction had no significance effect on the oil adsorption capacity of the protein concentrates. The oil adsorption capacity of the protein concentrates was higher than 178.7% reported in *Cirinia forda* (Osasona & Olaofe, 2010) and 233% reported in Yellow Mealworm protein extract (Zhao *et al.,* 2016). The difference in the availability of the non- polar side chains to bind fat could potentially explain the observed difference in oil adsorption capacity (Al-Kahtani *et al.,* 1993). The considerably high oil adsorption capacity exhibited by the protein concentrates shows the potential of protein concentrates in enhancing flavour characteristics of processed foods (Osasona & Olaofe, 2010). The ability of proteins to absorb oils is very important since the oil retains flavour (Kinsella, 1976). Flavour is one of the attribute that makes food palatable.

Aqueous extraction showed higher values for foaming capacity and foam stability with AE Pellet recording the highest values while HE recorded the least values. The foaming capacity and foam stability of the protein concentrates were considerably lower than 61% and 21.2% respectively obtained in soy protein isolates (Chove *et al.,* 2007). This explains the undesirable foaming capacity and foam stability of the protein concentrates. Foam stability is highly influenced by protein structure, protein concentration and ionic strength (Yi *et al.,* 2013). The fact that the protein concentrates had lower protein content compared to pure protein isolates could explain the impact of protein concentration on the foam stability of the protein concentrates. Additionally the protein concentrates had fat and this could also explain the low foaming capacity (Lomakina & Mikova, 2006). The high percentage of globulin could explain the reason for low foaming capacity (Table 4.4) since globular proteins are difficult to surface denature thus exhibiting low foam capacity (Grahams & Phillips, 1976). The protein concentrates showed considerably low values for foaming capacity and foam stability and therefore the protein concentrates would not be suitable in processing products such as cakes and ice creams where foaming is of importance (Wu *et al.,* 2009). The ability to form foams is governed by the type of protein, pH, processing method, surface tension and viscosity (Yasumatsu *et al.,* 1972). The foaming ability of *Cirina forda* was found to be about 7% while the foam stability was found to be about 3%. The foaming of proteins is attributed to desirable characteristics in food which include consistency, texture and appearance of foods (Akubor & Chukwu, 1999).

4.5 Effect of pH on Protein solubility of the protein concentrates

Protein solubility is an important functional property that has an influence on other functional properties. There was a general decrease in protein solubility from pH 2 to pH 8 followed by a significant increase at pH 12 among the protein concentrates (Figure 4.1).

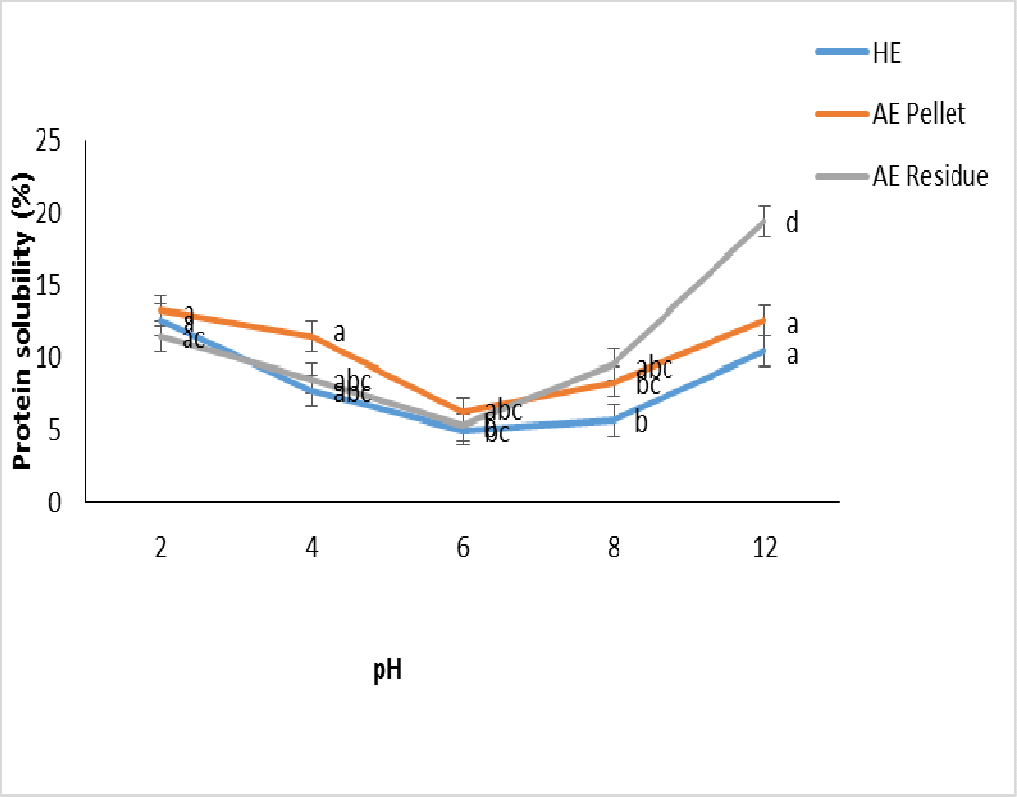

Figure 4.1: Effects of pH on protein solubility (n =3).

The protein concentrates significantly differed at pH 12 while no significant difference was observed at Ph 2, 4, 6 and 8. The highest solubility was recorded at pH 12 with AE residue having the highest value while HE and AE Pellet had the least. The protein solubility of *Cirinia forda* powder was recorded highest at pH 11 (Omotoso, 2006). Similarly the protein solubility of the palm weevil *Rhynchophorus phoenicis* powder was highest at pH 11(Omotoso & Adedire, 2007). The high protein solubility at high pH could be attributed to unfolding of the protein structure hence exposing hydrophilic groups hence promoting protein solubility (Yu *et al*., 2007).

Additionally the trend in protein solubility could be attributed to the prevalent charge of the amino acids at different pH regimes where low solubility was observed at the isoelectric point which was caused by reduced repulsion among the amino acids Protein solubility at a wider pH range shows the potential of utilization of *Acheta domesticus* protein concentrates in formulation of different food products (Lawal *et al.,* 2004). The solubility of proteins is governed by the amino acids composition and also by pH. The acidic amino acids ionize at high pH hence enhancing solubility (Alsohaimy *et al.,* 2007). The hydration and extent of hydrophobicity influence the solubility of proteins (Sathe & Salunkhe, 1981). Denaturation of proteins potentially results to decreased solubility of proteins as a result of increased exposure of the hydrophobic groups specifically in conditions of low pH (Oshodi & Ojokan, 1997). Additionally the isoelectric point play a critical role in influencing the solubility of the protein extracts.

4.6 Effect of NaCl and pH on water holding capacity of the protein concentrates

The water holding capacity of the protein concentrates was constant from 0% to 4% NaCl concentration followed by a decline upon increase of NaCl concentration up to 12% NaCl concentration (Figure 4.2).

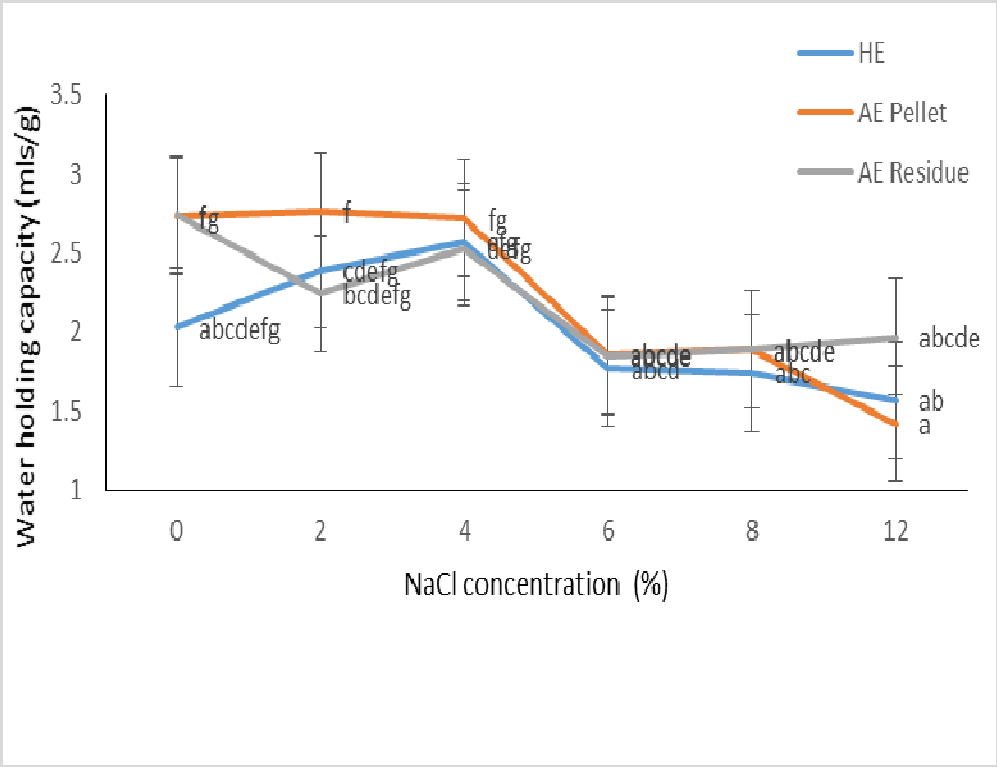

Figure 4.2: Effect of NaCl concentration on water holding capacity of the protein concentrates (n =3)

Equally Lawal *et al.,* (2004) observed a decline in water holding capacity of African locust bean protein isolate with increase in NaCl concentration, this observation could be attributed to the NaCl ions bounding more water and hence resulting to dehydration of the protein molecules thus reducing the water holding capacity. The trend could also be attributed to denaturation of the proteins at high salt concentration hence decrease in water holding capacity (Thorarinsdottir *et al.,* 2001). In addition the influence of NaCl on water holding capacity can be explained by the impact of salt on the protein- protein interaction (Langton & Hermansson, 1992). Particle size has been shown to have an influence on water holding capacity (Sangnark & Noomhorm, 2003). Therefore the observed unique trend observed in AE residue could be attributed to particle size difference since the residue had potential bigger particles since it was residue of the sieving process that used a 500 µm sieve.

pH had a significant influence on the water holding capacity of the protein concentrates (P= 0.0246) (Figure 4.3).

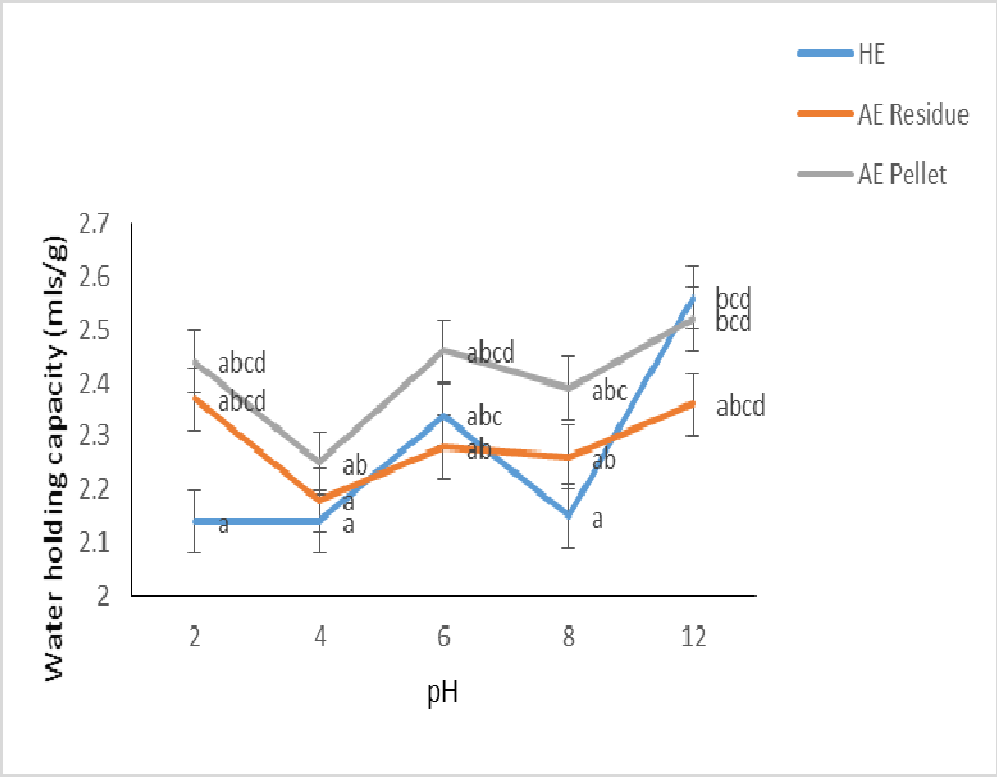

Figure 4.3: Effect of pH on water holding capacity of the protein concentrates (n =3)

The highest water holding capacity of the protein concentrates being observed at pH 12 and the least at pH 4. Similarly in meat proteins low water holding capacity was observed near the isoelectric point (pH 5) and tended to increase with either increase or decrease in pH. The considerably high water holding capacity at pH 2, pH 6 and pH 12 could be associated to increase in electrostatic repulsion and that created more space to hold more water molecules (Offer & Knight, 1988). The low water holding capacity observed at pH 4 could be explained by irreversible denaturation of the protein molecules hydrophilic groups (Wu *et al.,* 2009).

4.7 Effect of NaCl on emulsion capacity and emulsion stability of the protein concentrates

There was a general decrease in emulsion capacity with increase of NaCl concentration up to 6% followed by an increase with continuous increase in NaCl concentration (Figure 4.4).

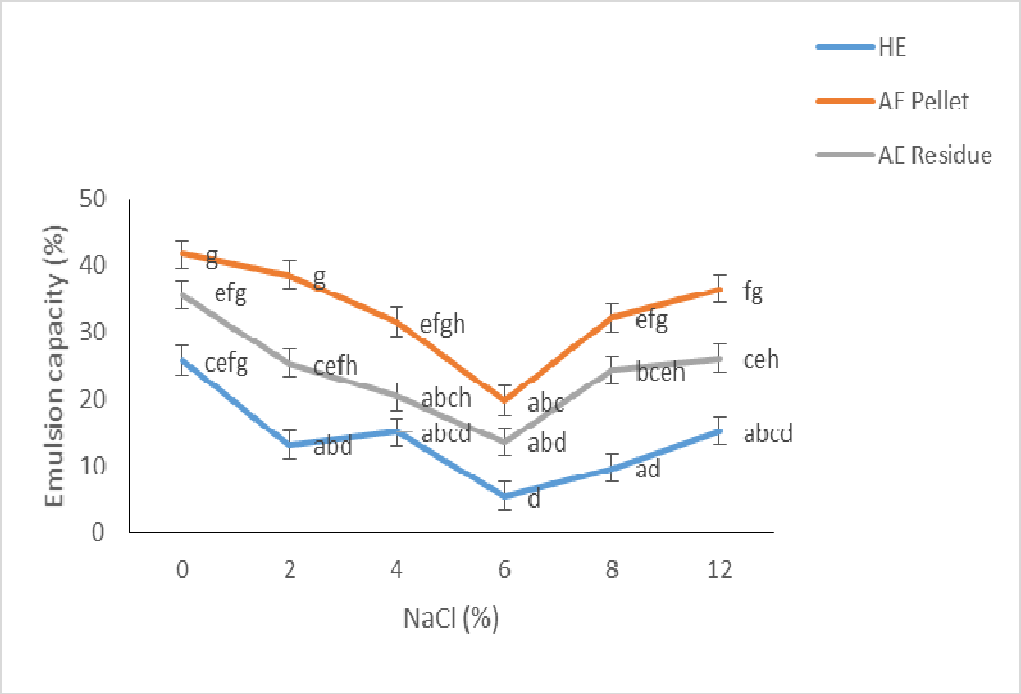

Figure 4.4: Effect of NaCl on emulsion capacity of protein concentrates (n =3)

Adeyeye, (2008) observed a decline in emulsion capacity of Silkworm larvae from 20% to 0% with the increase in NaCl concentration. Similarly Babiker *et al.,* (2007) observed a decline in emulsion activity of tree locust meal with increase in NaCl concentration. AE Pellet showed significantly high emulsion capacity compared to the other protein concentrates at 0%, 2%, 4%, 8% and 12% NaCl. Increase in protein concentration has been associated with increase in emulsion capacity of protein concentrates upto a certain limit above which emulsion capacity reduces due to accumulation of proteins in the aqueous phase (Lawal *et al.,* 2004). Therefore in this study the protein concentration of AE Pellet was the optimum concentration at which the best emulsion capacity could be achieved.

The effect of NaCl on emulsion stability of the protein concentrates is as shown in Figure 4.5.

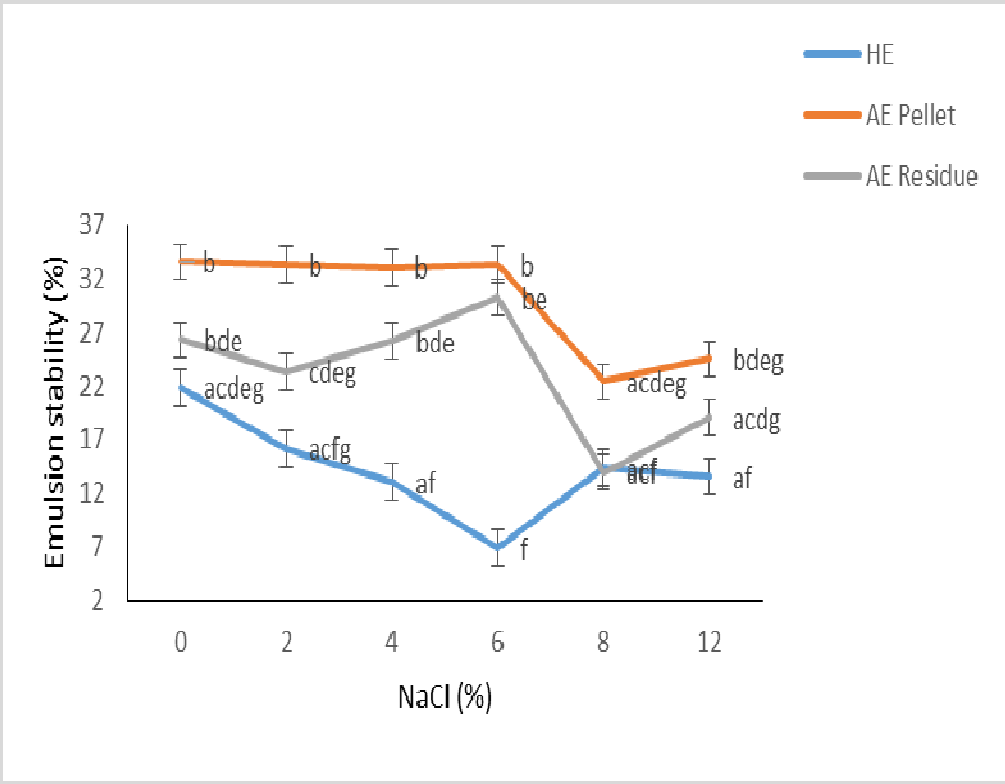

Figure 4.5: Effect of NaCl on emulsion stability of protein concentrates (n =3)

The AE Pellet exhibited considerably higher emulsion stability at 2% NaCl. Therefore AE Pellet was superior in emulsion stability in the NaCl concentration range. This could be attributed to the fact that emulsion activity is dependent on particle size difference (Sangnark & Noomhorm, 2003). The observed difference in trend among the protein concentrates could potentially be attributed to methods used in preparation.

4.8 Effect of pH on emulsion capacity and emulsion stability of the protein concentrates

pH significantly affected emulsion capacity and emulsion stability of the protein concentrates (P< 0.0001) (Figure 4.6).

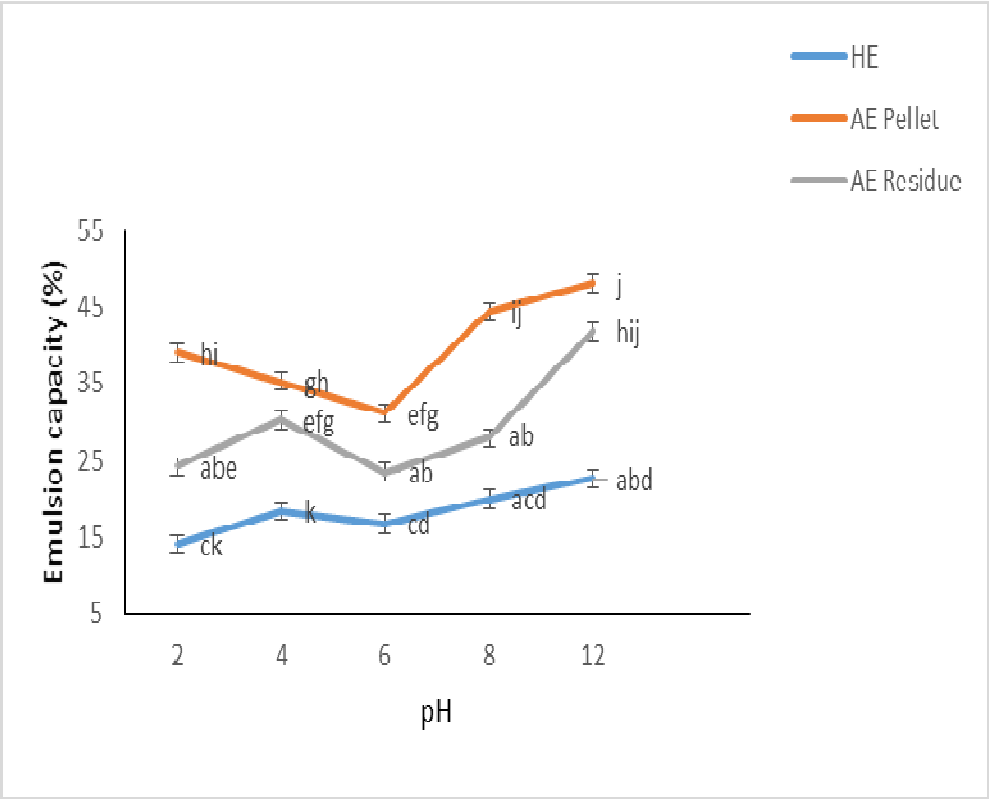

Figure 4.6: Effect of pH on emulsion capacity of the protein concentrates (n =3)

The highest emulsion capacity was obtained at pH 12 with AE Pellet and AE Residue recording the highest values while the least emulsion capacity was recorded at pH 6. Similarly defatted cashew nut exhibited high protein solubility and emulsion capacity at pH 12 and least at pH 4 (Ogunwolu et al., 2009). This trend could be explained by the observation that protein solubility was highest at pH 12 and the lowest at pH 6 (Figure 4.1). Potentially at pH 6 there could be less electrostatic repulsion forces of the protein molecules near the isoelectric point and therefore the proteins coalescenced and precipitated hence the low emulsion capacity (El Nasri & El Tinay, 2007). Additionally the observed low emulsion capacity at pH 6 could be explained

by the determination that protein adsorption at the oil water interface was diffusion controlled (Wu *et al.,* 2009).

Emulsion stability of HE seemed to have not been affected by pH however emulsion stability was highest at pH 2, 4 and 12 with AE Pellet having the highest values (Figure 4.7).

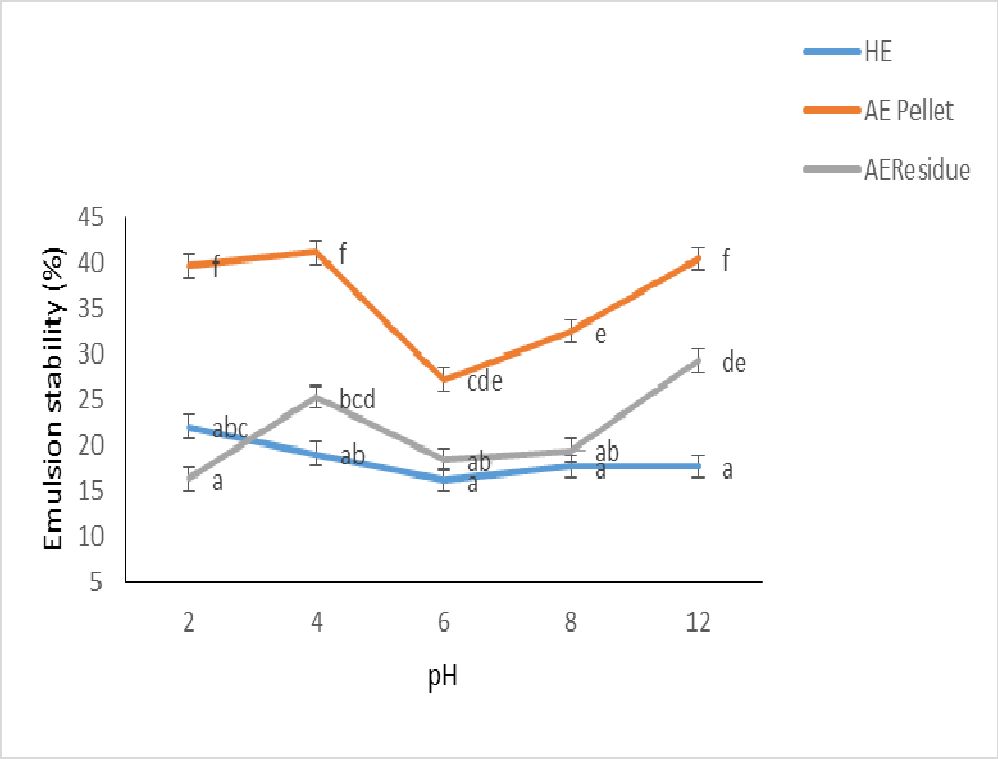

Figure 4.7: Effect of pH on emulsion stability of the protein concentrates (n =3)

AE Pellet showed similar results with fresh tree locust where the least emulsion stability (25.66%) was recorded at pH 6 with remarkable increase in emulsion stability at pH below and above pH 6. The trend could be explained by formation of charged layers around the fat globule resulting to mutual repulsion (Wu *et al.,* 2009). On the contrary highest emulsion stability was observed at pH 6 in boiled tree locust (Magzoub, 2015). The low emulsion stability observed at pH 6 could be presumably be due to increased protein-protein interaction (Lawal *et al.,* 2004).

4.9 Effect of NaCl on foaming capacity and foam stability of the protein concentrates

Foaming capacity of HE and AE Pellet was improved up to 4% NaCl, decreased at 6% NaCl, increased at 8% NaCl and finally decreased at 12% NaCl (Figure 4.8).

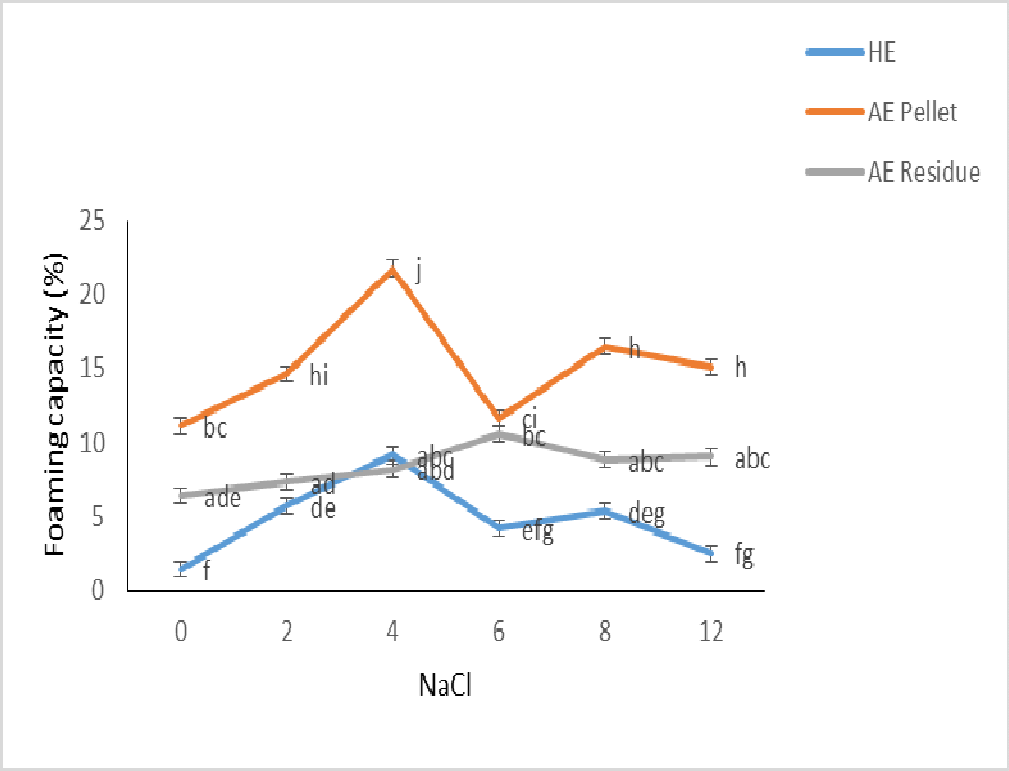

Figure 4.8: Effect of NaCl on foaming capacity of the protein concentrates (n =3)

Evidently the foaming capacity of the protein concentrates was significantly affected by NaCl ($P<0.0001$) with AE Pellet recording the highest foaming capacity at 4% NaCl. Similarly Babiker *et al.,* (2007) observed an increase in foaming capacity of tree locust meal with the addition of NaCl up to 0.4M. Similarly the foaming capacity of fried tree locust increased with increase of NaCl up to 0.4M followed by a decrease (Magzoub, 2015). The observed increase in foam capacity up to 4% NaCl could be as a result of enhanced protein solubility, diffusion and spreading at the air water interface by NaCl (Akintayo *et al.,* 1999). The initial increase in foam capacity

followed by a decrease could be explained by enhanced protein solubility and dispersion that enhanced foam capacity at first and occurrence of hydrophobic interaction between the protein molecules that reduced the flexibility of the protein molecules and consequently reduced the foam capacity (Lawal, 2004). The decrease in foaming capacity at 12% NaCl concentration could be attributed to the salting effect of NaCl (Idris *et al.,* 2003).

There was difference in foam stability among the protein concentrate. The foam stability of HE and AE Residue was not affected by NaCl concentration while the foam stability of AE Pellet was significantly affected by NaCl concentration ($P<0.0001$) (Figure 4.9).

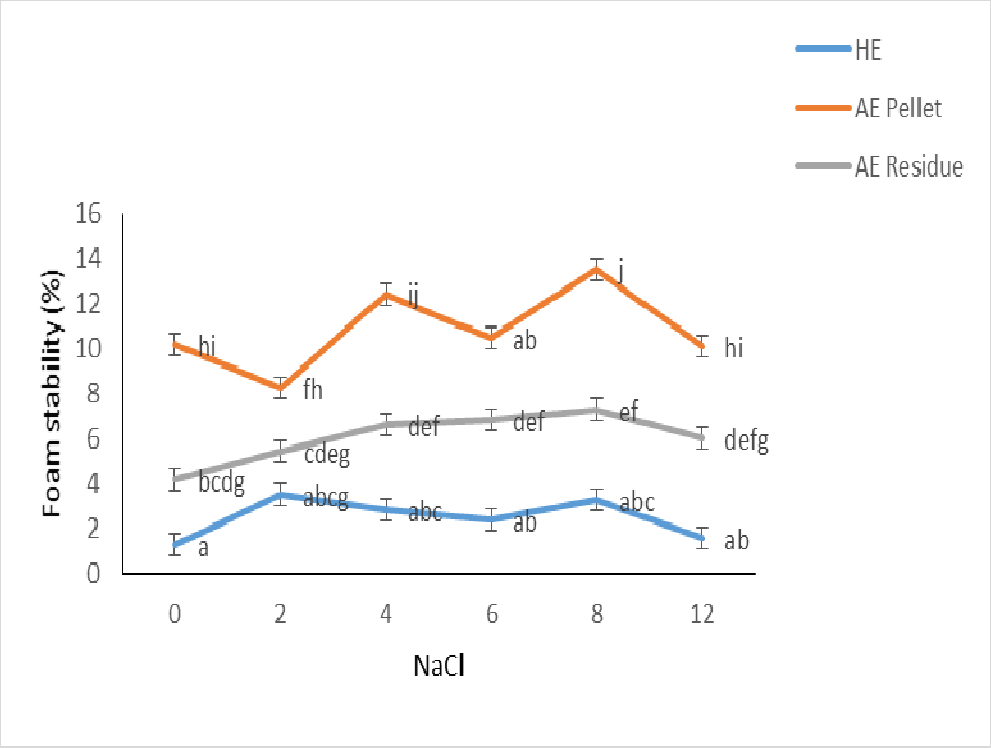

Figure 4.9: Effect of NaCl on foam stability of the protein concentrates (n =3)

The highest foaming stability was recorded in AE Pellet at 4% and 8% NaCl concentration. This is consistent with the results of foaming capacity (Fig 8) where

higher values were obtained at 4% and 8% NaCl. Similarly Wu *et al.,* (2009) observed that the foam stability of peanut protein product was enhanced with addition of NaCl concentration. The above observation could be attributed to enhanced protein to protein interactions that strengthened the foams (Damodaran & Kinsella, 1982).

4.10 Effect of pH on foaming capacity and foam stability of the protein concentrates

Foaming capacity improved up to pH 4 followed by a decline up to pH 8 and then an increase at pH 12. The foaming capacity of the protein concentrates differed at all the pH values with AE Pellet showing the highest values (Figure 4.10).

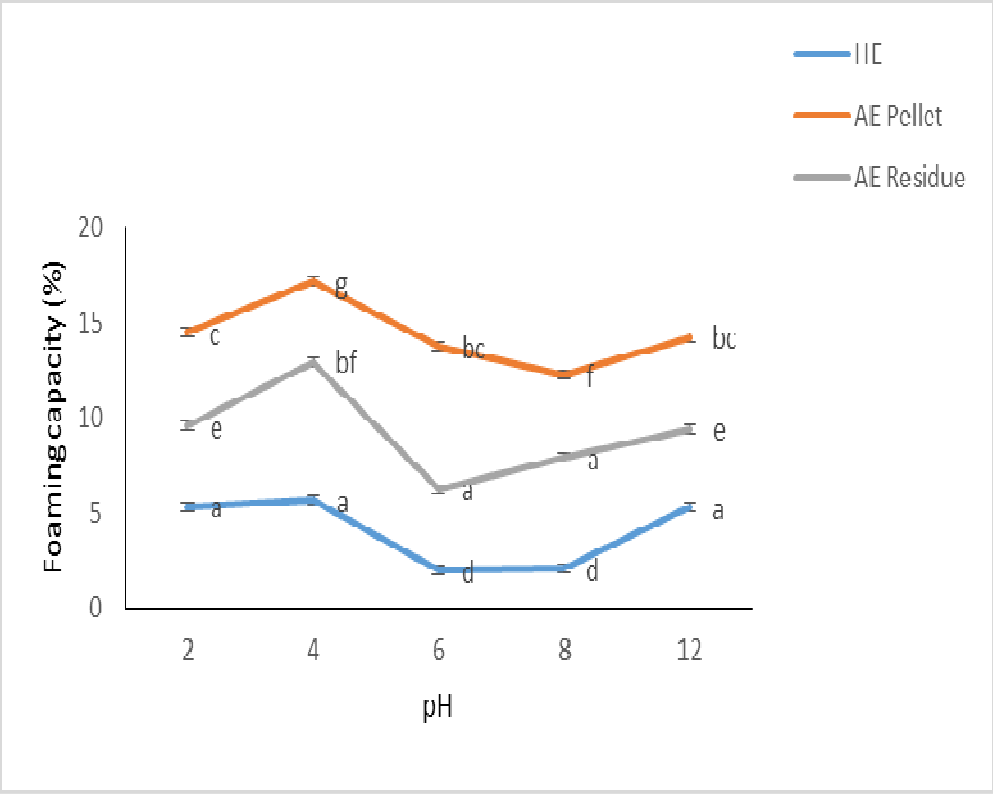

Figure 4.10: Effect of pH on foaming capacity of the protein concentrates (n =3)

All the protein concentrates demonstrated high values for foaming capacity at pH 4.The observed trend could be explained by an increase in net charge of the protein

molecules that resulted to weakening of hydrophobic interactions and increased protein flexibility (Ragab *et al.,* 2004), therefore the protein molecules were able to diffuse faster to the air water interface to encapsulate more air particles thus enhancing foaming capacity (Aluko & Yada, 1995).

The foam stability of the protein concentrates also differed at all the pH levels with AE Pellet having the highest values. The highest values for foam stability were observed at pH 4 among all the protein concentrates (Figure 4.11).

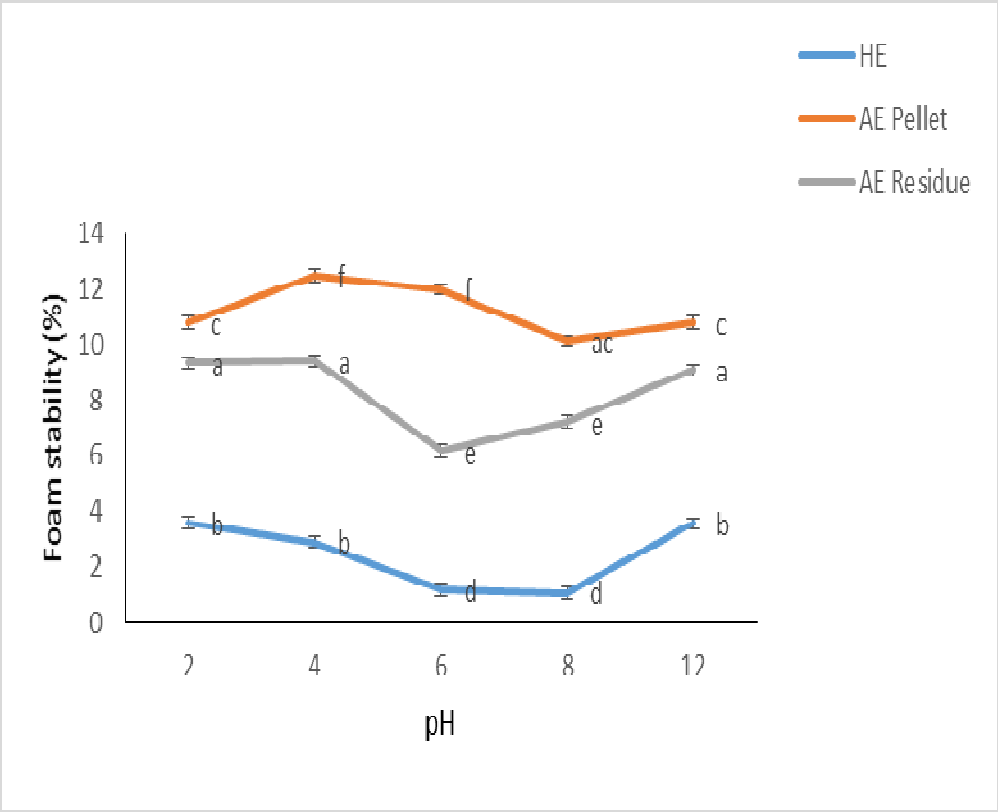

Figure 4.11: Effect of pH on foam stability of the protein concentrates (n =3)

Similarly high foam stability was recorded at pH 4 in protein concentrates of Bambarra groundnut (Lawal *et al.,* 2007). The high foam stability at pH 4 could be attributed to enhanced protein adsorption and viscoelasticity at air water interface, (Lawal *et al.,* 2007). Additionally the high foam stability at pH 4 could be explained

by enhanced protein solubility and surface activity of the soluble proteins (Ragab *et al.,* 2004).

4.11 Effect of NaCl and pH on least gelation concentration of the protein concentrates

Gelation was observed in the 30% AE Pellet at all the NaCl concentrations. At a concentration of 20% w/v none of the protein concentrates gelled, however there was formation of large aggregates (Table 4.5).

Table 4.5: Effect of NaCl on least gelation concentration of the protein concentrates.

NaCl	2%		4%		6%		8%		12%	
	20%	30%	20%	30%	20%	30%	20%	30%	20%	30%
HE	A	V	A	A	A	A	A	A	A	A
AE Pellet	A	O	A	O	A	O	A	O	A	O
AE Residue	A	O	A	A	A	A	A	A	A	A

Key

A: Aggregation, X: No gelation, V: Viscous fluid, O: Gelation

Yi *et al.,* (2013) concluded that protein concentration has significant effect on gelation and this explains why the least gelation concentration of the protein concentrates was 30%. The least gelation concentration could be affected by NaCl as increase in ionic strength has been shown to increase the least gelation concentration of African Locust bean protein isolate from 8% to 16% (Lawal, 2004). The observed gelation could also be attributed to enhanced protein solubility by NaCl which promotes overlapping of functional groups of adjacent protein molecules which is necessary for gel formation (Akintayo *et al.,* 1999).

Least gelation was also affected by pH with gelation occurring at pH 4, 6, 8 and 12 for AE Pellet. However weak gels designated as "viscous fluid" was observed in HE and AE Residue at pH 6 and pH 12 respectively (Table 4.6).

Table 4.6: Effect of pH on least gelation concentration of the protein concentrates

pH	2		4		6		8		12	
	20%	30%	20%	30%	20%	30%	20%	30%	20%	30%
HE	A	A	A	A	A	V	A	A	A	A
AE Pellet	A	V	A	O	X	O	X	O	X	O
AE Residue	A	A	A	A	A	A	A	A	A	V

Key

A: Aggregation, X: No gelation V: Viscous fluid, O: Gelation

Gelation occurred at 30% of the protein concentrates. Similarly gelation was observed in 30% *Acheta domesticus* protein concentrates at pH 7 and pH 10 (Yi *et al.,* 2013). This demonstrated that protein concentration and pH had a significant influence on gelation. The least gelation concentration of the *Acheta domesticus* protein concentrates was slightly higher than 0.5% to 25% which is the common concentration range for gels (Yi *et al.,* 2013). This implies that one will need higher amounts of the protein concentrates to form gels. Consequently this could have a cost implication however the protein concentrates shows potential of being utilized as gels. Gelation results to desirable texture of food product. Gelation is usually affected by pH, salt concentration, protein concentration and thermal treatment (Yi *et al.,* 2013).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the study it is clear that the extraction methods had an effect on the physico - chemical characteristics and functional properties of the protein concentrates. However HE exhibited more desirable results for yield, colour, crude protein, crude ash and available carbohydrate. The hexane extraction was very effective compared to aqueous extraction. On the other hand aqueous extraction demonstrated better emulsion capacity, emulsion stability, foaming capacity and foam stability.

Protein solubility, water holding capacity and emulsion capacity of the protein concentrates was optimum at pH 12 with AE Residue recording the highest value for protein solubility and AE Pellet having the highest value for emulsion capacity. NaCl significantly affected the water holding capacity of the protein concentrates. Consequently NaCl resulted to a decrease in emulsion capacity and stability of the protein concentrates. Foaming capacity was best at 4% NaCl with AE Pellet showing the highest values. Additionally foaming capacity and stability were at maximum at pH 4. Gelation was observed in AE Pellet at 30% concentration in the various NaCl and pH regimes.

Therefore, it is evident that the protein concentration method, NaCl, pH and protein concentration had significant effects on the functional properties of the protein concentrates. The considerable protein solubility at wide pH range shows the suitability of utilization of AE Pellet in formulation of wide range of products. pH 4 and 6% NaCl were optimum for foaming capacity and stability however the values were considerably lower than those of commonly used foaming agents therefore none of the protein concentrates was suitable to be utilized as a foaming agent. The least gelation concentration of AE Pellet was slightly higher than the range of common gels however there is still potential of utilization as a gelling agent.

5.2 Recommendations

I recommend:-

1. That food products from edible insects protein concentrates be developed and evaluated.
2. That economic feasibility study of the two extraction procedures be conducted.
3. Further purification and biochemical analysis of edible insect proteins.

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