PHYSICOCHEMICAL AND MICROBIOLOGICAL STABILITY OF SEMI-PROCESSED EDIBLE CRICKETS (*Acheta domesticus*) AND BLACK SOLDIER FLY LARVAE (*Hermetia illucens*) DURING STORAGE

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Physicochemical and Microbiological Stability of Semi-processed Edible Crickets (*Acheta domesticus*) and Black Soldier Fly Larvae (*Hermetia illucens*) during Storage

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my father, Alexander Wainaina and the rest of my entire family whose constant love and support has always motivated me to set higher targets for myself.

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ABBREVIATIONS

BSFL	Black soldier fly larvae
FAO	Food and Agricultural Organization
IV	Iodine Value
MUFA	Monounsaturated Fatty Acid
P-AV	P-anisidine Value
PE	Polyethylene
PL	Plastic
PP	Polypropylene
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
SFA	Saturated Fatty Acid
SV	Saponificaion Value
TVC	Total Viable Count
GAB	Guggenheim Anderson De Boer model
BET	Brannuer Emmet Teller model
A	Khun model constant
B	Khun model constant

C_b Enthalpy of sorption of monolayer

C_g Enthalpy of sorption of monolayer

K_g Enthalpy of sorption of multilayer

ABSTRACT

Edible insects are a dietary source of proteins for a significant part of rural population in Africa and Asia. Just like any other type of food, edible insects during storage are prone to microbial, chemical and physical changes influenced by both extrinsic and intrinsic factors. In this study the effect of storage time, storage environment and type of packaging were examined. Chemical, microbial and physical changes were all monitored during storage through commonly employed analyses including; peroxide value (PV) , p-anisidine value (P-AV), saponification value (SV), iodine value (IV) and fatty acid analyses for lipid oxidation and total viable count (TVC), yeasts and moulds, Enterobacteriaceae, *E.coli* and *Salmonella* counts for microbial stability. Moisture adsorption isotherms were also determined gravimetrically to help identify critical moisture contents of storage that can help optimize and predict the shelf-life of the semi-processed insects. Black soldier fly larvae (BSFL) and adult house cricket collected from specific rearing sites were boiled, solar dried, ground into powder and packaged in polypropylene (PP), plastic (PL) and polyethylene (PE) packages. These were then stored in either ambient or refrigerated conditions for a period of 6 months where by quality assessments were done after every 45 days. Temperature and relative humidity in the trial sites were monitored using a data logger. The PV, P-AV and SV increased ($P<0.05$) overtime while IV decreased ($P<0.05$) overtime in all the samples regardless of packaging treatment and type of storage environment. The changes were however significantly higher ($P<0.05$) in samples stored at ambient conditions than the refrigerated samples. The PV, P-AV and SV of both samples in all the sampling stages were in the order of PP>PE>PL while IV was in the order of PL>PE>PP. The polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids decreased overtime while the saturated (SFA) fatty acids increased. This was in the order of PP>PE>PL for both insects in both storage environments. All the microorganisms detected at the baseline level also showed a general significant increase ($p<0.05$) during storage. Yeasts and moulds were not detected at the baseline level in both insect species but were present in all the other subsequent sampling periods. Salmonella was not detected in the

adult house cricket but was present in BSFL. Three types of fungi; *Aspergillus spp.*, *Alternarie spp.* and *Penicillium spp.* were isolated in all the packages during storage in both insect species. Microbial counts among the different packages was in the order PP>PE>PL. The BSFL sample showed a sigmoidal form of degradation where by most drastic changes were seen at 45-90 days and 135-180 days while in cricket most drastic changes were seen in the period 0-45 days. The moisture adsorption isotherms were of type II according to Brunauer classification and the sorption capacities decreased with increasing temperatures. Cricket had a higher sorption capacity whereas BSFL was more sensitive to temperature changes. Transition from bound to free water in both insect sample begins at approximately 5 g/100 g. Shelf life of up to one year can be achieved if the cricket and BSFL powders are dried to approximately 6.5 g/100 g and 6 g/100 g respectively and packaged in 80µm bags. The results suggest that when the semi-processed insect powders are stored in packages with low water vapour and gas permeability and at lower temperatures, degradation during storage is reduced and a longer shelf life can be achieved. Measures should be put in place to avoid contamination during processing, packaging and storage so as to achieve a longer shelf life.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The world population is growing at an alarming rate and it is estimated that by the year 2050 the planet earth will be home to about 9 billion people (Van Huis et al., 2013). This implies that food production will have to be almost double the current production so as to be able to feed everyone (Van Huis et al., 2013). The resources that are currently being used in food production are already under stress and are impacting negatively on the environment (Klunder et al., 2012; Van Huis et al., 2013). There has been a general decline in the production of agricultural produce and meat and meat products (Dinar and Mendelsohn, 2011). In order to be able to satisfy the rising food demand currently and in the future, there is need to re-evaluate what we eat and how we produce it. Edible insects can be an alternative and reliable source of food.

Edible insects are widely consumed in different parts of the world including Asia, Africa and America (Committee, 2015; Klunder et al., 2012; Obopile & Seeletso, 2013; Van Huis et al., 2013). Consumption of insects both as human food and feed for animals has been around for millennia with some of the earliest citings being found in biblical literature (Van Huis et al., 2013). There are about 1800 species of insects that are used for human consumption globally (Jongema, 2011). Evidence from different researchers reveal that insects are rich in fat, protein and vitamins although this is highly variable within and among different species (Braide et al., 2011; Esther et al., 2015; Kinyuru & Kenji, 2010; Obopile & Seeletso, 2013; Van Huis et al., 2013). Their nutrient content is comparable to that of conventional livestock. Apart from nutritional benefits, insects have other merits including; lower greenhouse gas emissions, higher reproduction rate than conventional livestock, require lesser space for breeding, and have a higher feed conversion rate (Ramos-Elorduy, 2008; Wilkinson, 2011)

Despite the relatively widespread consumption and utilization of insects, information and research regarding the food safety aspect of both processed and raw insects during storage remains limited. In Kenya, entomophagy is dominant in the western part of the country although in small-scale (Kinyuru & Kenji, 2010). In order to improve insect palatability, the local communities subject these insects to partial processing such as boiling, sun drying and toasting (Ayieko & Oraria, 2008; Kinyuru & Kenji, 2010) after which they are packaged in different types of packages including; plastic containers, polyethylene bags, polypropylene bags and metallic cans. With lack of proper packaging materials and storage conditions, any surplus tends to be wasted due to deteriorative effects. There is limited information on insects' shelf life with regard to changes in nutritional, chemical and microbial quality upon storage, hence the need for this research.

1.2 Problem statement

Lack of appropriate preservation and storage facilities are one of the major factors that contribute to food shortages in Africa. Many farmers lack knowledge on the most appropriate methods in preserving their products. Storage facilities that have incorporated modern technology like freezing are not available resulting in colossal postharvest losses. There has been a steady increase in the world population coupled with economic growth and urbanization that has led to an upsurge in the demand of quality protein. Multitudes of people have moved into the middle class and can afford quality protein. This has resulted in a drastic upsurge in demand for quality protein that is proving difficult to satisfy. Protein energy malnutrition is one of the most common problems in many developing countries necessitating the need for various interventions to ameliorate the situation. Insects are widely consumed and evaluated as food supplements in many regions in the world. Nevertheless little attention has been given to their storage-stability and shelf life under different storage conditions. In the western Kenyan region where entomophagy is dominant, insects are rapidly consumed after harvesting rarely being stored for more than a week due to deterioration of quality. Commercialization of the insects where large masses need to be accumulated for

production in both the food and feed industry has been difficult due to lack of appropriate preservation and storage conditions.

1.3 Justification of the study

Edible insects are a prime source of essential nutrients that lead to improved health. These insects are rich in proteins, fat and minerals (Van Huis 2003). There is need to safe guard quality protein and ensure its access to consumers throughout the year (Van Huis 2003). This is especially important to continents like Africa where there is a rise in under nutrition and chronic malnutrition among children under the age of five years (Virginia et al. 2011).

Nutrients from insects can be obtained by consuming them directly or by consuming animals fed on these insects. Small-scale rearing of the edible insects which is currently being practiced can cater for household consumption. Large-scale rearing of insects can cater for both household and animal needs. This can also help reduce the rising cost of animal feed. With a shift to large-scale mini-livestock rearing for both food and feed, there is need to research on appropriate packaging materials and storage conditions that can be applied so as to maintain the nutritive quality of the insects over a long period of time. Insects have a high microbial instability after semi-processing (Klunder et al., 2012). There is also need for research on how to minimize any microbial contamination throughout the storage period.

1.4 Objectives

1.4.1 Main Objective

To assess the physicochemical and microbial stability of semi-processed adult house crickets and black soldier fly larvae flours during storage

1.4.2 Specific objectives

i. To determine the influence of different storage temperatures (conditions) on the chemical, nutritional and microbial quality of semi-processed insects
ii. To evaluate the effect of different packaging materials on the nutritional, chemical and microbial quality of semi-processed insects.
iii. To establish the moisture sorption characteristics of the semi-processed insects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Entomophagy

Entomophagy is the practice of using insects as part of the human diet (Van Huis et al., 2013). Usage of insects as either food/feed has been ongoing for millions of years with the earliest citing being found in biblical literature (Committee, 2015; Van Huis et al., 2013). Utilization of insects since ancient times until now is not surprising since their population on earth is superior to that of any other species and thus they represent a significant amount of biomass (Esther et al., 2015; Van Huis et al., 2013). Out of the 1.4 million species that have been described, about 1 million of these species are utilized in different parts of the world including Asia, South and Central America and Africa (Committee, 2015; Esther et al., 2015; Van Huis et al., 2013). Existing literature shows that insects are consumed all over the world as shown in Table 2.1. Some of the most commonly consumed insects include locusts, termites, beetles, termites, cicadas, grasshoppers, weevils and various caterpillars (Akinnawo & Ketiku, 2000). Contrary to popular beliefs insects are not 'famine' foods eaten in times of drought or when purchasing food becomes difficult, many people actually eat them out of choice due to their palatability and established place in local food cultures (Akpossan & Due, 2015). Some people such as the Yukpa of Colombia and Venezuela and the Pedi of South Africa prefer certain traditional insects to fresh meat (Obopile & Seeletso, 2013).

Table 2.1: Number of edible insects per continent and number of consuming countries

Continent	Number of species recorded	Percent of total	Number of consuming countries
Asia	349	20	29
Australia	152	9	14
Africa	524	30	36
Americas	679	39	23
Europe	41	2	11
Total	1745	100	113

The world total is actually 1681; some species occur in more than one continent, hence the higher total. Source; Ramos-Elorduy (2005)

Rearing and utilization of edible insects can simultaneously address environmental, socio-economic and health concerns thus making insect farming for food and feed an attractive and very timely opportunity. Rearing of insects as an alternative food/feed source has a couple of advantages including; insect rearing emits less greenhouse gases and ammonia as compared to cattle and pig farming, has a lesser environmental footprint than meat and requires less land. Insect rearing can be done in small scale even in the urban centers. It requires little startup capital and therefore even the poor can take part and improve their living standards (DeFoliart, 1997; Klunder et al., 2012).

2.2 Insects as food and feed

Currently promotion of insects as food for humans and feed for livestock has been recognized by a wide range of organisations including Food and Agricultural Organization of the United Nations, academics, the private sector and even the media (DeFoliart, 1997). Insects are in many ways a basic component of the diet of humans and animals (Esther et al., 2015) and they are available in high quantities without the risk of extinction.

The world population is increasing at an alarming rate and the pressure of producing adequate amount of proteins for everyone with shrinking resources is being felt now more than ever thus necessitating the need for an alternative protein source (Van Huis et al., 2013). Global food production must increase if we are to feed the growing population. However this might not be achieved if radical food production technologies are not introduced in our systems. Despite the natural resources being more and more limited e.g. to feed the world in 2014 required us to dedicate 40% of world ice free land to agriculture. 70% of the total agricultural land was used for grain production and 30 % of the grains that were produced were fed to farm animals. Agriculture in 2014 consumed 70% of fresh water and was responsible for 14 to 17% of greenhouse gas emissions. To achieve global food security it is very crucial to be able to produce more animal proteins for the growing populations more efficiently and with minimal environmental impact. Farming insects on organic wastes presents viable alternatives (DeFoliart, 1997)

The Food and Agricultural Organization (FAO) reported that an estimated 870 million people were undernourished in the period 2010-2012 indicating that about 12.5% of the global population or one in eight people were affected. This occurred despite global increase in food production over the past years. Undernourishment is more pronounced in Sub-saharan Africa where 27% of the people are undernourished (Durst et al., 2013). To reduce the amount of undernourished people globally, there is need to look at edible insects as a healthy nutritional source for both humans and animals. Insects have basically always been in our diets either directly or indirectly i.e. insects are already part of the natural diets for farm animals such as free roaming chickens, pigs and carnivore fish (Van Huis et al., 2013) which we then slaughter and eat.

Edible insects as a healthy food need to be integrated into the agenda of many food agencies and this will require a more comprehensive understanding of the nutritional value of the insects in particular impacts to consumers health, food safety, environmental impact and risk assessments of using the insects in the food chain. National and international poverty alleviation agencies need to be made aware of the

usage of insects as an alternative protein source. Policy makers and legislators in different countries worldwide are called upon to include insects in the policy and legal frame works covering the food, health and feed sectors (Van Huis et al., 2013).

2.3 Cricket

Crickets are insects that belong to the insect order Orthoptera. They are black or brown in color and have two sets of wings and a long antenna (Legendre et al., 2010). The house cricket (*Acheta domesticus*) belongs to the Gryllidae family (Bello et al., 2013). The house cricket is a cosmopolitan omnivorous insect (Parajulee et al., 1993) which is mostly preferred compared to other cricket species due to its soft body. It is reared and consumed as human food in countries such as Thailand, Cambodia and Kenya (Ayieko et al., 2016; Van Huis et al., 2013). Out of the known 900 cricket species in the world, the most common species eaten in the western part of Kenya are *Acheta domesticus* and *Gryllus bimaculatus* (Ayieko et al., 2010). The adult house cricket contains about 64.38% protein, 13.41% fat, 9.55% fibre and 3.85% ash (Rumpold & Schluter, 2013). The crickets can also be incorporated in modern products such as muffins (Ayieko et al., 2010) to ease in their consumption for those who cannot consume them directly.

2.4 Black soldier fly larvae

The black soldier fly, *Hermetia illucens (Linnaeus)* is a common fly belonging to the Stratiomydae family (Park, 2015). Its life cycle has five stages including egg, larvae, prepupal, pupae and adult (Alvarez, 2012). Once the adult lays eggs they remain in this stage for about 4.5 days after which they hatch and the larvae are formed. The larvae immediately start to seek whatever waste they can find and consume it (Alvarez, 2012; Park, 2015). The larvae stage of the black soldier fly is what many scientists are interested in due to its exemplary ability to convert feedstuff/manure into valuable biomass that is nutritionally rich. The larvae is able to feed and digest wastes such as municipal solid waste, fish renderings, waste water treatment among others (Alvarez, 2012). Analysis of the larvae has revealed that it contains about 42.1% protein, 34.8%

lipids and 14.6% ash which can be manipulated based on the diet its feeding on (Park, 2015).

Fish farming industry provides about 45% of sea food consumed worldwide but in the recent past has been facing problems due to reducing amount of feed protein (Alvarez, 2012). Through economic evaluations it has been ascertained that the black soldier fly larvae can help lower the costs of feeds which account for about 40-50% of total operational costs (Park, 2015).

2.5 Nutritional value of insects

Insects contain essential amounts of nutrients that are required by both animals and humans. Many studies have shown that insects are highly digestible and contain considerable amounts of high quality protein, fat, vitamins and minerals especially iron and zinc (Akinnawo & Ketiku, 2000; Ekpo et al., 2009; Esther et al., 2015; Opara et al., 2012; Van Huis et al., 2013). It has been documented that insects have the same amino acid requirements as man and therefore they actively accumulate these nutrients which are readily available for utilization by man (Ekpo et al., 2009). Insects are an important dietary component in numerous developing countries. They can provide a good source of protein, minerals, vitamins and energy to poor rural communities which compares favourably well with that of commonly consumed meat (Table2.2) and thus help reduce incidences of malnutrition (Obopile & Seeletso, 2013). Womeni et al. (2009) found that these insects were rich in polyunsaturated fatty acids and frequently contained the essential linoleic and linolenic fatty acids that are fundamental for the healthy growth and development of children and infants (Akpossan & Due, 2015). Structural analysis of unsaturated fatty acids reveal that they are mainly palmitoleic, oleic, linoleic and linolenic acids (Ekpo et al., 2009). Insects also contain other substances such as antibacterial enzymes and hormones that are good for human health (Durst et al., 2008).

The nutritional value of edible insects varies due to the wide variety of species (Van Huis et al., 2013). Furthermore, nutritional variation can also be present within the same species of insects. This variation is dependent on the metamorphosis stage as seen in insects with complete metamorphosis such as ants, bees and beetles. The habitat and the diet of the insects also influences the nutritional value. In addition, processing and preparation methods such as boiling, frying and drying will greatly influence the nutritional value of the edible insects (Van Huis et al., 2013).

Table 2.2: Comparison of average proteins between insects and commonly consumed meat

Animal group	**Species and common names**	**Edible part**	**Protein content (g/100g fresh weight)**
Insects (raw)	Locusts and grasshoppers: *Locusta migratoria, Ruspolia differens*)	Larva	14-18
	Silk worm (*Bombyx mori*)	Caterpillar	10-17
	Palmworm beetles: *Rhynchophorus palmarum*, *Rhynchophorus phoenics*	Larva	7-36
	Crickets	Adult	8-25
	Termites	Adult	13-28
Cattle		Beef (raw)	19-26
Fish (raw)	Finfish	Tilapia	16-19
		Mackerel	16-28
		Catfish	17-28

Source: Van Huis et al., 2013

2.6 Processing and packaging of edible insects

Insects intended for human/animal consumption can be consumed as either raw whole insect, processed insect in some powder or paste or as an extract such as a protein isolate (Van Huis et al., 2013). African insects are usually processed to tasty food products which can be immediately consumed or they can be used as flavour intensifiers in soups and stews (Nabayo et al., 2012). Some of the common processes include; removal of appendages, dewinging, degutting, beheading, washing with water to remove any dirt, steaming, boiling, roasting, toasting, deep frying, shallow frying, smoking and sun drying. Examples of some common processing methods applied to some edible insects are as shown in Table 2.3.

Table 2.3: Processing methods of different edible insect species consumed in different countries

Insect	Processing	Reference
Cirina Forda (westwood) larvae	Sun drying	(Akinnawo & Ketiku, 2000)
African palm weevil larvae	Boiling, roasting or frying	(Opara et al., 2012)
long winged termites (*macrotermes subhylanus*)	Toasting and solar drying	(Kinyuru & Kenji, 2010)
edible caterpillar (*Bunae alcinoe*)	Roasting and sun drying	(Braide et al., 2011)
Grasshopper (*Ruspolia differens*)	Toasting and solar drying	(Kinyuru & Kenji, 2010)
Ecosternum delegorguei (edible stinkbug)	Roasting	(Makore et al., 2015)
house crickets	Boiling, solar drying and grounding	(Klunder, et al., 2012)

Packaging enables a food to be transported safely, enable the product to have an extended shelf life by protecting it against harmful microorganisms, contamination and degradation that would otherwise occur (Siracusa, 2003). According to Siracusa, (2003), the most important characteristics for packaging dried foods/feeds are barrier properties to water vapour, oxygen and light in addition to physical strength required to maintain the integrity of the package. Dried products under storage are very sensitive to water (moisture) absorption as this would cause significant changes in texture and quality. Permeation of oxygen or light into the package containing a dried product with a high fat content could induce oxidation leading to rancidity and associated off flavors (Kaleem et al., 2015). Cricket breeders in Vietnam normally package their produce in plastic bags after boiling in water for one minute and thereafter frozen or chilled for transport to the local markets. In Lao People's Democratic Republic (PDR) semi cooked crickets are packaged in plastic bags in quantities of between 0.5-3.0 kg and frozen or chilled for storage, transport and marketing. Vacuum packaging is recommended for product before chilling to extend its shelf life (Van Huis et al., 2013).

2.7 Storage stability of insects

Edible insects like any other type of food are prone to microbial, chemical and physical changes during storage. These changes are majorly influenced by the intrinsic factors like water activity and nutrient content or extrinsic factors such as temperature and humidity. Edible insects are comparable to meat and meat products where the major deterioration mechanisms are microbial growth and oxidation that leads to changes in appearance, rancidity and off flavors (Kilcast & Subramaniam, 2000).

2.7.1 Microbial stability of processed edible insects

Bacteria, yeasts and moulds have been reported in several cases to be the major cause of spoilage in edible insects. These include microbes such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli*, Enterobacteriaceae, bacterial spores, *Proteus mirabilis*, Aspergillus, Mucor, Penicillium and Fusarium (Braide et al., 2011; Klunder

et al., 2012; Mpuchane et al., 2000; Mujuru et al., 2014; Opara et al., 2012). A big percentage of these are soil-borne that contaminate the product during farming. They can also be reintroduced in the products during handling, processing and storage (Braide et al., 2011). Certain strains *Escherichia coli* and Aspergillus are toxin and mycotoxin producers respectively that present health risks when consumed by either humans or animals. *Mucor* species are responsible for food deterioration and eventual spoilage (Braide et al., 2011).

Edible insects are rich in nutrients such as proteins, fats, vitamins and minerals (Rumpold & Schluter, 2013) and thus provide suitable grounds for microbes to flourish and proliferate. A heating step prior to storage was recommended as it was found to be effective in eliminating Enterobacteria but not bacterial spores (Klunder et al., 2012). In Kenya termites are fried in their own fat. In Uganda termites are steamed in banana leaves while the larvae of the *Rhynchophorus phoenicis* are traditionally smoked and sundried in West Africa before consumption or storage (Nabayo et al., 2012). The heating step helps in reduction of microbial flora, water activity and prevents from enzymatic spoilage (Klunder et al., 2012).

Temperature and moisture have to be carefully monitored during storage. The product should be dried preferably to a water activity of less than 0.6 where most microorganisms cannot grow. According to Klunder et al., (2012) cricket samples that were boiled and stored spoilt rapidly due to high water activity Although the total plate count of boiled dried and sautéed dried grasshoppers was seen to increase during a 12 week period, they portrayed microbial stability with a log cfu/g count of less than five (Ssepuuya et al., 2016). Microbial count of dried and ground crickets remained fairly stable during storage at room temperature (Klunder et al., 2012). Storage should be done in suitable packages that can protect the product. Apart from moulds and bacteria, insects were also responsible for quality deterioration of the phane edible caterpillar of the emperor moth (Mpuchane et al., 2000).

2.7.2 Chemical stability

Macro and micro nutrient losses in foods might occur during harvesting and distribution, processing, handling at home or at the industrial level or during storage due to their sensitivity to light, heat, oxygen or a combination of these (Severi et al.,1997). Chemical changes are majorly attributed to reaction between food components or reaction between food and other external agents such as oxygen (Kilcast & Subramaniam, 2000). Edible insects are comparable to meat and meat products where the major chemical deterioration mechanisms are due to oxidation that leads to changes in appearance, rancidity and off flavors (Kilcast & Subramaniam, 2000).

Fat is the second largest nutrient in edible insects having a mean monounsaturated and polyunsaturated fatty acid fraction of about 30.83% to 41.97% and 15.95% to 39.76% respectively in different insect orders (Rumpold & Schluter, 2013). Thus, they are highly susceptible to both oxidative and hydrolytic rancidity. Proteins can also react with hydroperoxides resulting from oxidation leading to denaturation (Frankel, 1980). Processed ready to eat and vacuum packed *Ruspolia nitidula* that were stored at room temperature for 12 weeks, showed an increase in acid value stabilizing at 3.2 mg KOH/g which was higher than the recommended value of 2 mg KOH/g (Ssepuuya et al., 2016)

Foods should ideally be stored at freezing (-18^0) and refrigeration temperatures to encourage nutrient retention (Severi et al., 1997). Flour derived from *Rhychophorus phoenics* larvae that had been subjected to preservation methods such as boiling, sun drying and smoking was stored in a freezer for 90 days and refrigerator for one week. It showed a relatively high oil absorption and protein solubility level which could be used in agro industrial applications. It was recommended that the flours be stored for less than three days in refrigeration and less than a week in a freezer so as to maintain a suitable water absorption capacity (Nabayo et al., 2012).

2.8 Moisture sorption isotherms

Prior to storage edible insects are mostly processed and stored in their dried form either as a whole or in pulverised form. These food are dry and can reabsorb moisture from the environment thus reducing their shelf life. To understand the stability of a food during storage the relationship between moisture content and water activity needs to be established. Moisture sorption isotherms help to identify critical moisture contents of storage, that can help optimize the shelf-life of stored products.

Moisture sorption isotherms describe the relationship between the moisture content of a material and the moisture level of its surrounding at a constant temperature. These isotherms are fundamental in design and optimization of food processing operations such as drying, packaging and prediction of moisture changes and storage stability (Jha et al.,2014). Over 200 models have been proposed by different researchers to help simulate sorption behaviours of numerous food matrices (Goneli et al.,2016). Every food has its own unique interaction with moisture depending on its composition and thus it is difficult to find mathematical models that describe accurately sorption isotherms over the entire range of water activity and for different types of foods (Akoy et al., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental material

Adult cricket and black soldier fly larvae (BSFL) were obtained from International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. The insects were blanched in boiling water for five minutes and dried in a solar drying tent to a constant weight. An El-USB-1 data logger (Lascar electronics Inc. Pennsylvania, USA) was placed in the solar dying tent to record the temperature and relative humidity. The temperature and relative humidity varied from 50-60°C and 15.5-24.5% respectively. The dried insect samples were then pulverised using a kitchen blender to form the insect meals.

3.2 Experimental design

Randomized block design with destructive sampling after every 45 days for 180 days was used in this study. Blanched and solar dried of black soldier fly larvae and house cricket were subdivided into a 100g samples and bagged in small polypropylene sacks, plastic containers and polyethylene bags. The packages containing each of the samples were then randomly selected and stored at either ambient (25^0C) or refrigerator (4^0C) conditions. Samples were analysed after every 45 days over a period of 6 months.

3.3 Bagging, storage and sampling of the insect meals

The processed insect flours were randomly filled into small polypropylene sacks (PP), plastic containers (PL) and polyethylene (PE) packages as shown in Figures 1, 2 and 3. Twelve packages including 4 PP, 4 PE and 4 PL packages were randomly selected and stored in each of the storage environments; ambient or refrigerated conditions. An EL-USB-2 data logger (Lascar electronics Inc. Pennsylvania, USA) programmed to record data after every one hour was placed in each of the storage environments to record

temperature and relative humidity. The plastic containers were tightly sealed using their screw lids while the polypropylene and polyethylene packages were firmly twisted in the open end and fastened.

Sampling was done during trial set up to establish the baseline data and subsequently as described in 3.2 above. In this study, the samples stored in refrigerated polypropylene packages were only stored up to 90 days and analysed only during trial set up and at the 45th day. To obtain samples for the assessment of different parameters at every sampling stage, each of the different types of packages from the two different storage environments were taken, thoroughly mixed, and divided into two portions for the different type of chemical, microbial and physical analyses including; peroxide (PV) , p-anisidine (P-AV), saponification (SV), iodine (IV) values and fatty acid analyses for lipid oxidation and total viable count (TVC), yeasts and moulds, Enterobacteriaceae, *E.coli* and *Salmonella* counts for microbial stability. Moisture adsorption isotherms were also determined gravimetrically at the beginning of the experiment.

Figure 3.1: Polypropylene (PP) packages used in storage of black soldier fly larvae and house cricket meal

Figure 3.2: Plastic (PL) packages used in storage of black soldier fly larvae and house cricket meal

Figure 3.3: Polyethylene (PE) packages used in storage of black soldier fly larvae and house cricket meal

3.4 Proximate analyses

Moisture, protein, fat, crude fibre and ash were determined according to AOAC (1999) methods.

3.4.1 Determination of moisture content

The moisture dishes were washed and placed in a cabinet drier at 105°C for one hour. They were then placed in a desiccator to cool and the initial weight of the dishes

recorded afterwards (W_1). Two grams of sample were taken and placed in the moisture dish and weight recorded (W_2). The dishes were then placed in a cabinet drier for 3 hours. After the dying time the dishes were removed from the drier, cooled in a desiccator and the final weight recorded (. The moisture contents of the samples was calculated as shown below;

Moisture content (%) = $(W_3 - W_1)/ (W_2 - W_1) \times 100$

3.4.2 Determination of crude protein

One gram of the sample was weighed into a digestion flask together with a catalyst composed of 5 g of K_2SO_4 and 0.5 g of $CuSO_4$ and 15ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest color turned blue signifying the end of the digestion process. The digest was cooled, transferred into a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. Ten (10) ml of diluted digest was transferred into a distilling flask and washed with about 2 ml of distilled water. Distillation was done to a volume of about 60 ml distillate. The distillate was titrated using 0.02N-HCL to an orange color of the mixed indicator which signified the end point.

Calculations were done using the formulae below;

Nitrogen%= $(V_1 - V_2) \times N \times f \times 0.014 \times 100/V \times 100/S$

Where; V_1= Titre for the sample (ml); V_2-titre for the blank

N=Normality of standard HCL solution

F= Factor of standard HCL solution

V= Volume of diluted digest taken for distillation (10ml)

S= Weight of sample taken (g)

Protein %= Nitrogen × protein factor

3.4.3 Determination of crude fat

The soxhlet extraction method which gives intermittent extraction of oil with excess of fresh organic solvent was used. Five grams of samples were weighed into extraction thimbles and the initial weights of the extraction flask taken. Fat extraction was done using petroleum ether in soxhlet extraction apparatus for 16 hours. The extraction solvents were evaporated and the extracted fat dried in an oven for about 15 min before the final weights of the flasks with the extracted fat were taken.

Calculations were done using the formula below;

$$\text{Crude fat (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where;

W_1= Weight of sample before extraction

W_2=Weight of sample after extraction

3.4.4 Determination of crude fibre

Two grams of sample was weighed into a 500 ml conical flask. 200 ml of boiling 1.25% H_2SO_4 was added and boiling done for 30 minutes under a reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter and the residue washed to completely remove the acid with boiling water. 200 ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 minutes. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCL and again washed with boiling water to rinse acid from the residue. The residue was washed twice with ethanol and thrice with pet ether. It was them dried in an oven at 105°C in a porcelain dish with a constant weight (W_1). Incineration was done in a muffle furnace at 550°C for 3 hours, the dish was then cooled in a desiccator and the final weight (W_2) taken.

Calculations were done as shown below;

$$\text{Crude fibre (\%)} = \left(\frac{W_1 - W_2}{W}\right) \times 100$$

Where;

W_1= Weight of acid and alkali digested sample

W_2= Weight of incinerated sample after acid and alkali digestion

W= Weight of sample

3.4.5 Determination of ash

Samples weighing 2 g were first weighed and put into clean and dry crucibles The samples were the charred by flame to eliminate organic material before being incinerated at 550°C in a muffle furnace to the point of white ash. The residues were cooled in desiccators and weights taken.

Calculations were done as shown below;

$$\text{Crude ash (\%)} = \left(\frac{\text{Weight of ash}_{(g)}}{\text{Weight of sample}_{(g)}}\right) \times 100$$

3.4.6 Total available Carbohydrates

The content of total carbohydrate was calculated by subtracting the sum of moisture, protein, fat, ash and crude fibre from 100% as shown below.

Total availble carbohydrate = 100%- (moisture + protein + fat + ash + crude fibre)%

3.5 Analysis of fatty acid composition

The fatty acid profile was determined by gas chromatography. Lipid extraction was done using the modified Bligh and Dyer method (1959). 1g sample were placed in a glass stoppered centrifuge tube and denatured at 100°C for three minutes. 2ml of

water and 7.5 ml of methanol-chloroform (2:1 v/v) was added and the mixture shaken overnight. The samples were centrifuged and the supernatant decanted and the residue resuspended in 9.5 ml of methanol-chloroform-water (2:1:0.8) and the homogenate centrifuged.The chloroform phase was extracted and dried in a vacuum rotary evaporator at 40°C. The residue was completely dried in a desiccator over KOH pellets. Then the lipid was methylated by placing 2 mg of the sample in a flask and refluxing with 2 ml of 95% methanol-HCL for 1 hour. The methyl esters were extracted with three portions of hexane (1 ml) and then washed with distilled water (3 ml). The hexane layer was dried in a vacuum rotary evaporator and the residue redissolved in a small drop of hexane. Then 0.2 µl was injected into the GC (Shimadzu GC-9 A) with a capillary column (supelcowax 30m × 0.53 mm); injection/detection temperatures, 220°C under a flame ionization detector. Identification of the fatty acid methyl esters was by comparison of retention times with standards and expressed as percentages of total methyl esters.

3.6 Determination of chemical characteristics

Saponification value, iodine value, peroxide value and P-anisidine values were determined by AOAC (1996) methods Chemical changes in stored black soldier fly larvae (BSFL) were assessed using peroxide value, saponification value, iodine value and p-anisidine values. The lipids were first extracted using Bligh and Dyer method. Approximately 2g of a well ground BSFL sample were extracted with 20.0 ml of chloroform-methanol (2:1 v/v) containing 10mg/L of butylated hydroxyl toluene (BHT). The mixture was centrifuged (Kokusan, H-2000C) at a 1000 RPM for 5 minutes at room temperature to give an aqueous top phase and an organic bottom. The bottom organic phase was carefully withdrawn (about 90%) by using a Pasteur pipette. The solvents were removed from the organic phase by use of a rotary evaporator so as to be left with a pure lipid. The extracted lipids were used for the chemical analyses of the other parameters. Oxidation rate during storage was assessed by periodic determination of peroxide value (PV) and p-anisidine value (P-AV). AOAC official methods (AOAC, 1999) was used to determine the peroxide values. This method is based on the

measurement of iodine produced from potassium iodide by peroxides present in a sample using iodometric titration. 2.5 grams of oil sample was weighed into a glass stoppered flask. 25 ml of acetic acid:chloroform mixture was then added to all the flasks containing the oil samples and a blank. 1 ml of saturated potassium iodide was added and the sample gently stirred for one minute. The sample was then placed in the dark for 30 minutes after which 30ml of water was added and shaken well. About 1ml of starch solution was added and titration was done with 0.01N-sodium thiosulphate until the blue colour disappeared. The peroxide value was calculated as follows:

$PV = ((A-B) \times F \times 10)/\text{Weight of fat used}$

Where;

A=Volume of 0.01N-sodium thiosulphate required for the sample (ml)

B=Volume of 0.01N-sodium thiosulphate required for the blank (ml)

F=Factor of 0.01N- sodium thiosulphate

P-AV was determined according to IUPAC official method. This method is based on the reaction between aldehyde compounds and p-anisidine where the products of this reaction are spectrophometrically determined. 0.3g of the oil sample was measured directly into a 10 ml flask. The sample was then dissolved in iso-octane in the 10 ml flask. The absorbance (A_1) from 2.5 ml sample was measured in glass cuvettes and placed in the dark for 10 minutes before a new spectrophometric measurement (A_2) was made. Measurements were performed in batches of three samples and one blank at a time to avoid oxidation due to delays. The anisidine values were calculated as follows;

$P\text{-}AV = 10\ ml \times (1.2 \times (A_{S2} - A_{B2}) - (A_{S1} - A_{B1}))/\ \text{Weight of fat used}$

Where;

10ml = Volume of isooctane used to dissolve sample

1.2= Correction factor for dilution of sample solution with 1ml of anisidine reagent dissolved in acetic acid

A_{S1} and A_{S2}= First and second spectrophometric measurement of samples

A_{B1} and A_{B2}= First and second spectrophometric measurements of blanks

Iodine value (IV) and saponification value (SV) were determined gravimetrically according to the AOCS method. In the determination of iodine value, 2g of oil sample was weighed into a glass stoppered flask. 10 ml of carbon tetrachloride was added and a blank test done at the same time. 25ml of wijs solution was then pipetted into all the flasks which were then placed in the dark for one hour. 20ml of 10% potassium iodide and a 100 ml of distilled water were then added and mixed well. The solution was titrated with 0.1N-sodium thiosulphate solution until the colour changed to yellow and then a few drops of starch solution were added and the titration continued until the blue colour disappeared. IV was calculated as follows;

$IV= ((B-A) \times F \times 126.9 \times 10^{-3})/ \text{weight of fat used}) \times 100$

Where;

B= volume of 0.1N-sodium thiosulphate required for the blank (ml)

A=volume of 0.1N-sodium thiosulphate required for the sample (ml)

F=Factor of 0.1N-sodium thiosulphate

In the determination of SV, 2g of the oil samples were weighed into different flasks. 25ml of 0.5N-alchoholic potassium hydroxide was pipetted into each of the flasks and a blank test done at the same time. The samples were then boiled under a reflux condenser for 30 minutes. A few drops of phenolphthalein were then added and the solution titrated with 0.5N-hydrochloric acid (HCL) until the pink colour disappeared. The SV was calculated as follows;

$SV= ((B-A) \times F \times 28.05)/ \text{weight of fat used})$

Where;

B=Volume of 0.5N HCL required for the blank (ml)

A= Volume of 0.5N HCL required for the sample (ml)

F=Factor of 0.5N HCL

3.7 Determination of color

The color of the insect samples was determined using a hunter lab color difference meter (Minolta, Tokyo, Japan). The instrument was standardized each time with a white a black ceramic plate. The color was directly measured at different points of the sample. Reflected color L*, a* and b* values was determined directly while the derived color parameters (hue angle and chroma) were calculated.

3.8 Microbiological analysis

A 5 g sample was mixed with 45 ml sterile peptone water and homogenized in a stomacher at normal speed for 1 minute. The homogenate was then diluted with sterilized peptone water to decimal dilutions of between 10^{-1}-10^{-4} before either pour plating or surface plating

Microbiological procedures conducted on stored samples of adult house cricket and black soldier fly larvae included; total aerobic plate count using nutrient agar, fungal count using potato dextrose agar, *E.coli* count using mac conkey agar, presence of *Salmonella* species count using Salmonella Shigella agar and identification using triple sugar iron (TSI) slants and Enterobacteriaceae count using violet red bile agar. The procedures used here were those recommended by AOAC (1996) as described below.

3.8.1 Enumeration of *Enterobacteriaceae*

These were enumerated in pour plates of violet red bile glucose agar (VRBGA) (Oxoid Ltd., United Kingdom) with an overlay of the same medium and incubated at 37°C for 24 hours.

One milliliter of each of the decimal dilutions was transferred to a sterile petri dish. About 15ml of the molten violet red bile glucose agar tempered at 45°C in a water bath was poured in each of the petri dishes. Time elapsing between the end of preparation of the initial suspension and the time when the medium was poured did not exceed 15 minutes.

The inoculum was then carefully mixed with the medium and the mixture allowed to solidify. After complete solidification, overlay of 10-15 ml of VRBGA medium tempered at 45°C was added onto the surface of the inoculate medium and allowed to solidify. The prepared dishes were then inverted and placed in an incubator at 37°C for 24 hours.

3.8.2 Enumeration of Aerobic mesophilic micro-organisms

This were enumerated by total viable count using plate count agar (PCA) (Oxoid Ltd., United Kingdom) incubated at 32 ±2°C for 48 hours.

One milliliter of the sample diluent mixture was aseptically transferred into the sterile petridishes. 18-20ml of molten tempered non-selective plating media was then be added in the sterile petri dishes. The plates were rotated to ensure complete mixing and dispersal of the sample. Thereafter the agar was allowed to solidify at room temperature. For aerobic mesophilic micro-organisms counts the non-selective plates were incubated at 32 ±2°C for 48 hours. After incubation the plates were examined for growth and all the visible colonies counted.

3.8.3 Enumeration of Yeasts and moulds

This were enumerated by surface plating on Potato dextrose agar (PDA) (Oxoid Ltd., United Kingdom) and incubated at 22-25^{0}C for 5 days.

One mL of the sample diluents was aseptically transferred onto the surface of already solidified PDA agar that had already been prepared 24 hours in advance. This was then evenly spread on the surface by use of a glass rod. Samples were incubated for 5 days and all the visible colonies counted.

3.8.4 Enumeration of E.coli

This were enumerated by surface plating Mac conkey agar (Oxoid Ltd., United Kingdom) and incubated at 37°C for 24 hours after which positive samples were expected to have pink colonies.

One milliliter of each of the decimal dilutions was transferred to a sterile petri dish. About 15-20 ml of the molten Mac Conkey (Oxoid Ltd., United Kingdom) agar tempered at 45°C in a water bath was poured in each of the petri dishes. Time elapsing between the end of preparation of the initial suspension and the time when the medium was poured did not exceed 15 minutes. The inoculum was then carefully mixed with the medium and the mixture allowed to solidify. The prepared dishes were then inverted and placed in an incubator at 37°C for 24 hours after which the pink colonies in the plates were counted.

3.8.5 Enumeration of Salmonella

A sample of 10 g was pre-enriched in a non-selective media (nutrient broth) for 24 hours at 35°C was used. The sample was then transferred in a selective media (tetrathionate broth) for selective pre-enrichment for 18-24 hours at 35°C. The sample was then streaked onto Salmonella-Shigella agar (selective differential agar) and incubated at 35°C for 24 hours. *Salmonella* grew as colorless to tan or at times colonies with a black

center. The colonies were then inoculated (stab butt and the slants streaked) into slants of tripple sugar iron agar for 24 hours at 35°C.

3.9 Moisture sorption analysis

The adsorption isotherms of the ground samples were determined using standard gravimetric technique (Wolf et al., 1985). The samples were first dried in an oven at 105^0C for 24 hours. Triplicate samples of about 0.5 g weighed to three or four decimal points were placed in small aluminium caps with open top, and then introduced into airtight desiccators in which saturated salt solutions were placed to maintain a fixed relative humidity. The desiccators were then placed in an incubator maintained at either 25°C, 30°C or 35°C. The samples were then allowed to equilibrate. During the first 24 hours sample weights were measured after every 12 hours on a sensitive weighing balance (reading at least to three decimal points) and towards the end of the experiment sample weights were taken at half day intervals. Equilibrium moisture content was attained when the difference between two consecutive measurements was less than 0.5% of sample weights/ or <0.001 g. Equilibrium moisture content was then be determined through the oven drying method. The following salts (Table 3.1) were used to maintain different relative humidity levels.

Table 3.1: Water activity of saturated salts

	Temperature		
Materials/Salts	25^0C	30^0C	35^0C
Lithium Chloride (LiCl)	0.11	0.11	0.11
Potassium Acetate (CH_3CO_2K)	0.23	0.22	0.22
Magnesium Chloride ($MgCl_2$)	0.33	0.32	0.32
Potassium Carbonate (K_2CO_3)	0.43	0.43	0.43
Magnesium Nitrate ($Mg(NO_3)_2$)	0.53	0.51	0.50
Potassium Iodide (KI)	0.69	0.68	0.67
Sodium Chloride (NaCl)	0.75	0.75	0.75
Potassium Chloride (KCl)	0.84	0.84	0.83
Potassium Sulphate (K_2SO_4)	0.97	0.97	0.97

Source; (Greenspan, 1977)

Experimental data was modelled using Guggenheim-Anderson-de Boer (GAB) model, Brunauer-Emmet-Teller (BET) model, Caurie model, Smith model, Oswin model and Khun model at all three temperatures studied. Model parameters were obtained by linear or non-linear regression analysis of the linear or quadratic forms of the expressions using Microsoft excel 2013.

Table 3.2: Isotherm model equations applied to obtained experimental data on semi-processed house cricket and black soldier fly larvae

Model	Model Equation	Reference
Brunauer-Emmet-Teller (BET)	$M_{eq} = \frac{M_o \times C_b \times a_W}{(1-a_W)(1+(C_b-1)a_W)}$ (1.)	(Brunauer et al., 1938)
Guggenheim-Anderson-de Boer (GAB)	$M_{eq} = \frac{M_o \times C_g \times K_g \times a_W}{(1-K_g a_W)(1+(C_g-1)K_g a_W)}$ (2.)	(Anderson, 1940)
Oswin	$M_{eq} = A\left[\frac{a_w}{1-a_w}\right]^B$ (3.)	(Oswin, 1946)
Smith	$M_{eq} = A + B\ln(1-a_W)$ (4.)	(Smith, 1947)
Khun	$M_{eq} = \frac{A}{\ln a_W} + B$ (5.)	(Khun, 1967)
Caurie	$\ln(M_{eq}) = \ln A - B \times a_w$ (6.)	(Caurie, 1981)

3.10 Statistical analyses

All the data was subjected to analysis of variance using Stata SE version 12 (StataCorp LP, Texas, USA). Furthermore due to the inherent limitations of ANOVA in describing differences in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was also applied to test effects of storage duration, storage environment type of package and the interaction effects. When the coefficient of the interaction term was significant ($P<0.05$), it was concluded that there was a significant difference between treatments over the storage period. Means were separated using Boniferroni adjustment at 95% confidence level.

CHAPTER FOUR

RESULTS AND DISCUSSION.

4.1 Storage stability of black soldier fly larvae meal

4.1.1 Effect of storage time, storage environment and type of package on the chemical stability of black soldier fly larvae meal (*Hermetia illucens)*

Black soldier fly larvae are nutritionally rich i.e. contain about 30% fat (Park, 2015) and thus are highly susceptible to oxidative deterioration during storage depending on the conditions present. In the present study, the lipid deterioration rate of black soldier fly larvae meal was determined under different storage conditions and in different packages. Peroxide value (PV), p-anisidine value (P-AV), saponification value (SV) and iodine value (IV) were used to monitor the oil quality with increase in storage duration. Changes in PV, P-AV, SV and IV values of stored adult house cricket meal are as shown in Figures 4.1, 4.2, 4.3 and 4.4. Results show that freshly boiled, solar dried and ground black soldier fly larvae meal had a PV, SV, IV and P-AV value of 22.2 meq O^2/Kg, 230.7 mg KOH/g, 90.6 mg I_2/g and 1.4 P-AV units respectively. The IV of the black soldier fly larvae meal compared well with that of fresh *Tenebrio molitor* (yellow mealworm beetle) grease of 96 g/100 g but was slightly higher than that recorded for black soldier fly larvae grease of 84 g/100 g (Zheng et al., 2013). This could suggest that the oil sample in this study was slightly more unsaturated. The SV was higher than that recorded for fresh yellow mealworm beetle grease, black soldier fly larvae grease and processed (dried and ground) *Imbrasia oyemensis* larva of 162.0, 157.0 and 184.2 mg KOH/g (Akpossan & Due, 2015; Zheng et al., 2013). The black soldier fly larvae meal had already undergone some oxidation during processing (boiling and solar drying) which could have led to increase in fatty acid molecular weight and formation of compounds such as aldehydes and ketones that resulted in an increased SV. The PV were much higher than those found in fresh *Macrotermes subhylanus* and *Ruspolia differens* (green and brown) that were ranging between 0.13-0.19 meq O^2/Kg (Kinyuru

& Kenji, 2010). This can be attributed to heat processing that may have increased the oxidation rate, exposure of the samples during drying to oxygen and also microbial rancidity where micro-organisms use their enzymes to breakdown chemical structures in the oil (Kaleem et al., 2015).

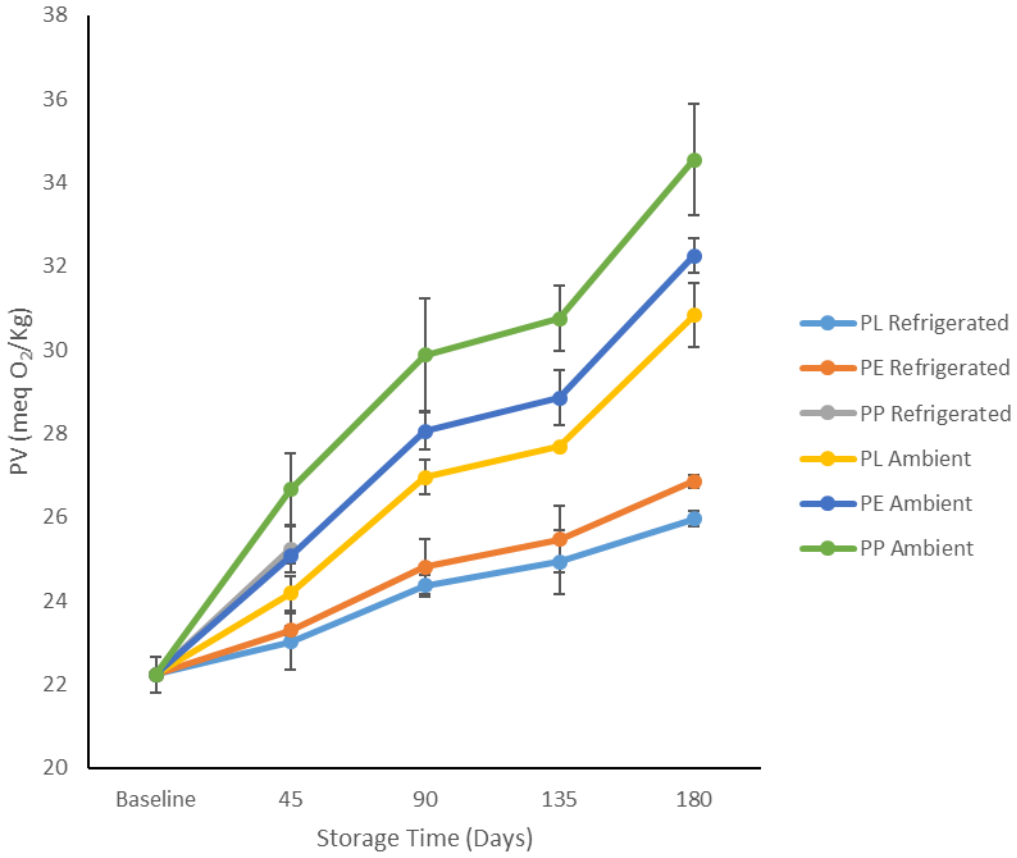


Figure 4.1: Peroxide values (PV) of boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in PV of semi-processed black soldier fly larvae meal is as shown in Figure 4.1. The PV seems to have continually increased during the entire storage period. PV is a measure of primary oxidation in oils and increases as the amount of primary oxidation products produced increases (Kaleem et al., 2015). The increase was significantly higher ($P<0.05$) in ambient storage than in refrigerated storage. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and thus oxidation at ambient conditions was expected to be higher than at refrigeration. Under ambient storage, increase in PV at the different sampling periods was in the range of; 8.7-19.9% at 0-45 days, 21.2-34.4% at 45-90 days, 24.5-38.3% at 90-135 days and 38.7-55.4% at 135-180 days of storage. Increase in PV in refrigerated storage was in the range of; 3.5-13.5% at 0-45 days, 9.6-11.6% at 45-90 days, 12.1-14.6% at 90-135 days and 16.7-20.8% at 135-180 days. At all the different sampling stages in both storage environments increase in PV was in the order PP>PE>PL. Most drastic increases in PV were noted to be in the period between 45-90 days and 135-180 days of storage. In refrigerated storage the increase was in the range of 5.9-6.5% and 4.1-5.4% at 90 and 180 days respectively. In ambient storage the increase was in the range of 11.5-12.1% at 45 days and 11.4-12.4% at 180 days respectively. At the end of the storage period, PV of samples in ambient storage was significantly higher ($P<0.05$) than in the refrigerated samples. In ambient storage, sample stored in PL package had the lowest ($P<0.05$) PV than samples stored in PE and PP where there was no significant difference between the two packages. In refrigerated storage, sample stored in PL package had the lowest PV although it was not significantly different from the sample stored in PE package in the same environment. The PV in both storage environments among the different packages at the end of the storage period was in the order PP>PE>PL. The interaction effect between storage time, type of packaging and storage environment was also highly significant ($P<0.001$).

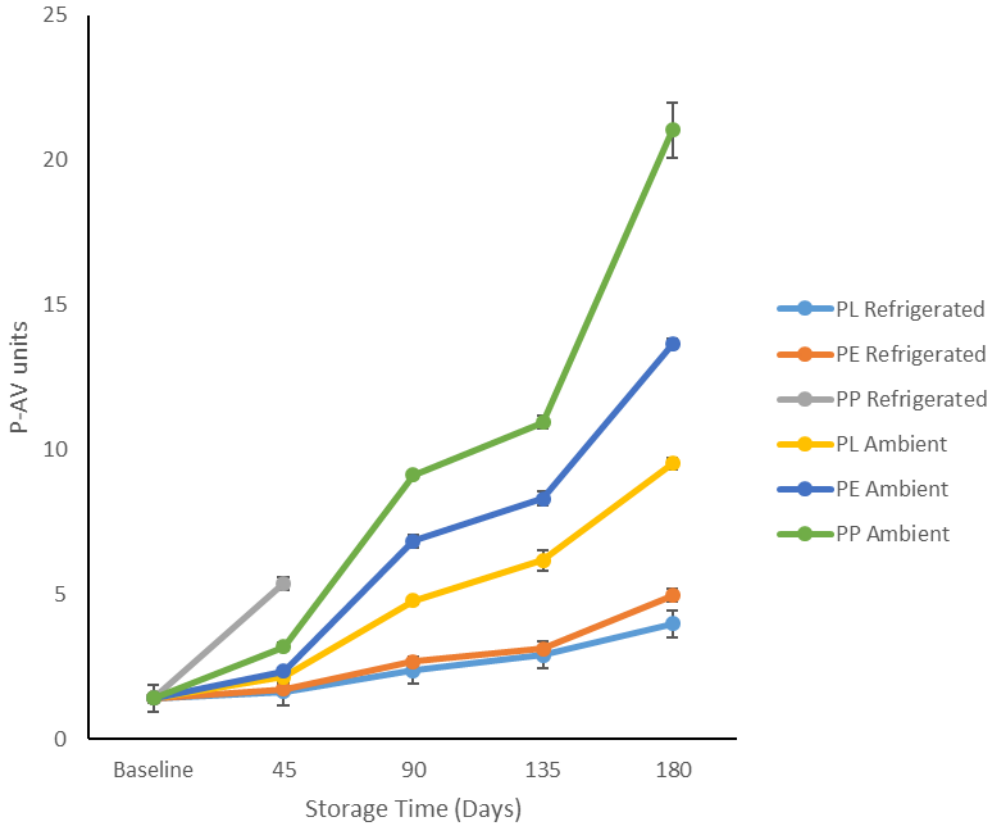


Figure 4.2: P-anisidine (P-AV) values of boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL)

Change in p-anisidine value during storage of black soldier fly larvae meal is as shown in Figure 4.2. The P-AV value continually increased in all the packages in both storage environments. The p-anisidine value is a measure of the secondary products of lipid oxidation including aldehydes and other carbonyl compounds in oxidized lipids (Shahidi et al., 1994). Increase in p-anisidine value during storage of a product can be attributed to the activity of lipase activity originating from the microorganisms tissue (Boran et al., 2006) or to the conversion of primary oxidation products to secondary products

(Maionese et al., 2004).The increases were higher in ambient storage than in refrigerated storage. In ambient storage the increases were ranging from 52.5-125.5% at 0-45 days, 239.0-547.5% at 45-90 days, 338.3-676.6% at 90-135 days and 575.2-1392.2% at 135-180 days. In refrigerated storage the increases were ranging from 15.6-22.7% at 0-45 days, 67.4-90.1% at 45-90 days, 105.0-120.6% at 90-135 days and 183.0-252.5% at 135-180 days of storage. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and therefore breakdown of primary oxidation to secondary oxidation products was higher at ambient conditions than in refrigeration. The increase in P-AV in both storage environments and in all the packages seems to have been most drastic in the period between 45-90 and 135-180 days of storage. At 45-90 days, in ambient storage the increases were in the range of 122.3-187.1% and at a 135-180 days; 54.1-92.2%. In refrigerated storage the increases were in the range of 44.8-54.9% and 38.1-59.8% at 45-90 and 135-180 days respectively. At the end of the storage period, significantly ($P<0.05$) higher P-AV were recorded in ambient storage than in refrigeration. The P-AV values in both storage environments decreased in the order PL<PE<PP where there were significant ($P<0.05$) differences among all the packages. Samples packaged in PP package in ambient storage had a significantly higher P-AV than samples stored in the other two packages at the end of storage. The interaction effect between the storage environment, type of package and storage duration was highly significant ($P<0.001$) on the P-AV value. Results (Figure 5) show that refrigerated sample in PL package had the least ($p<0.05$) P-AV value at the end of the storage period than in the other packages.

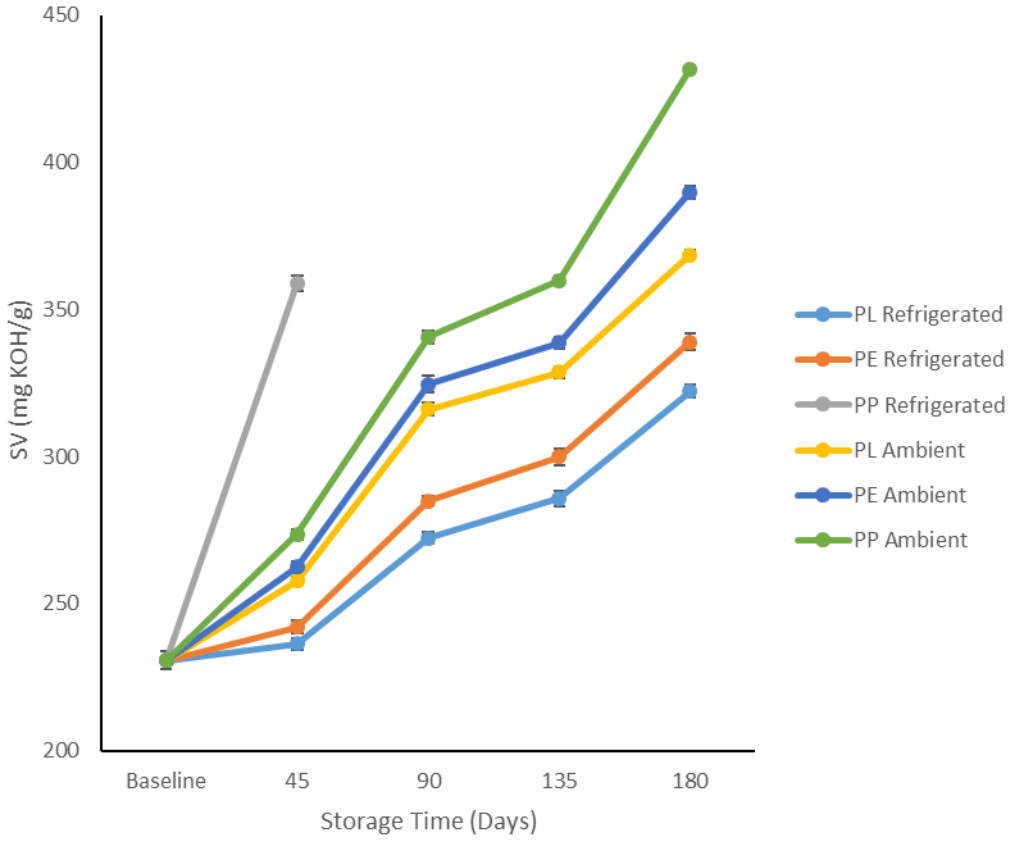


Figure 4.3: Saponification values (SV) of boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in SV during storage of the black soldier fly larvae meal is as shown in Figure 4.3. The SV continually increased during the entire storage duration in all the packages and in both storage environments. SV is a measure of the molecular weight and is defined as the amount of alkali required to saponify fatty acids in a given weight of oil. Hydrolysis and oxidation brings about lipid breakdown, forming free fatty acids or aldehydes and ketones as the end products. Increase in SV is not normally expected during this time but it is possible that the end products of oxidation such as aldehydes and ketones may contribute to increase in SV (Boran et al., 2006). Refrigerated samples

recorded significantly ($P<0.05$) lower SV than samples in ambient storage. A low SV corresponds to a higher amount of long chain fatty acids in a particular sample. Most of the long chain fatty acids are unsaturated and thus refrigerated samples that showed lower SV's, had undergone less oxidation than samples in ambient storage that had significantly higher SV's. Increase in SV at the different sampling points was higher for samples stored in ambient conditions than the refrigerated samples. Under ambient conditions, increase in SV at the different sampling points was in the range of; 11.7-18.6% at 45 days, 37.0-47.7% at 45-90 days, 42.4-56.0% at 90-135 days and 59.8-87.2% at 135-180 days of storage. In refrigerated storage the increase was in the range of; 2.4-55.5% at 0-45 days, 18.0-23.5% at 45-90 days, 23.8-30.0% at 90-135 days and 39.7-46.9% at 135-180 days of storage. Increase in SV in both storage environments at the different sampling points was in the order PP>PE>PL. Most drastic increases in SV in both storage environments were seen in the periods between 45-90 days and 135-180 days of storage. This increase in ambient storage was in the range of 22.7-24.5% and 12.2-20.0% at 90 and 180 days respectively. In refrigerated environment, the increase was ranging from 15.3-17.8% at 45-90 days and 12.8-13.0% at 135-180 days. At the end of the storage period, samples in ambient conditions had significantly higher ($P<0.05$) SV than the refrigerated samples. Sample packaged in PL package under refrigerated conditions had the lowest SV ($P<0.05$) among all the packages. The interaction effect between storage time, type of packaging and storage environment was also highly significant ($P<0.001$).

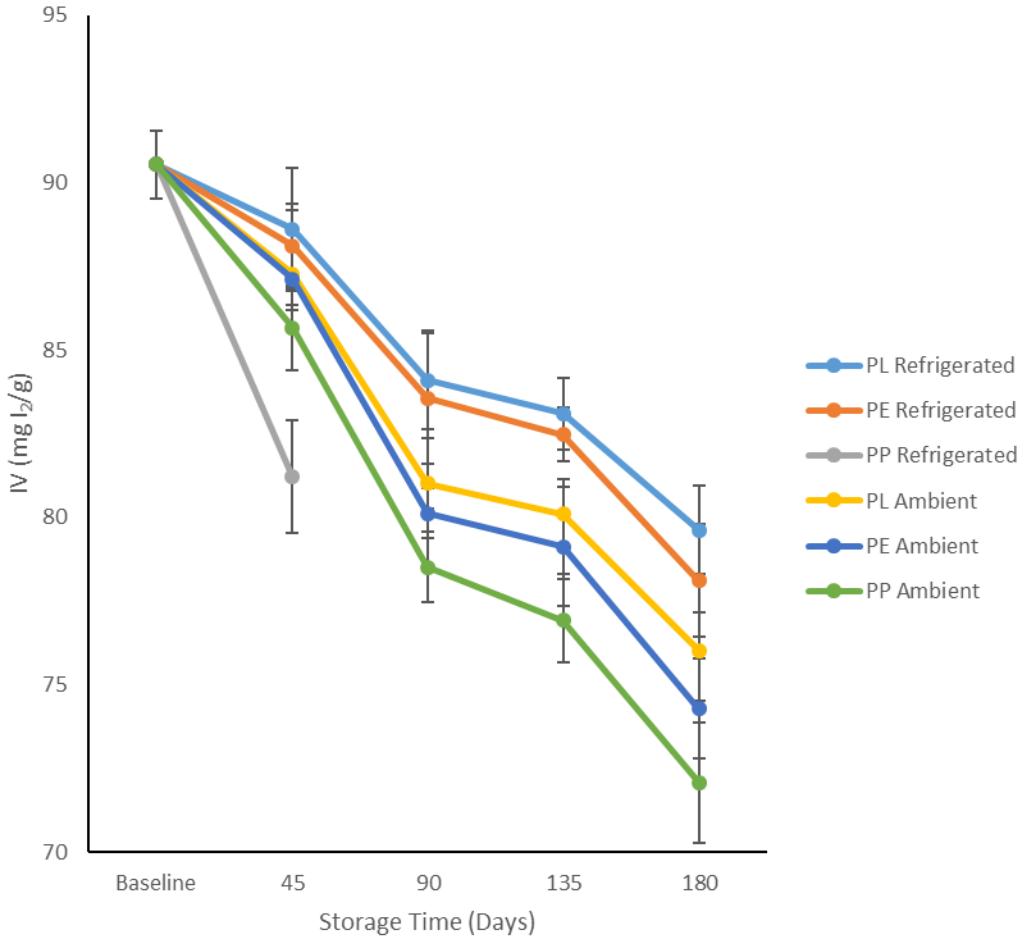


Figure 4.4: Iodine values (IV) of boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in IV of stored black soldier fly larvae meal is shown in Figure 4.4**.** The IV continually decreased during the entire storage duration. The iodine value is related to the degree of unsaturation of the oil and a decrease in the iodine value shows a decrease in the level of unsaturation of the oil (Boran et al., 2006). Samples stored in ambient storage recorded lower IV values than samples in refrigerated storage. IV reflects the amount of double bonds in a lipid. Lipid oxidation causes a decrease in IV which reflects a reduction in the number of double bonds in the lipid. Lipid oxidation rates are

directly related to temperature (Flick et al., 1992) and thus oxidation at ambient conditions was expected to be higher than at refrigeration. In refrigerated storage, decrease in IV at the different sampling periods was in the range of; 2.1-10.3% at 0-45 days, 7.1-7.7% at 45-90 days, 8.2-8.9% at 90-135 days and 12.1-13.7% at 135-180 days of storage. Under ambient conditions the decrease in IV was ranging from; 3.6-5.4% at 0-45 days, 10.5-13.3% at 45-90 days, 11.5-15.1% at 90-135 days and 16.1-20.4% at 135-180 days of storage. In both storage environments, at the different sampling stages, the IV was in the order PP>PE>PL. During storage, most drastic change in IV at both storage environments was seen in the period between 45-90 days and 135-180 days of storage. This decrease in refrigerated storage was ranging from 5.1-5.2% at 45-90 days and 4.2-5.3% at 135-180 days. Under ambient conditions, this decrease was in the range of 7.2-8.4% and 5.1-6.3% at 45-90 and 135-180 days respectively. At the end of the storage period, refrigerated sample in PL had the highest IV although it was not significantly different from IV of sample stored in PE package in the same environment. Sample stored in PP package under ambient conditions had the lowest IV value although there were no differences in significance among the packages in this environment. The storage time, type of packaging and storage environment all had a significant ($P<0.001$) effect on the IV. The interaction effect between storage time, type of packaging and storage environment was also highly significant ($P<0.001$). The results of this study were similar to those of (Boran et al., 2006) who noted that increase in PV, P-AV and SV and decrease in IV was higher at 4°C as compared to -18°C.

During storage most drastic change in all the chemical parameters in both storage environments were seen at 45-90 days and 135-180 days of storage. Oxidation in lipids usually includes three phases; initiation, propagation and termination (Choe & Min, 2006). Slow oxidation rates at the periods; 0-45 days and 90-135 days can be attributed to the initiation period where a hydrogen molecule is abstracted from the fatty acids. The process requires a lot of energy and thus occurs at a slower rate. High oxidation rates in the periods between 45-90 days and 135-180 days can be attributed to the propagation period where there is a high amount of primary oxidation products. Once the primary

oxidation products increase in concentration, the propagation phase which occurs through a radical chain mechanism begins and occurs at a faster rate (Kaleem et al., 2015). This correlates well with the drastic increase in PV recorded at the same period. Drastic increase in TVC could have also lead to an increased rate of microbial rancidity at the same time.

Lipid oxidation in the different packages was occurring in the order of PP>PE>PL. This can be attributed to differences in the rates of diffusion of both gases and water vapor across the packages. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of oxidation. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages as shown in Table 4.1. Refrigerated samples stored in PP packages were analyzed upto the 45^{th} day after which they were considered to be spoilt. Due to the high relative humidity in the refrigerator (97%), the samples packaged in polypropylene bags adsorbed a lot of water. This resulted in a vast growth of moulds on the sample which could only be stored for 45 days.The results of this study are similar to those of Adebola & Nusa Halima, (2014) who also found microbial deterioration in stored Garri (cassava product) to decrease in the order polypropylene bags (PP)> polyethylene bags (PE)> plastic buckets (PL).

Table 4.1: Permeability of high density polyethylene and plastic (polypropylene) package to oxygen and water vapor

	Package permeability		
Package	**Oxygen**	**Water vapor**	**Reference**
High density polyethylene (PE)	2100-7100 $cm^3/mm^2/day/atm$ at 23°C, 0% RH	7-24 $cm^3/mm^2/day/atm$ at 23°C, 0% RH	(Allahvaisi, 2012)
	500 MM/100 cm^2 in 24 h and 25°C	1-1.5 mm/100 cm^2 in 24h &37.8°C & 90% RH	(Allahvaisi, 2012)
Plastic (PL)-Polypropylene	2000-2500 $cm^3/mm^2/day/atm$ at 23°C, 0% RH	7 $cm^3/mm^2/day/atm$ at 23°C, 0% RH	(Allahvaisi, 2012)
	160 MM/100 cm^2 in 24 h and 25°C	0.25 mm/100 cm^2 in 24h &37.8°C & 90% RH	(Allahvaisi, 2012)

4.1.2 Effect of storage time, storage environment and type of package on the fatty acid composition of black soldier fly larvae meal (*Hermetia illucens*)

Degradation of fatty acid composition of semi-processed black soldier fly larvae meal is as shown in Figures; 4.5, 4.6 and 4.7. Freshly boiled and solar dried black soldier fly larvae meal was composed of 38.5% saturated fatty acids (SFA), 18.2% monounsaturated fatty acids (MUFA) and 19.8% polyunsaturated fatty acids (PUFA). The most dominant SFA were; lauric acid (21.6%) and palmitic acid (12.6%). The major MUFA was oleic acid (18.2%) while the major PUFA was linoleic acid (13.4%). This is in agreement with other studies (Makkar et al., 2014; Oonincx et al., 2015) who found that the major SFA, MUFA and PUFA in black soldier fly larvae were palmitic acid,

oleic acid and linoleic acid respectively. The larvae also contained appreciable amounts of n3 PUFA i.e. EPA (1.2%) and DHA (2.7%). This was in contrast with other studies involving fatty analysis of the black soldier fly larvae where long chain fatty acids were not detected (Makkar et al., 2014; Oonincx et al., 2015). Differences in fatty acid composition can be attributed to their different diets and habitats as suggested by Van Huis et al. (2013).

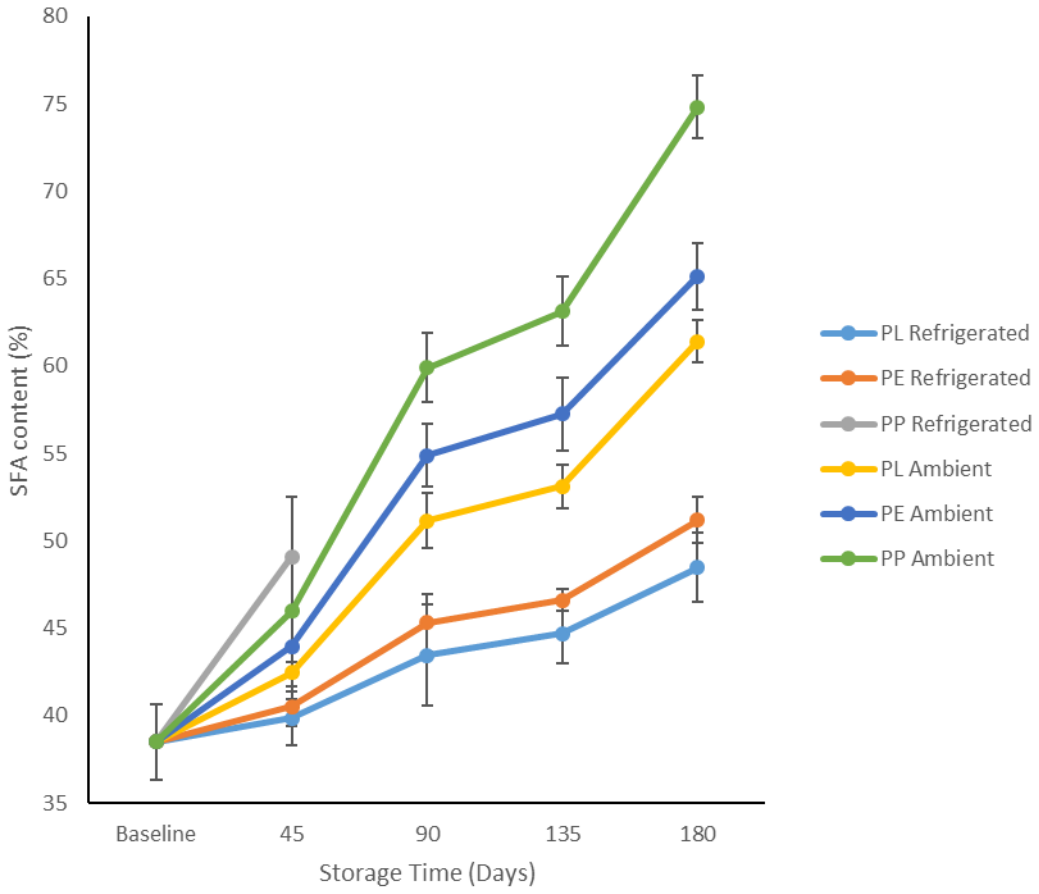


Figure 4.5: Saturated fatty acid (SFA) content in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in the saturated fatty acid content of semi-processed black soldier fly larvae is as shown in Figure 4.5. The SFA content seems to have increased during storage in all the packages. This included an increase in lauric acid, myristic acid, palmitic acid and stearic acid contents.

Increase in saturated fatty acids during storage was attributed to decrease in unsaturated (MUFA and PUFA) fatty acids during storage (Isdell et al., 2003). The increase at the different sampling periods was higher for samples stored at ambient conditions than the refrigerated samples. This can be attributed to higher temperatures at ambient storage where auto-oxidation and decomposition of hydroperoxides is higher (Choe & Min, 2006). Effect of dissolved oxygen also increases with increase in temperature (Choe & Min, 2006) leading to a higher breakdown of unsaturated fatty acids and an increase in SFA content. In refrigerated storage the increase was ranging from; 3.6-27.5% at 0-45 days, 12.9-17.7% at 45-90 days, 16.1-21.1% at 90-135 days and 25.9-33.0% at 135-180 days. In ambient storage the increase was ranging from; 10.3-19.4% at 0-45 days, 32.9-55.6% at45-90 days, 38.0-64.0% at 90-135 days and 59.5-94.3% at 135-180 days of storage. Increase in SFA content among the different packages was in the order PP>PE>PL. During storage, most drastic increases in SFA content were seen in the periods between 45-90 days and 135-180 days of storage. At 45-90 days, the increase was in the range of 9.0-11.8% and 20.5-30.4% in refrigerated and ambient storage respectively. At 135-180 days, the increase was in the range of 8.5-9.8% and 15.6-18.5% in refrigerated and ambient storage respectively. At the end of storage, samples stored in ambient conditions had significantly ($P<0.05$) higher SFA content than the refrigerated samples. Sample packaged in PP and stored in ambient conditions had a significantly higher SFA content than all the other samples. Although refrigerated samples in PL package had the least SFA content, it was not significantly different from sample in PE package in the same environment. The interaction between storage time, storage environment and type of package was also highly significant ($P<0.001$) on the change in SFA content during storage.

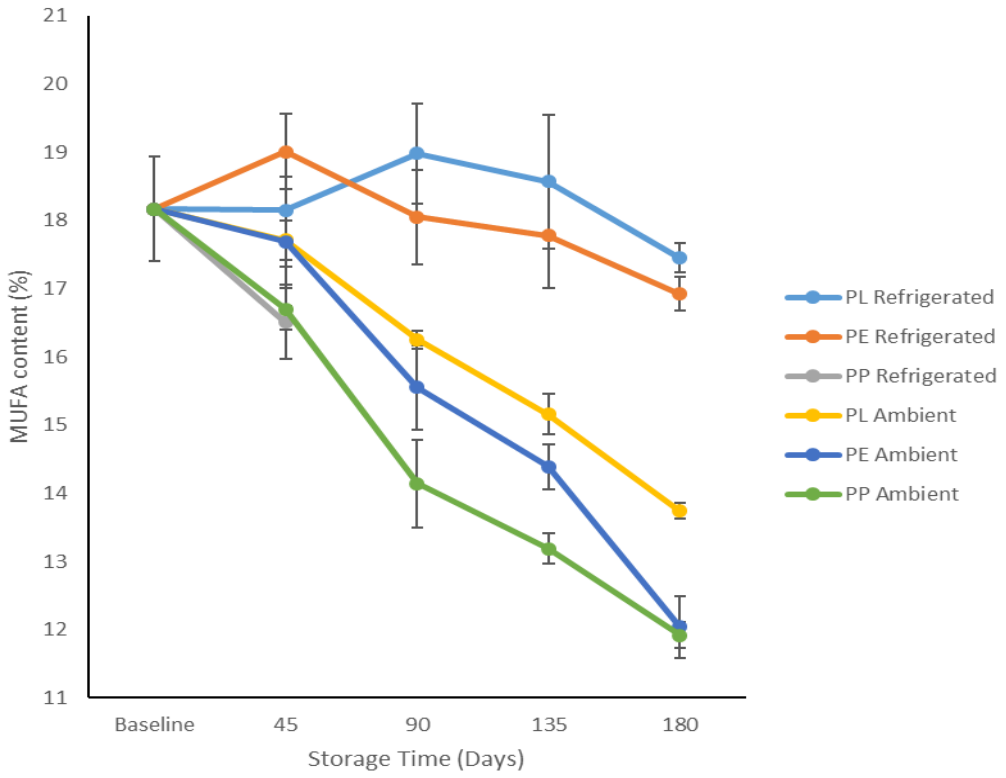


Figure 4.6: Monounsaturated fatty acid (MUFA) content in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in the MUFA content of semi-processed black soldier fly larvae meal is as shown in Figure 4.6 above**.** The MUFA content of the larvae gradually decreased throughout the storage period. Decrease in MUFA content was attributed to oxidation that was taking place during storage (Bhulaidok et al., 2010). The decrease was apparently higher in samples stored in ambient conditions than in the refrigerated samples. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and effect of dissolved oxygen also increases with increase in temperature (Choe & Min, 2006). At the different sampling periods the decrease was ranging from 2.5-8.1% at 45 days, 8.2-15.3 at 90 days, 6.7-7.5% at 135 days and 9.6-16.3% at 180 days in ambient

storage. In refrigeration the decreases were ranging from 0.1-9.1% at 45 days, 4.6-5.1% at 90 days, 1.5-2.2% at 135 days and 4.8-6.0% at 180 days. During the storage duration most drastic changes in the MUFA content were seen in the period between 45-90 days and 135-180 days in storage. At the end of the storage period, although refrigerated samples had a higher MUFA content, there were no significant differences between the two storage environments. The MUFA content was decreasing in the order PP<PE<PL although the variation between these packages was not significant (P>0.05). The storage environment, time and type of packaging did not have a significant (P>0.05) influence on the MUFA content of the black soldier fly larvae meal. The interaction between storage time, storage environment and type of package was also not significant (P>0.05)

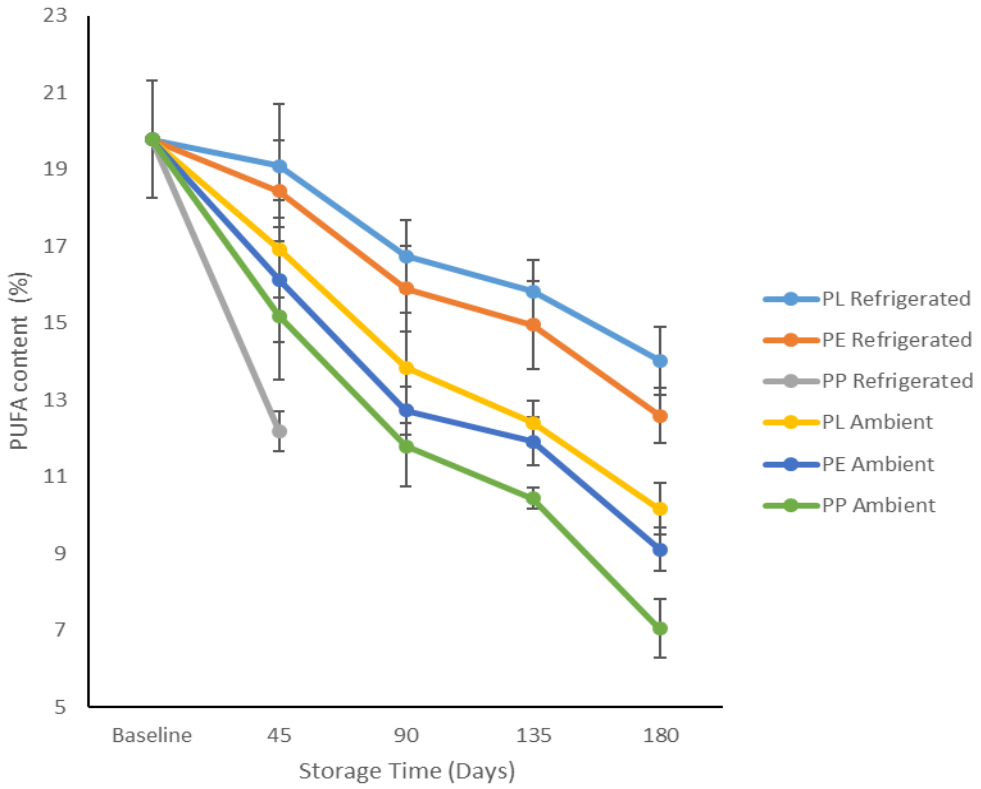


Figure 4.7: polyunsaturated fatty acid (PUFA) content in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in the PUFA content of stored black soldier fly larvae meal is as shown in Figure 4.7**.** The PUFA content seems to have continually decreased throughout the storage period in all the samples. Decrease in PUFA content was due to oxidation that was taking place during storage (Bhulaidok et al., 2010). The decreases were significantly higher ($P<0.05$) for samples stored in ambient conditions than the refrigerated samples. This can be attributed to higher temperatures at ambient storage where auto-oxidation and decomposition of hydroperoxides is higher (Choe & Min, 2006). Effect of dissolved oxygen is also increased with increase in temperature (Choe & Min, 2006) . At the different sampling periods the decrease was ranging from 3.5-38.4% at 45 days, 15.4-19.7% at 90 days, 20.0-24.5% at 135 days and 29.2-36.4% at 180 days in refrigerated storage. In ambient conditions, the decrease was in the range of 14.5-23.3% at 45 days, 30.1-40.4% at 90 days, 37.3-47.2% at 135 days and 48.7-64.4% at 180 days. The PUFA content was decreasing ($p<0.05$) in the order PL<PE<PP in all the sampling stages. During the storage period, most drastic decrease in the PUFA content in all the packages was seen in the period between 45-90 days and 135-180 days of storage. The decrease was ranging from 12.3-13.8% and 11.4-15.8% at 90 and 180 days of storage in refrigeration. In ambient storage the decrease was in the range of 18.3-22.3 and 18.1-32.6% at 90 and 180 days respectively. Sample packaged in PP package in ambient storage had a significantly lower PUFA content than all the other samples. Refrigerated sample in PL had the highest PUFA content at the end although it was not significantly different from the sample packaged in PE package in the same environment.

During storage although the increase in SFA and decrease in MUFA and PUFA contents were seen throughout the storage period, most drastic changes were seen to occur in the periods 45-90 days and 135-180 days. Less drastic changes were seen between the periods of 0-45 days and 90-135 days of storage. Oxidation in lipids usually includes three phases; initiation, propagation and termination (Choe & Min, 2006). Higher rates of oxidation at 45-90 days and 135-180 days can be attributed to the propagation period where there are high numbers of fatty acid radicals and oxidation occurs at a faster rate.

Increased microbial activities during these periods could also have led to microbial rancidity in which bacteria and yeast use their enzymes to breakdown chemical structures in the oil leading to production of unwanted flavors and odors (Kaleem et al., 2015).

Fatty acid oxidation in the different packages was occurring in the order PP>PE>PL. This can be attributed to differences in the rates of diffusion of both gases and water vapor across the packages. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of oxidation. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages as shown in Table (3.2).

4.1.3 Effect of storage time, storage environment and type of package on the microbial stability of black soldier fly larvae meal *(Hermetia illucens)*

Black soldier fly larvae like most meat products are rich in nutrients and may provide a favourable environment for the growth and proliferation of microorganisms (Klunder et al., 2012). Five microbial parameters including TVC, Enterobacteriaceae, *E-coli*, yeasts and moulds and Salmonella were analysed during storage of semi-processed black soldier fly larvae meal at refrigeration and ambient conditions. The results of this are as shown in Figures; 4.8, 4.9.4.10, 4.11 and in Table 4.2. TVC, Enterobacteriaceae and *E-coli* counts in freshly boiled, solar dried and ground larvae were to 5.5, 3.0 and 1.4 log cfu/g respectively. Yeasts and moulds were not detected at this stage (during experimental set up) but the samples were found to be positive for Salmonella. Bacterial loads detected were in agreement with other studies (Braide et al., 2011; Mujuru et al., 2014) who reported total bacterial counts, *E.coli*, Enterobacteriaceae and moulds in processed (degutting, salting, solar drying and roasting) *Gonimbrasia belina* (Mopani worms) and processed (roasted and sundried) *Bunae alcinoe*. (Osinubi, 1989) also isolated Salmonella from dried fish. Although the levels of microorganisms in the freshly boiled, solar dried and ground larvae were higher than expected they were still within an acceptable range as compared to products such as cured meats and food stuffs

that require further cooking where bacterial counts of up to 6.0 log cfu/g are acceptable (Stannard, 1997). Presence of bacteria could be attributed to contamination during processing and packaging (Gardiner, 2005). Klunder et al., (2012) reported that boiling crickets and mealworm larvae for five minutes was sufficient to eliminate all the Enterobacteriaceae in insect samples. Although the samples in this study were subjected to a similar process, Enterobacteriaceae were still present suggesting that there could have been some contamination. Contamination could have arisen through exposure to the environment (dust and air), unhygienic human contact, processing and post handling practices as has been reported by Mujuru et al. (2014) and Opara et al. (2012)

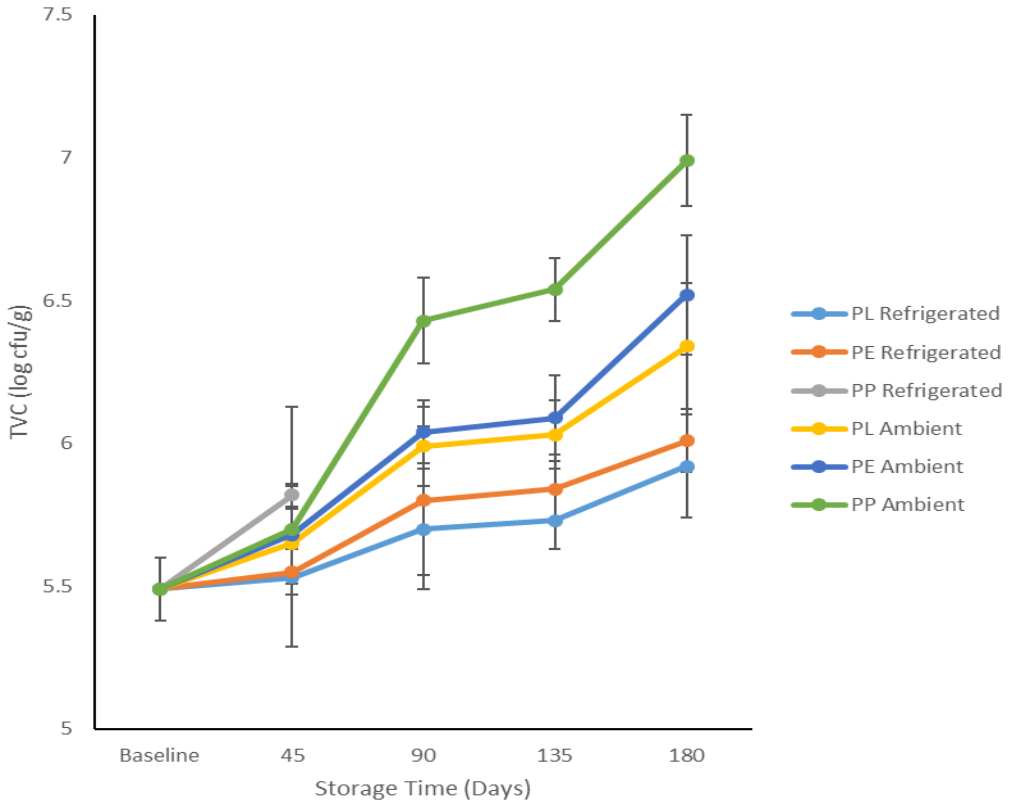


Figure 4.8: Total viable count (TVC) in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in total viable count (TVC) of semi-processed black soldier fly larvae during storage is as shown in Figure 4.8**.** The TVC count steadily increased during the entire storage period in all the samples. The black soldier fly larvae provides a nutrient rich environment (Alvarez, 2012; Park, 2015) where different types of microorganisms can be able to grow and multiply. The increase in TVC was higher in ambient storage than in refrigeration. Growth rates of most microorganisms increase with increase in temperature up to an optimum level after which they are killed (Mossel et al., 1995). Furthermore, at low temperatures reaction rates of different enzymes in the microorganisms become much slower and the fluidity of the cytoplasmic membrane is reduced interfering with transport mechanisms (Mossel et al., 1995) thus reducing the growth rate. In ambient storage at the different sampling points the increase was ranging from 0.19-0.2 log cfu/g at 0-45 days, 0.5-0.9 log cfu/g at 45-90 days, 0.5-1.1 log cfu/g at 90-135 days and 0.9-1.5 log cfu/g at 135-180 days. In refrigerated storage the increase was ranging from 0.04-0.3 log cfu/g at 0-45 days, 0.2-0.3 log cfu/g at 45-90 days, 0.2-0.4 log cfu/g at 90-135 days and 0.4-0.5 log cfu/g at 135-180 days of storage. The TVC count was increasing in the order PL<PE<PP in both storage environments at the different points. During storage, most drastic increases in TVC in both storage environments and in all the packages were seen at 45-90 days and 135-180 days. In ambient storage this increase was ranging from 0.3-0.7 log cfu/g at 45-90 days and 0.3-0.5 log cfu/g at 135-180 days. In refrigeration the increase was ranging from 0.2-0.3 log cfu/g at 0-45 days and 0.2-0.2 log cfu/g at 135-180 days of storage. At the end of the storage period, refrigerated samples had a significantly lower ($P<0.05$) TVC count than samples in ambient storage. Sample stored in PP recorded significantly higher ($P<0.05$) TVC count then refrigerated sample in PL recorded the lowest count ($p<0.05$). The interaction effect between storage time, storage environment and type of package significantly ($P<0.05$) affected the change in TVC.

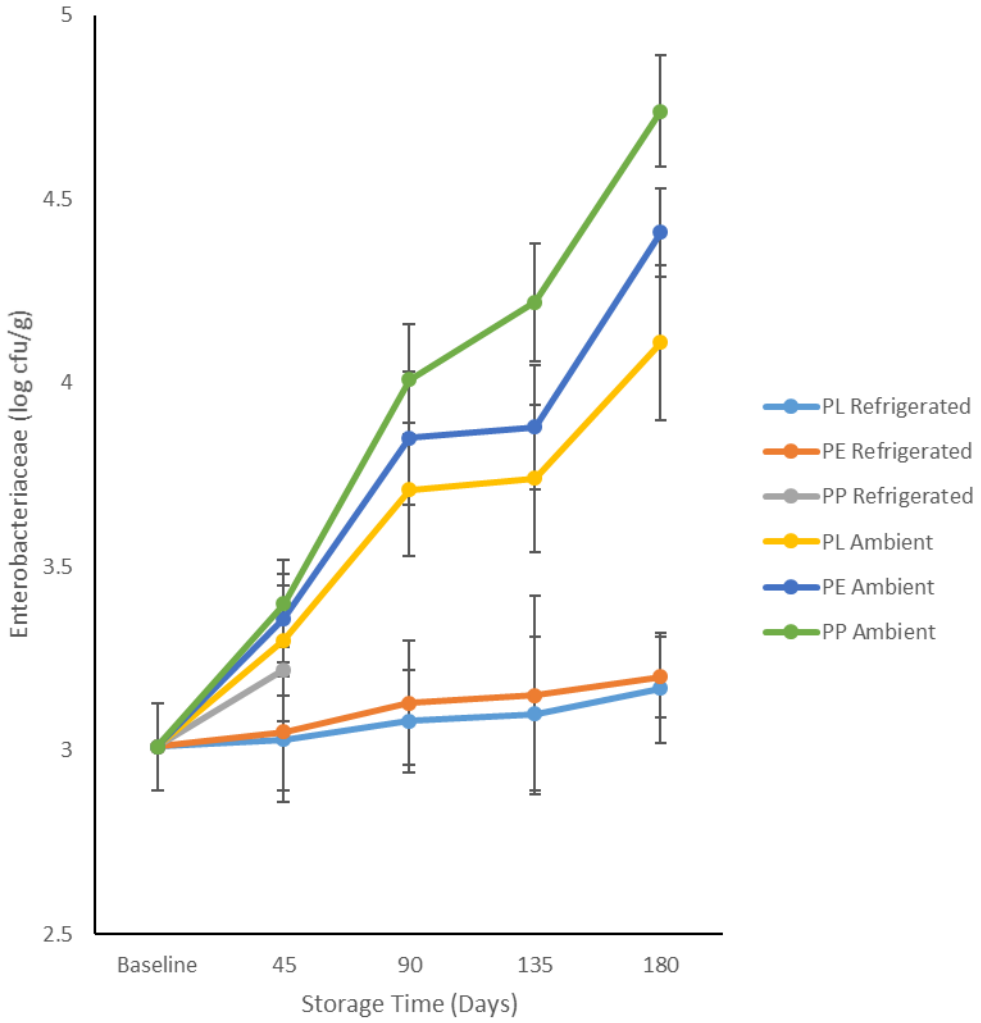


Figure 4.9: *Enterobacteriaceae* in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in Enterobacteriaceae count in stored black soldier fly larvae is as shown in Figure 4.9. The Enterobacteriaceae count gradually increased during the storage period in all the packages. The increases were higher in ambient storage than in refrigeration. Growth rates of most microorganisms have been observed to increase with increase in temperature. In ambient storage, the increase was ranging from; 0.3-0.4 log cfu/g at 0-45 days, 0.7-1.0 log cfu/g at 45-90 days, 0.7-1.2 log cfu/g at 90-135 days and 1.1-1.7 log

cfu/g at 135-180 days. In refrigerated storage the increase was ranging from 0.02-0.2 log cfu/g at 0-45 days, 0.1-0.12 log cfu/g at 45-90 days, 0.1-0.14 log cfu/g at 90-135 days and 0.16-0.19 log cfu/g at 135-180 days. The increase in the Enterobacteriaceae count at all the sampling stages was in the order PP>PE>PL. During the storage period, the most drastic increase occurred in the periods between 45-90 days and 135-180 days of sampling. In ambient storage this increase was ranging from 0.4-0.6 log cfu/g at 90 days and 0.4-0.5 log cfu/g at 180 days of storage. At refrigerated conditions, this increase was ranging from; 0.1-0.1 log cfu/g at 45 days and 0.1-0.1 log cfu/g at 180 days. At the end of the storage period, Enterobacteriaceae count in samples in ambient storage was significantly higher ($P<0.05$) than refrigerated samples. Sample packaged in PP recorded the highest Enterobacteriaceae count at the end of storage which was significantly higher ($P<0.05$) than in PL package in the same environment. Sample stored in PL package and refrigerated had the lowest Enterobacteriaceae although there was no variation in significance between PL and PE packages in the same environment. The interaction effect between storage time, storage environment and type of package was also highly significant ($P<0.001$) on the change in Enterobacteriaceae count during storage.

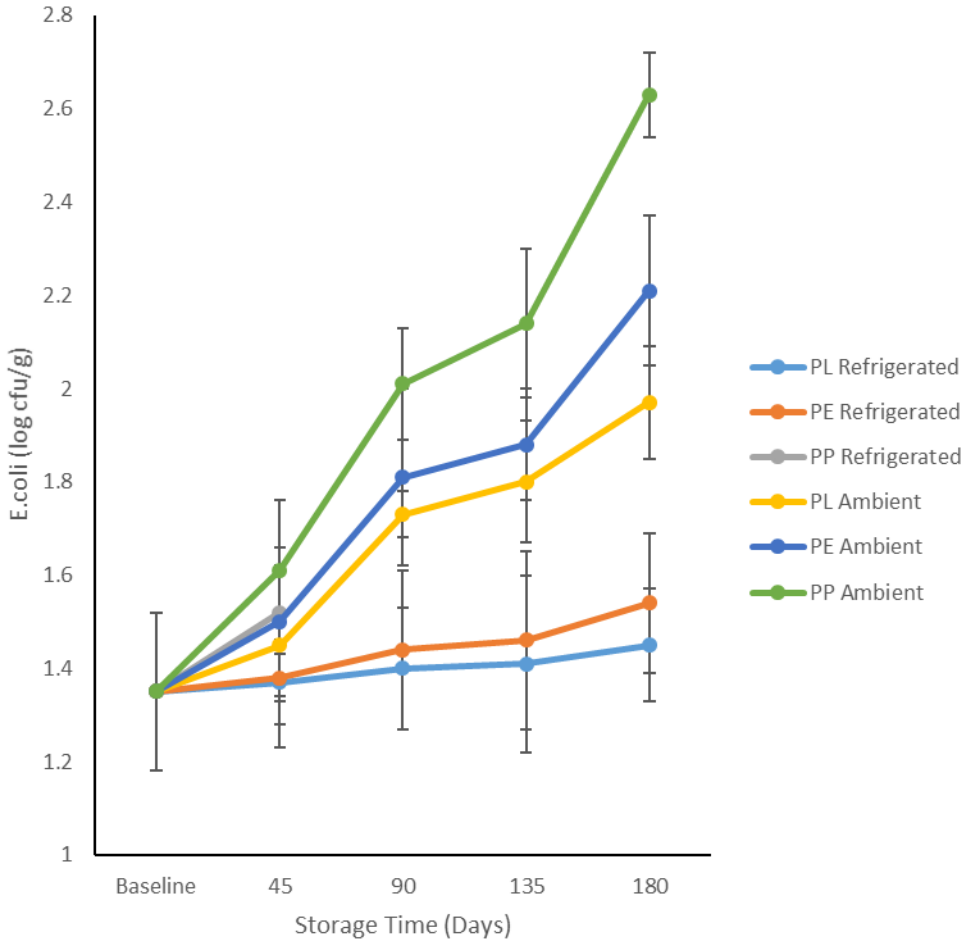


Figure 4.10: E.coli count in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PE) and plastic (PE))

Variation in *E-coli* count during storage of black soldier fly larvae is as shown in Figure 4.10**.** The *E-coli* count steadily increased throughout the storage period in all the samples. Continuous increase in *E-coli* count could be attributed to the nutrient rich environment provided by the BSFL meal (Alvarez, 2012; Park, 2015). The increase was higher in ambient conditions than in refrigeration. The increase in ambient storage was ranging from 0.1-0.3 log cfu/g at 0-45 days, 0.4-0.7 log cfu/g at 45-90 days, 0.5-0.8 log cfu/g at 90-135 days and 0.6-1.3 log cfu/g at 135-180 days in ambient storage. Under

refrigerated conditions, the increase was ranging from; 0.02-0.2 log cfu/g at 0-45 days, 0.05-0.1 log cfu/g at 45-90 days, 0.1-0.1 log cfu/g at 90-135 days and 0.1-0.2 log cfu/g at 135-180 days. In both storage environments, increase in *E-coli* count was in the order PP>PE>PL. During storage, most drastic increase in *E-coli* count was seen at 45-90 days and 135-180 days. The increase was in the range of 0.03-0.1 log cfu/g and 0.04-0.1 log cfu/g at 45-90 days and 135-180 days of storage in refrigeration. Under ambient conditions the increase was in the range of; 0.3-0.4 log cfu/g and 0.2-0.5 log cfu/g at 45-90 days and 135-180 days of storage respectively. At the end of the storage period, refrigerated samples had a significantly ($P<0.05$) lower *E-coli* count than samples in ambient storage. Sample stored in PP package under ambient conditions had significantly higher ($P<0.05$) *E-coli* count than all the other packages in both storage environments. Refrigerated sample in PL had the lowest *E-coli* count although it was not significantly different from that in PE package. The interaction effect between storage time, storage environment and type of package was highly significant ($P<0.001$) on the change in *E-coli* content during storage.

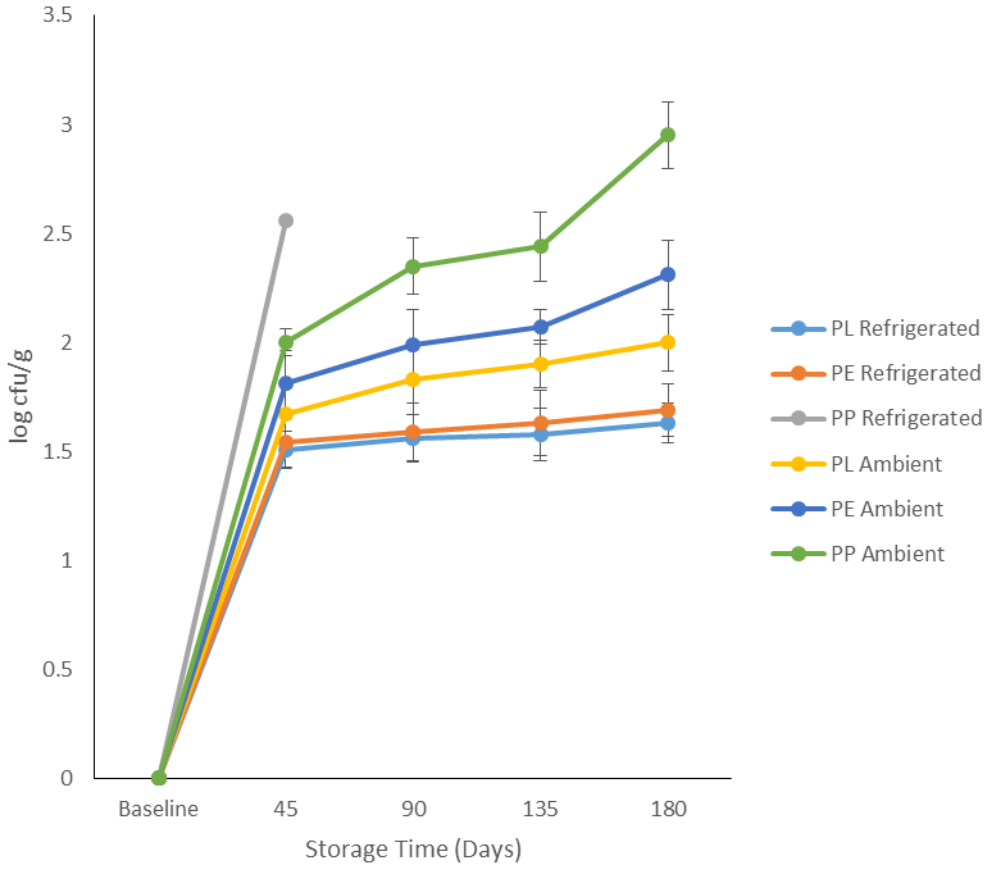
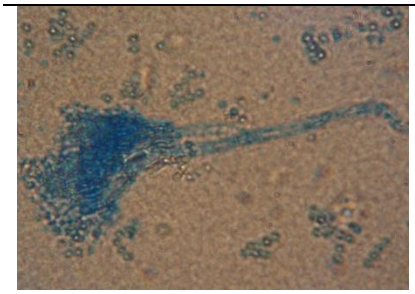
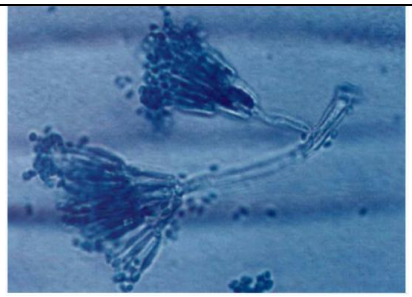


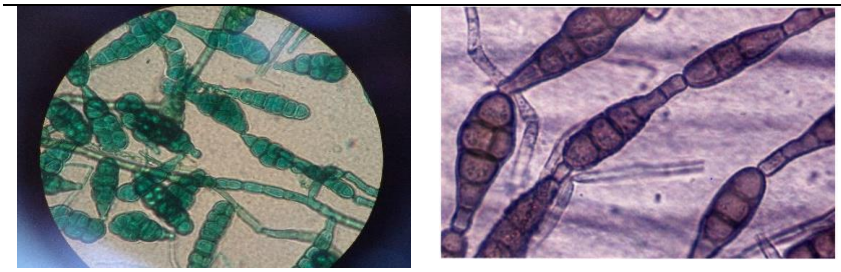
Figure 4.11: Yeasts and moulds count in boiled and solar dried black soldier fly larvae meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in yeast and mould count during storage of black soldier fly larvae is as shown in Figure 4.11**.** Yeasts and moulds were not detected in the samples at the baseline level although later on the samples were positive. Fungi and their spores are widely distributed in air and may have contaminated the samples through the deposit of spores when the samples were exposed to air during processing and packaging (Braide et al., 2011). The yeast and mould count was higher in samples stored at ambient conditions than in refrigerated samples. Growth rates of most microorganisms increase with increase in temperature. During storage, the yeast and mould count gradually increased

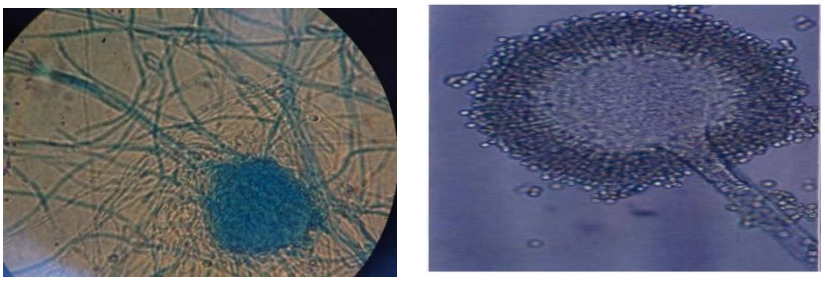
in all the packages. Under ambient storage, the increase was ranging from 1.7-2.0 log cfu/g between 0-45 days, 1.8-2.4 log cfu/g between 45-90 days, 1.9-2.4 log cfu/g between 90-135 days and 2.0-3.0 log cfu/g between 135-180 days. Under refrigeration, the increase ranged from; 1.5-2.6 log cfu/g between 0-45 days, 1.6-1.6 log cfu/g between 45-90 days, 1.6-1.6 log cfu/g between 90-135 days and 1.6-1.7 log cfu/g between 135-180 days of storage. The increase in both storage environments was in the order PP>PE>PL. During storage, most drastic increase in yeast and mould was observed at 45 days of storage in both storage environments. The increase was ranging from 1.7-2.0 log cfu/g and 1.5-2.6 log cfu/g in ambient and refrigerated conditions respectively. After 180 days of storage, sample stored in PP package under ambient conditions had the highest ($P<0.05$) count among all the other packages (Figure 4.11). Refrigerated samples did not show variation in significance although PL package had a lower count than PE package. The interaction effect between storage time, storage environment and type of package was highly significant ($P<0.001$) on the change in yeasts and moulds during storage.

Aspergillus, *Alterneria* and *Penicillium* were the major groups of moulds that were identified as shown in Plate 4.1. *Aspergillus* and *Penicillium* species have also been isolated in degutted Mopani worms (Mujuru et al., 2014).

Experimental data	**Reference pictures from literature** **Source;**(Navi et al., n.d.)
	 Penicillium species



Alternaria species



Aspergillus species

Plate 4.1: Types of moulds isolated during storage of semi-processed Black soldier fly larvae in PL. PE and PP packages in both ambient and refrigerated storage.

Black soldier fly larvae samples were found to be contaminated with *Salmonella* species as shown in Table 4.2 Presence of *Salmonella spp*. indicated that the lot from which the sample was obtained was not safe for consumption. *Salmonella spp.* have not been isolated in other studies involving processed and stored insects (Braide et al., 2011; Klunder et al., 2012; Mpuchane et al., 2000; Mujuru et al., 2014; Opara et al., 2012; Van Huis et al., 2013) indicating that this was likely a post/pre harvest contamination. Pre-harvest contamination could have arisen from the waste products that the insects were fed on before harvesting. Contamination could have arisen through exposure to the environment (dust and air), unhygienic human contact, processing and post handling practices (Mujuru et al., 2014; Opara et al., 2012).

Table 4.2: Salmonella content of black soldier fly larvae during storage

Storage	Ambient conditions			Refrigerated conditions		
Days	PP	PE	PL	PP	PE	PL
0	+	+	+	+	+	+
45	+	+	+	+	+	+
90	+	+	+	Nd	+	+
135	+	+	+	Nd	+	+
180	+	+	+	Nd	+	+

+ - Positive sign indicates the sample tested positive for the presence of Salmonella. Nd- Not determined

Increase in microbial count in the microorganisms analyzed was most drastic in the periods between 45-90 and 135-180 days of storage. The increase in the micro-organisms formed a sigmoidal kinetic curve where two periods of stability were observed from 0 to 45 days and from 90 to 135 days of storage. This results suggest a decrease in the growth and multiplication rate of microorganisms at these periods. At the start of the experiment there seems to be a lag phase where growth and multiplication rate of microorganisms is usually low. At 45 days the log phase begins where growth rate is usually higher and the general microbial population increases. At 90 to 135 days the growth rate was a bit reduced and this could be attributed to competition among the micro-organisms present. There may have been competition for nutrients, attachment sites, oxygen/carbon dioxide or a combination of these factors (Foods, n.d.) leading to the reduction of microbial growth and multiplication. Insufficient supply of nutrients and other necessities at this stage could have led to the inactivation (death) of some of the microbes (Foods, n.d). Afterwards from 135 days the growth rate increased. There was reduced competition and thus microorganisms present could grow at a faster rate.

Increase in TVC, Enterobacteriaceae, *E-coli* and yeast and moulds in the different packages was in the order PP>PE>PL. At the end of the storage period refrigerated sample in PL had the least number of microorganisms while sample stored in PP under

ambient conditions had the highest number of microbes. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of microbial growth. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages (Allahvaisi, 2012). Permeability to relative humidity among the packages could have led to an increased water activity of the samples thus promoting microbial growth (Foods, n.d.). Refrigerated samples packaged in PP bags were analyzed only upto the 45th day of storage. The samples adsorbed a lot of water while in the refrigerator (relative humidity 97%) owing to the high permeability of PP to water vapour and gases. This resulted in an increase in water activity of the sample promoting a colossal amount of bacterial growth. Consequently, the shelf life of the product was shortened to 45 days afterwhich the samples in these packages were considered as spoilt.

Efforts should be taken to improve on the quality of insects that are meant for consumption either by humans or animals to avoid contamination. Different types of micro-organisms when present in food pose detrimental health risks; some strains *E.coli* produces toxins in foods which cause diarrhoea and severe abdominal pains (Nester et al., 1998), *Penicillium* and F*usarium* species are known to produce various mycotoxins in foods and feeds in fields or under storage. These toxins might be nephrotoxic or carcinogenic. *Aspergillus* produce aflatoxins that might induce hepato-cellular carcinoma (Braide et al., 2011).

4.1.4 Effect of storage time, storage environment and type of package on the colour of Black soldier fly larvae meal *(Hermetia illucens)*

Changes in color shade, saturation and total color change of the stored black soldier fly larvae meal were monitored through the LAB system and are as shown in Table 4.3.

Table 4.3: Color changes in semi-processed BSFL stored in PE, PL and PP packages in ambient and refrigerated conditions for 180 days

Packaging Material	Storage time (Days)	Hue angle		Chroma		Total color change	
		4^0C	25^0C	4^0C	25^0C	4^0C	25^0C
PE	0	84.3±1.7^{a}	84.3±1.7^{a}	13.9±0.5^{a}	13.9±0.5^{a}	0.0^{c}	0.0^{c}
	45	84.8±0.3^{a}	85.5±1.0^{a}	14.6±0.3^{a}	14.9±0.3^{a}	2.6±0.2^{ab}	1.6±1.2^{ac}
	90	85.0±0.4^{a}	83.3±0.5^{ac}	14.4±1.6^{a}	14.3±0.3^{a}	2.2±1.1^{abc}	2.2±0.2^{abc}
	135	85.9±3.1^{a}	84.6±0.5^{a}	13.0±0.1^{a}	12.3±2.1^{a}	1.8±0.6^{abc}	3.4±1.4^{ab}
	180	76.9±0.6^{bc}	75.8±1.5^{b}	13.4±0.5^{a}	12.7±0.6^{a}	3.5±0.7^{ab}	3.5±1.4^{ab}
PL	0	84.3±1.7^{a}	84.3±1.7^{a}	13.9±0.5^{a}	13.9±0.5^{a}	0.0^{c}	0.0^{c}
	45	82.5±1.1^{ac}	83.9±2.0^{a}	13.1±0.2^{a}	12.4±1.4^{a}	1.2±0.4^{ac}	3.5±1.9^{ab}
	90	85.6±1.6^{a}	85.5±4.1^{a}	12.8±1.2^{a}	12.2±1.8^{a}	3.7±1.1^{ab}	3.8±0.2^{ab}
	135	75.1±0.8^{b}	84.8±1.4^{a}	13.0±1.6^{a}	12.1±0.8^{a}	3.2±0.9^{ab}	4.9±0.7^{b}
	180	84.6±2.8^{a}	73.4±3.2^{b}	11.7±1.3^{a}	11.8±0.8^{a}	3.3±1.5^{ab}	4.4±1.2^{ab}
PP	0	84.3±1.7^{a}	84.3±1.7^{a}	13.9±0.5^{a}	13.9±0.5^{a}	0.0^{c}	0.0^{c}
	45	84.3±0.7^{a}	86.1±0.9^{a}	14.6±1.1^{a}	13.1±0.6^{a}	1.8±0.1^{abc}	2.2±0.8^{abc}
	90	Nd	86.6±2.1^{a}	Nd	12.6±1.3^{a}	Nd	2.7±1.0^{ab}
	135	Nd	74.2±1.7^{b}	Nd	12.6±0.8^{a}	Nd	3.8±0.2^{ab}
	180	Nd	82.4±1.0^{ac}	Nd	11.9±1.2^{a}	Nd	5.7±0.4^{ab}

PE= Polyethylene package, PL=Plastic package, PP=Polypropylene package. Nd-not determined.

The hue angle and chroma give a more comprehensive three dimensional view of the color change with increase in storage duration (Isdell et al., 2003). The hue angle and chroma values of freshly boiled and solar dried black soldier fly larvae were 83.3 and 13.9 respectively. In all the packages in both storage environments, the hue angle did not change significantly for the first 90 days of storage. This shows that there was no change in the color shade of the samples. In ambient storage, significant decrease in the hue angle was seen in the period between 135-180 days in PE and PL packages and at 90-

135 days in PP package. In refrigerated storage, significant decrease ($P<0.05$) in the hue angle was seen at 135-180 days in PE and 90-135 days in PL. Combined effect of storage time, storage environment and type of package was highly significant ($P<0.001$) on the hue angle. The chroma values were constant in all the packages throughout the entire storage period and did not show any significant change in any of the packages. This shows that the vividness of the black soldier fly larvae color did not change in all the packages in both storage environments. The combined effect of storage time, environment of storage and type of package was not significant ($P>0.05$). The total color change which is an indicator of the overall color change seems to have increased in all the packages throughout the entire storage period. The increase was higher at ambient storage than in refrigerated storage although no significant differences were seen. This can be attributed to a higher temperature that accelerates degradation reactions in the sample including oxidation thus leading to a greater color change. Similar results were noted in packaged beef, where samples stored at a higher temperature had a greater degree of color degradation (Isdell et al., 2003). Color change in PP package was higher than in PE and PL packages and this can be attributed to a superior gas and water vapor permeability in PP than in the other two packages. The results compared well with change in color of cooked ham where color deterioration was higher in oxygen permeable packages (Isdell et al., 2003). The combined effect of storage time, environment of storage and type of package was not significant ($P>0.05$) on the overall color change of the samples.

4.2 Storage stability of the house cricket meal

4.2.1 Effect of storage time, storage environment and type of package on the chemical stability of adult House cricket meal (*Acheta domesticus*)

Adult house crickets are rich in unsaturated fatty acids and therefore highly susceptible to oxidation during storage. In the present study, the lipid deterioration rate of adult house cricket was determined under different storage conditions and in different packages. Commonly employed chemical analyses including PV, P-AV, SV and IV

were used to monitor the oil quality with increase in storage duration. Changes in PV, P-AV, SV and IV values of stored adult house cricket meal are as shown in Figures 4.12, 4.13, 4.14 and 4.15. Results show that freshly boiled, solar dried and ground house cricket meal had a PV, SV, IV and P-AV value of 16.9 meq O_2/Kg, 216.5 mg KOH/g, 129.0 mg I_2/g and 12.4 P-AV units respectively. The IV of semi-processed adult house cricket compare well with those of fresh *Oryctes rhinoceros* larvae and *Rhynchophorus phoenics* that were ranging between 123.6-140.0 mg I_2/g (Ekpo et al., 2009) suggesting that the cricket oil had a fairly high degree of unsaturation. The SV was higher than 184.2 mg KOH/g recorded for dried and ground *Imbrasia oyemensis* larva (Akpossan & Due, 2015) showing that the cricket oil had already undergone some degree of oxidation. This can be attributed to heating during semi-processing that accelerated the rate of oxidation (Kaleem et al., 2015). The PV were much higher than those found in fresh *Macrotermes subhylanus* and *Ruspolia differens* (green and brown) that were ranging between 0.13-0.19 meq O_2/g (Kinyuru & Kenji, 2010). This can also be attributed to heat processing that increased the oxidation rate, exposure of the samples during drying to oxygen and also microbial rancidity where micro-organisms use their enzymes to breakdown chemical structures in the oil (Kaleem et al., 2015).

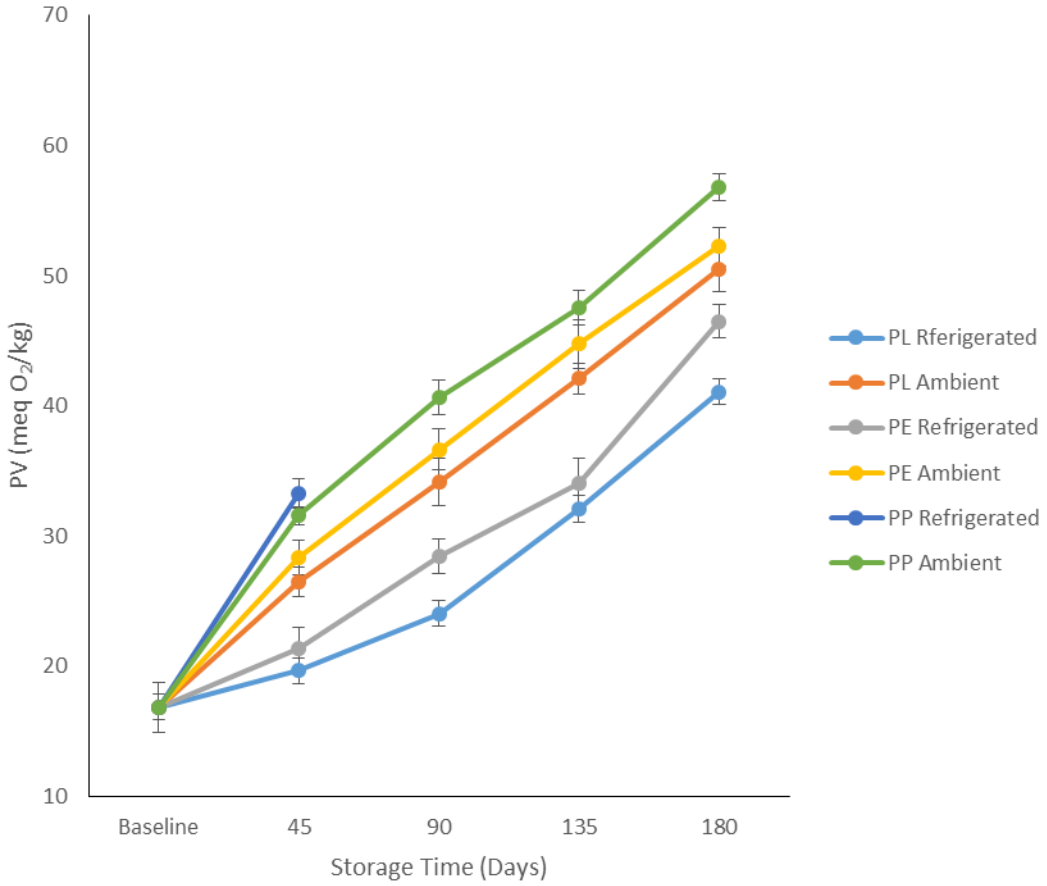


Figure 4.12: Peroxide value (PV) of boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in the PV of adult house cricket meal is as shown in Figure 4.12**.** The PV of the samples seems to have progressively increased throughout the storage period. PV is a measure of primary oxidation in oils and increases as the amount of primary oxidation products produced increases (Kaleem et al., 2015). Samples in ambient storage recorded higher ($P<0.05$) PV values than refrigerated samples. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and thus oxidation at ambient conditions was expected to be higher than at refrigeration In ambient storage the increases were ranging from 56.9-87.1% at 0-45 days, 102.5-141.1% at 45-90 days, 149.6-181.9% at 90-135

days and 199.4-236.9% at 135-180 days of storage. In refrigerated storage the increases' were ranging from 16.5-97.1% at 0-45 days, 42.6-68.4% at 45-90 days, 90.2-102.1% at 90-135 days and 143.6-175.6% at 135-180 days. The increases in PV at all the sampling stages were in the order of PP>PE>PL in the different packages. In ambient storage, the percentage increase in PV was most drastic after 45 days of storage in all the packages [PL (56.9 %< PE (68.1 %< PP (87.1%)] while in refrigerated storage the increase was highest at the 180^{th} days sampling period in both PL (28.0%) and PE (36.4%)**.** At the end of the storage period, samples in ambient storage had a higher PV value than refrigerated samples. The PV value increased in the order of PL<PE<PL, where PV value in the PP package was significantly higher than that in the PL package but neither showed significant difference from the PV value of the sample packaged in PE package. The interaction between storage time, storage environment and type of package was significant ($P<0.001$). Results show that refrigerated storage and PL packaging contributed to the least PV.

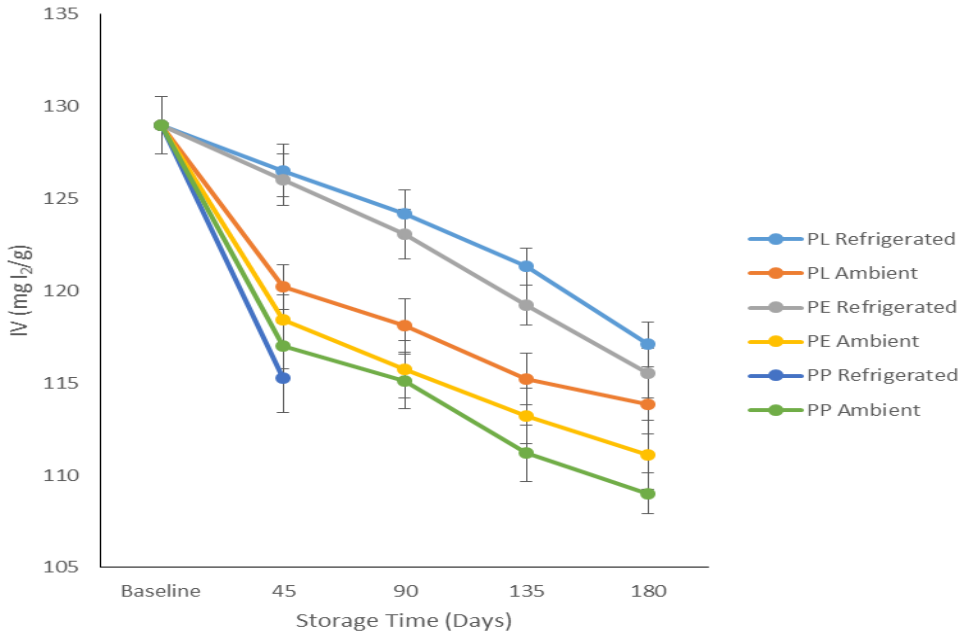


Figure 4.13: Iodine value (IV) of boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in the iodine value of stored adult house cricket meal is as shown in Figure 4.13. The iodine value of the samples continually decreased during the entire storage period. The iodine value is related to the degree of unsaturation of the oil and a decrease in the iodine value shows a decrease in the level of unsaturation of the oil (Boran et al., 2006). The decrease was higher ($P<0.05$) for samples stored in ambient conditions than the refrigerated samples. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and thus oxidation at ambient conditions was expected to be higher than at refrigeration. In ambient storage the decrease was ranging from 6.8-9.3% at 0-45 days, 8.4-10.8% at 45-90 days, 10.7-13.8% at 90-135 days and 11.7-15.5% at 135-180 days. In refrigerated storage the decrease was ranging from 1.9-6.0% at 0-45 days, 3.7-4.6% at 45-90 days, 6.0-7.6% at 90-135 days and 9.2-10.4% at 135-180 days of storage. At all the sampling stages the iodine value was decreasing in the order of PL<PE<PP. Most drastic change in IV during refrigerated storage was seen at 135-180 days where it was

ranging from 2.3-3.5% in both PE and PL packages. Most drastic storage for the samples stored under ambient conditions was seen at 0-45 days and was in the range of 6.8-9.3%. At the end of the storage period, samples in refrigerated storage had significantly ($P<0.05$) lower iodine values than sample in ambient storage. Refrigerated sample stored in PL package had the lowest iodine ($P>0.05$) value although there was no significant difference between this sample and the one stored in PE package in the same environment. PP package in ambient storage had the lowest iodine value although there was no variation in significance among the samples stored in this environment. The interaction between storage time, storage environment and type of package was significant ($P<0.001$) on the change in IV.

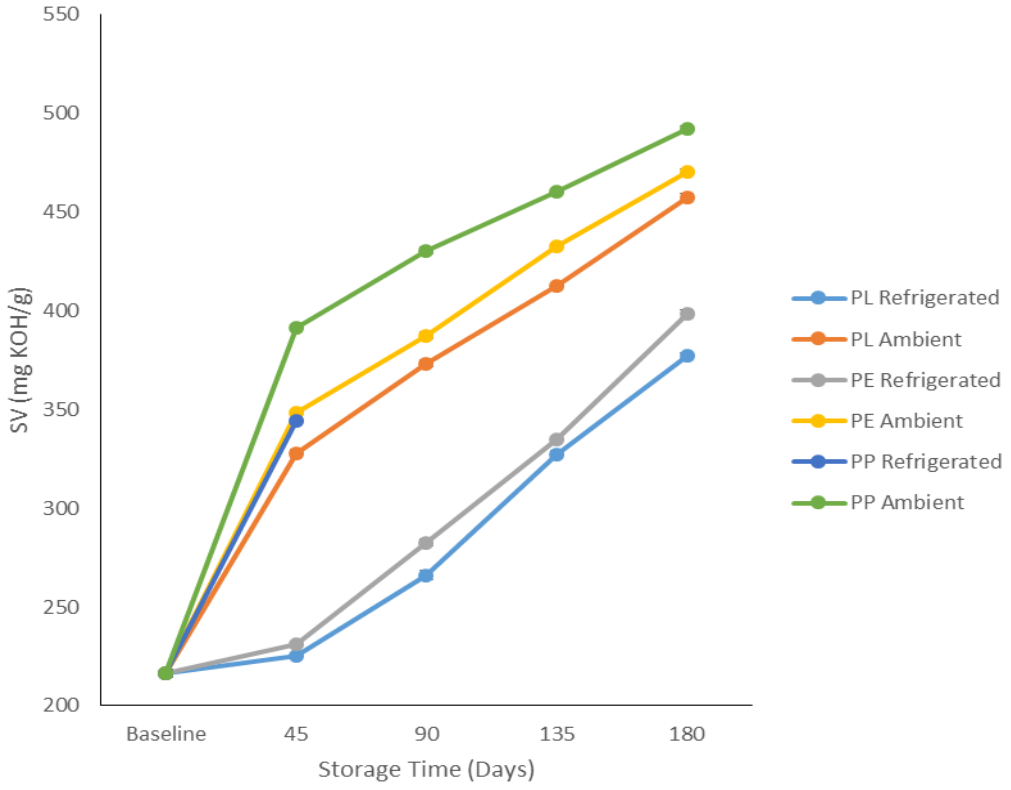


Figure 4.14: Saponification value (SV) of boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in saponification of semi-processed adult house cricket meal is as shown in Figure 4.14. The SV continually increased throughout the storage period. SV is a measure of the molecular weight and is defined as the amount of alkali required to saponify fatty acids in a given weight of oil. Hydrolysis and oxidation brings about lipid breakdown, forming free fatty acids or aldehydes and ketones as the end products. Increase in SV is not normally expected during this time but it is possible that the end products of oxidation such as aldehydes and ketones may contribute to increase in SV (Boran et al., 2006) The increase was higher in the different sampling periods in samples stored in ambient storage than in refrigerated samples. This was attributed to a higher temperature (Flick et al., 1992) that resulted in higher oxidation rate at ambient conditions than in refrigeration In ambient storage the increase was ranging from; 51.4-80.9% at 0-45 days, 72.5-98.9% at 45-90 days, 90.6-112.8% at 90-135 days and 111.4-127.4% at 135-180 days. In refrigerated storage the increase was ranging from 4.2-59.0% at 0-45 days, 23.0-79.0% at 45-90 days, 51.2-84.1% at 90-135 days and 74.4-84.1% at 135-180 days. The increase in SV in both storage environments was in the order PL<PE<PP. In ambient storage the increase was most drastic in the period between 0-45 days of storage in all the packages. Increase in PP, PE and PL packages was 80.9%, 61.0% and 51.4%. In refrigerated storage the increase was most drastic at 90-135 days for PL (23.0%) and at 45-90 days for PE (22.1%). At the end of the storage period, refrigerated samples had significantly ($P<0.05$) lower SV than samples in ambient storage. Sample stored in PL package and refrigerated had the least ($P<0.05$) SV while sample stored under ambient conditions in PP package had a significantly higher SV than all the other packages. The interaction between storage time, storage environment and type of package was highly significant ($P<0.001$) on the change in SV during storage.

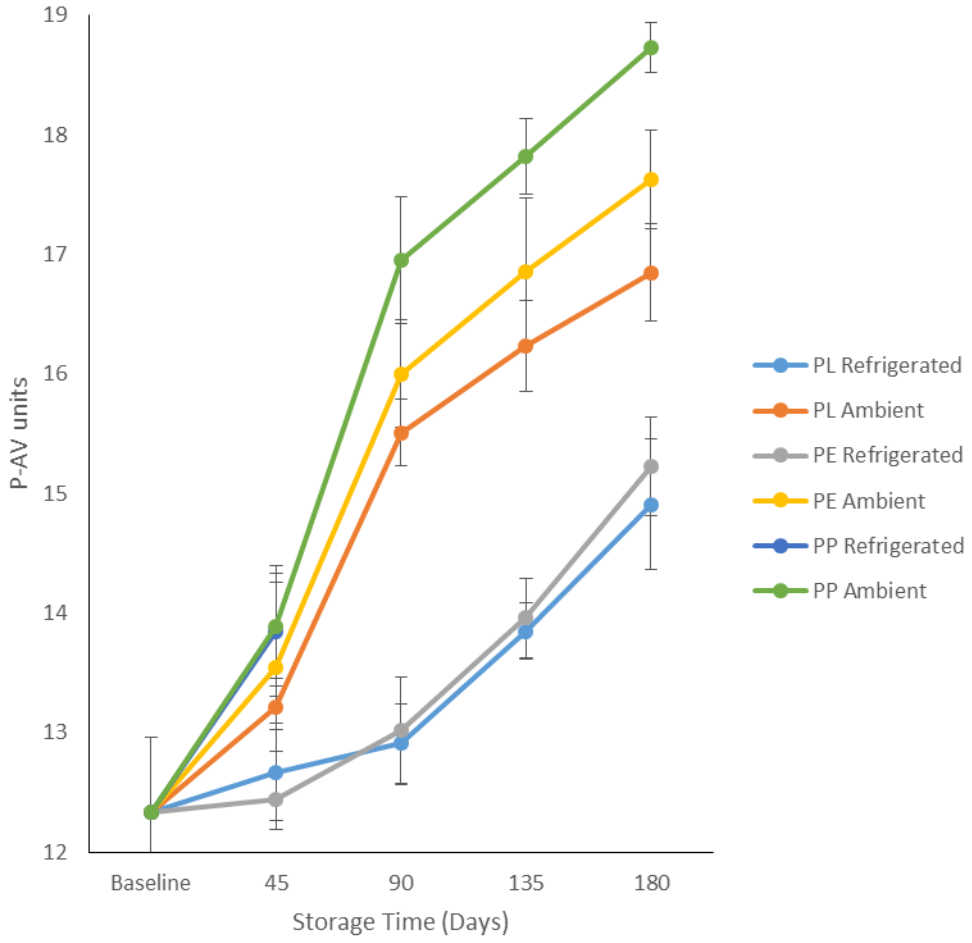


Figure 4.15: P-anisidine value (P-AV) of boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in the p-anisidine value of adult house cricket meal while in storage is as shown in Figure 4.15. The p-anisidine value progressively increased throughout the storage period. The p-anisidine value is a measure of the secondary products of lipid oxidation including aldehydes and other carbonyl compounds in oxidized lipids (Shahidi et al., 1994). Increase in p-anisidine value during storage of a product can be attributed to the activity of lipase activity originating from the biological or microorganisms tissue

(Boran et al., 2006) or to the conversion of primary oxidation products to secondary products (Maionese et al., 2004). Samples in ambient storage recorded higher ($P<0.05$) values than the samples in refrigeration**.** This can be attributed to higher temperatures that increased the lipid oxidation rates at ambient conditions (Flick et al., 1992). In ambient storage increase was ranging from 7.1-12.6% at 0-45 days, 25.7-37.4% at 45-90 days, 31.5-44.4% at 90-135 days and 36.6-51.8% at 135-180 days of storage. In refrigerated storage, the increase was ranging from 0.8-12.2% at 0-45 days, 4.6-5.5% at 45-90 days, 12.2-13.1% at 90-135 days and 20.8-23.4% at 135-180 days of storage. The increase in p-anisidine value in all the different sampling stages was in the order PP>PE>PL. In ambient storage most drastic increase in P-AV occurred at 45-90 (17.4-22.0%) days while in refrigeration most drastic increase occurred at 135-180 (7.7-9.1%) days. At the end of the storage period samples stored under ambient conditions had significantly higher P-AV values than the refrigerated samples. Refrigerated sample in PL package had the lowest p-anisidine although there was no significant ($P<0.05$) difference between this sample and the one stored in PE package under the same storage conditions. Sample stored in PP package under ambient conditions had the highest p-anisidine value. This value was significantly ($P<0.05$) higher than the one for the sample stored in PL package although it was not significantly different from the sample store in PE package in the same environment. The interaction between storage time, storage environment and type of package was significant ($P<0.001$) on the change in P-AV of the adult cricket meal.

The decrease in IV and increase in SV, PV and P-AV were higher at ambient temperatures than in refrigerated conditions. Lipid oxidation rates are directly related to temperature (Flick et al., 1992) and thus oxidation at ambient conditions was expected to be higher than at refrigeration. The results of this study were similar to (Boran et al., 2006) who noted that increase in PV, P-AV and SV and decrease in IV was higher at 4°C as compared to -18°C.

During storage most drastic change in IV, SV, PV and P-AV in ambient storage were seen at 0-45 days and at 135-180 days in refrigerated storage in all the packages. Drastic

changes in ambient storage at the start of the experiment indicate that oxidation rate was highest during this period. This can be attributed to heat treatment and solar drying at the start of the experiment that could have promoted free radical formation in the insects lipids thus facilitating the high oxidation rate (Toci et al., 2013). Drastic changes in refrigerated environment seemed to have been delayed to between 135-180 days in all the packages. Thus can be attributed to the reduced temperature that resulted in a reduced oxidation rate.

Lipid oxidation in the different packages was occurring in the order PP>PE>PL. This can be attributed to differences in the rates of diffusion of both gases and water vapor across the packages. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of oxidation. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages as shown in Table 5.The results of this study are similar to (Adebola & Nusa Halima, 2014) who also found microbial deterioration in stored stored Garri (food prepared from cassava roots) to decrease in the order of polypropylene (PP)> polyethylene bags (PE)> plastic buckets (PE).

4.2.2 Effect of storage time, storage environment and type of package on the microbial stability of adult House cricket meal (*Acheta domesticus*)

Adult house cricket are nutritionally rich and can provide suitable environment for the growth and proliferation of micro-organisms (Barroso et al., 2014; Rumpold & Schluter, 2013; Van Huis et al., 2013). Five microbial parameters including TVC, Enterobacteriaceae, *E-coli*, yeasts and moulds and *Salmonella* were analysed during storage of semi-processed adult house cricket meal at refrigeration and ambient conditions. The results of these are shown in Figures; 4.16, 4.17 and 4.18**.** Enterobacteriaceae, *E-coli* and *Salmonella* were not detected at the start of the experiment and during the entire storage period in all the samples. The results of this study were similar to Klunder et al., 2012 who also did not detect Enterobacteriaceae in boiled and dried house crickets and mealworm larvae. Absence of Enterobacteriaceae

can be attributed to the boiling step where they were all killed. There also seems to have been minimized contamination during processing, packaging and storage in this case as *E-coli* were not detected. Absence of Salmonella was in line with other studies involving insects and insect products (Braide et al., 2011; Klunder et al., 2012; Mpuchane et al., 2000; Mujuru et al., 2014; Opara et al., 2012; Van Huis et al., 2013) where no Salmonella were detected.

The total viable count for house cricket meal at the beginning of the experiment was found to be 5.2 log cfu/g. Although this was considerably high it was still the range of acceptability as compared to products such as cured meats and food stuffs that require further cooking where bacterial counts of up to 6.0 log cfu/g are acceptable (Stannard, 1997). Presence of bacteria even after processing can be attributed to contamination during processing, packaging and storage (Braide et al., 2011b; Mujuru et al., 2014; Opara et al., 2012). Yeasts and moulds were not detected at the beginning of the experiment although later on they were present. Fungi and their spores are widely distributed in air and may have contaminated the samples through the deposit of their spores when they were exposed to air during processing and packaging (Braide et al., 2011). This may also have been as a result of contamination through human contact and other post handling practices (Mujuru et al., 2014; Opara et al., 2012).

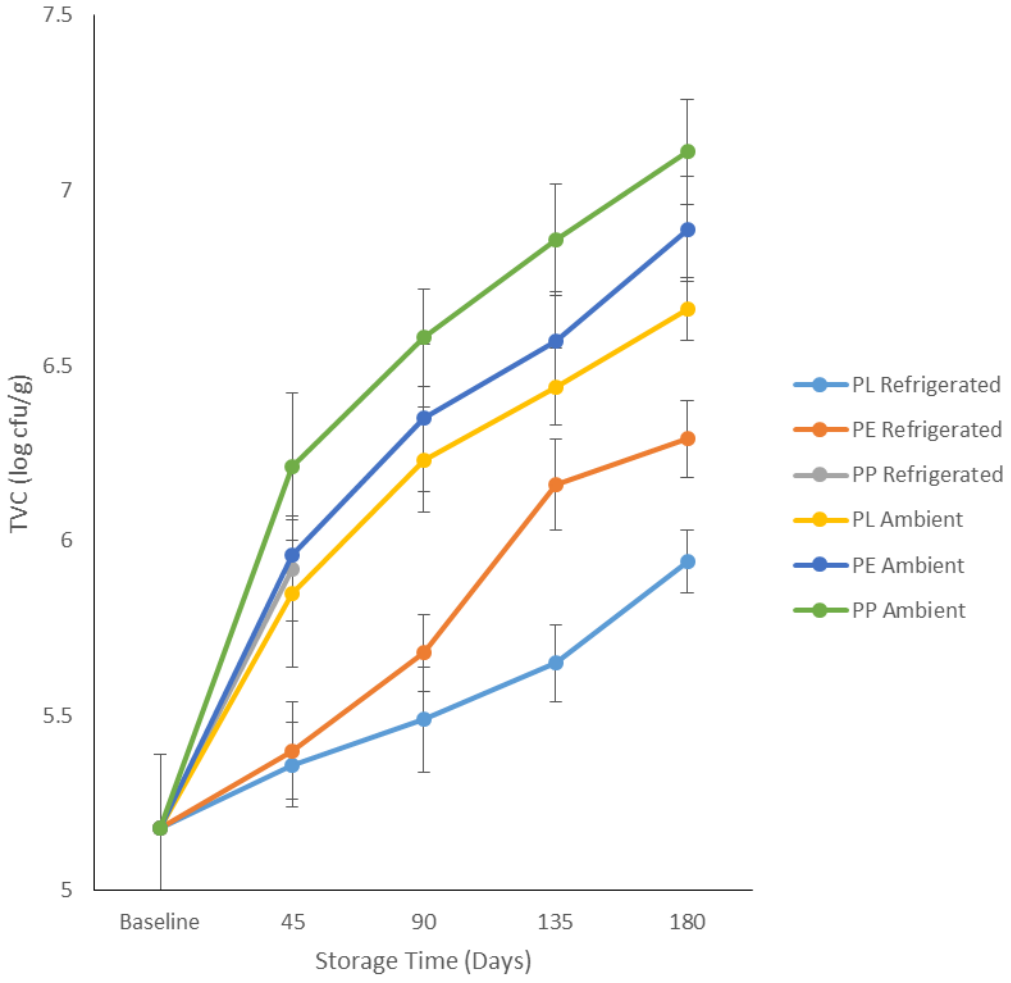


Figure 4.16: Total viable count in boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in the total viable count of stored house cricket meal is as shown in Figure 4.16. The TVC count steadily increased in all the packages throughout the storage period. House crickets are rich in nutrients and provide a suitable environment for the growth and proliferation of different microorganisms. The increase was higher in samples stored under ambient conditions than the refrigerated samples. Growth rates of most microorganisms are directly proportional to temperature and increase as temperature increases (Mossel at al., 1995). Under ambient conditions, the increase was

ranging from; 0.7-1.0 log cfu/g at 0-45 days, 1.1-1.4 log cfu/g at 45-90 days, 1.3-1.7 log cfu/g at 90-135 days and 1.5-1.9 log cfu/g at 135-180 days of storage. In refrigerated storage, the increase was ranging from; 0.2-0.7 log cfu/g at 0-45 days, 0.3-0.5 log cfu/g at 45-90 days, 0.5-1.0 log cfu/g at 90-135 days and 0.8-1.1 log cfu/g at 135-180 days. In both storage environments at the different sampling periods, TVC count was in the order PP>PE>PL. Under ambient conditions, most drastic increase in TVC was seen at 0-45 days. The increase in PP, PE and PL packages was 1.0, 0.8 and 0.7 log cfu/g respectively. In refrigerated environment most drastic increase occurred at 135-180 days (0.3 log cfu/g) in PL and at 90-135 days (0.5 log cfu/g) in PE package. At the end of the storage period, refrigerated samples had significantly lower TVC count than samples in ambient storage. Refrigerated sample in PL package had the lowest microbial count although it was not significantly ($P>0.05$) different from that in the PE package. Under ambient conditions, sample packaged in PP had the highest TVC count although there was no variation in significance among all the packages in this environment. The storage environment, time and type of packaging all had a significant ($P<0.001$) effect on the TVC of the adult cricket meal. The interaction between storage time, storage environment and type of package was however not significant ($P>0.05$) on the TVC count during storage.

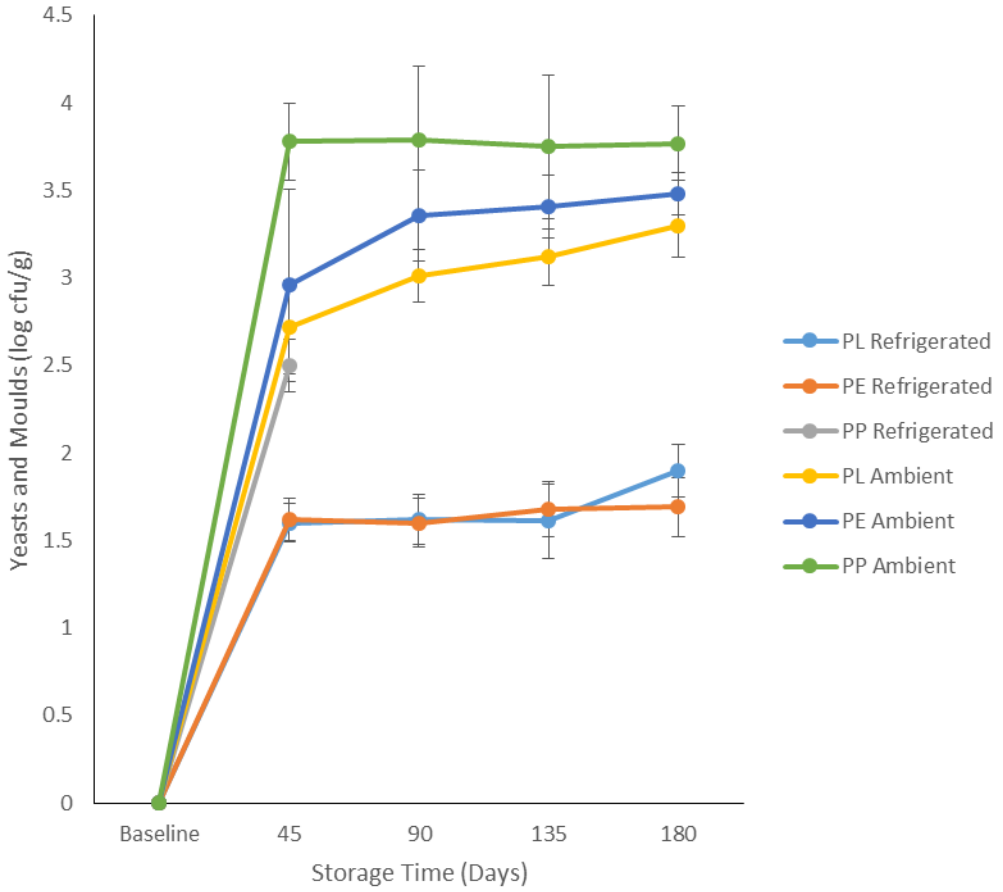


Figure 4.17: Yeasts and mould count in boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in yeasts and moulds during storage of house cricket meal is as shown in Figure 4.17. Yeasts and moulds were not detected at the start of the experiment although later on at the subsequent sampling stages they were detected. Fungi and their spores are widely distributed in air and may have contaminated the samples through the deposit of their spores when they were exposed to air during processing and packaging (Braide et al., 2011). Yeasts and moulds increased throughout the entire storage period owing to the rich nutrient content of the house cricket meal. The increases were higher in ambient

storage than in refrigeration. Increase in ambient storage at each of the sampling stages was ranging from; 2.7-3.8 log cfu/g at 0-45 days, 3.0-3.8 log cfu/g at 45-90 days, 3.1-3.8 log cfu/g at 90-135 days and 3.3-3.8 log cfu/g at 135-180 days. In refrigerated storage the increase was ranging from; 1.6-2.5 log cfu/g at 0-45 days, 1.6-1.6 log cfu/g at 45-90 days, 1.6-1.7 log cfu/g at 90-135 days and 1.7-1.9 log cfu/g at 135-180 days of storage. Yeast and mould count at each of the sampling stages in the two storage environments was in the order PP>PE>PL. At the end of the storage period, samples in ambient storage had a higher ($P<0.05$) yeast and mould count than the refrigerated samples. Under ambient storage sample stored in PP had the highest count although there was no variation in significance among the three packages in this environment. Refrigerated sample in PL had the lowest count although it was not significantly different from that in the PE package. The storage environment, time and type of packaging all had a significant ($P<0.001$) effect on the yeast and mould count of the adult cricket meal. The interaction between storage time, storage environment and type of package was however not significant ($P>0.05$) on the yeast and mould count during storage.

Aspergillus, Alterneria and penicillium were the major groups of moulds that were identified as shown in Plate 4.1. Aspergillus and Penicillium species have also been isolated in degutted Mopani worms (Mujuru et al., 2014)

During storage, most drastic increase in TVC under ambient conditions was seen at 0-45 days while in refrigeration the drastic increase was delayed to 90-135 (PE) days and 135-180 (PL) days. In ambient storage the first 0-45 days of storage seems to have coincided with the log phase where the growth and multiplication rate in microorganisms is usually very high. In the subsequent sampling stages the growth rate was reduced and this could be attributed to factors such as competition and reduction in nutrients (Foods, 2001). In refrigerated storage, the log phase seems to have been delayed to 135 days in PE and 180 days in PL packages. This may be due reduction in temperature that leads to an increase in the lag phase of microbial growth (Foods, 2001).

Among the different packages used, increase in microorganisms detected was in the order PP>PE>PL. At the end of the experiment, microbial count was higher in PP packages than in the other packages. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of microbial growth. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages (Allahvaisi, 2012). Permeability to relative humidity among the packages could have interfered (caused it to increase) with water activity of the samples thus promoting microbial growth (Foods, 2001). Sample packaged in refrigerated PP bags was analyzed only up to the 45^{th} day of storage (refer to section 4.1.3)

4.2.3 Effect of storage time, storage environment and type of package on the fatty acid composition of adult House cricket meal (*Acheta domesticus*)

Degradation in fatty acid profiles of boiled solar dried adult house cricket meal is as shown in Figures 4.18, 4.19, and 4.20. Freshly boiled, solar dried and ground samples were composed of 27.1% SFA, 21.0% MUFA and 49.6% PUFA. The most dominant SFA were; palmitic acid (20.6%) and stearic acid (4.7%), the major MUFA was oleic acid (21.0%) while the major PUFA was linoleic acid (40.4%). The samples also contained appreciable amounts of n3 PUFAS i.e. DHA (1.8%) and EPA (2.7%). This results were comparable to Finke, 2002 who also found that the major fatty acids in house crickets were palmitic, stearic, oleic and linoleic acids.

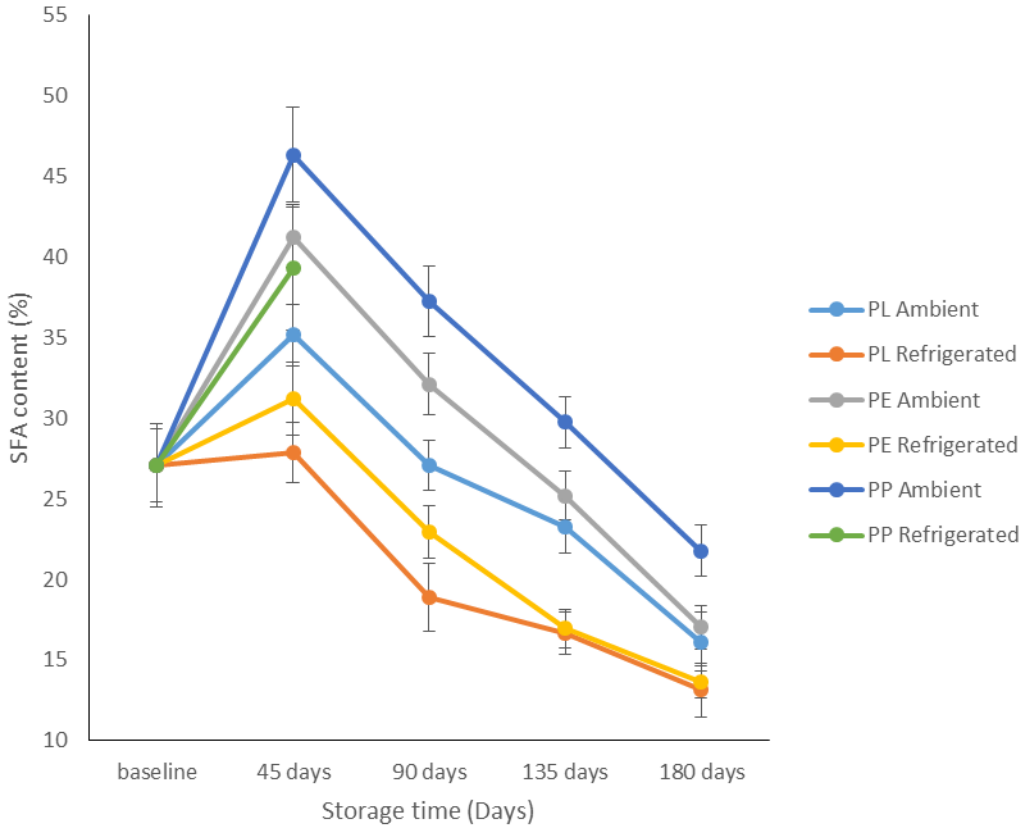


Figure 4.18: Saturated fatty acid content in boiled and solar dried adult house cricket meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in SFA content of semi-processed adult cricket meal during storage is as shown in Figure 4.18. The SFA content increased in the first 45 days of storage after which it continuously declined during the rest of the storage period. Increase in saturated fatty acids during storage has been deemed to be the effects of decrease in other fatty (MUFA and PUFA) acids (Isdell et al., 2003).The increase was higher in samples packaged in ambient storage than in refrigerated storage. This can be attributed to higher temperatures at ambient storage where auto-oxidation and decomposition of hydroperoxides is higher (Choe & Min, 2006). Effect of dissolved oxygen is also

increases with increase in temperature (Choe & Min, 2006). This implies that as the temperature increase, oxygen dissolved in lipids becomes more active and breaks down the fatty acid chain at a faster rate. Increase in ambient storage was 29.9%, 52.4% and 71.3% in PL, PE and PP packages respectively. Increase in refrigerated storage was 3.0 %, 15.3% and 45.3% in PL, PE and PP packages respectively. The increase in SFA in both storage environments was in the order PL<PE<PP. The increase was followed by a subsequent decrease during the rest of the storage period. The decrease was significantly higher ($P<0.05$) in ambient storage than in refrigeration. Decrease in ambient storage was ranging from; 7.0-13.9% at 90-135 days and 19.5-40.4%. In refrigerated storage the decrease was ranging from 15.2-30.3% at 90 days, 37.4-38.5% at 90-135 days and 49.5-51.5% at 135-180 days. During the storage period, most drastic changes in SFA content were seen in the first 45 days in ambient storage where SFA increased and at 90 days in refrigerated storage where the SFA decreased. After 180 days of storage, SFA content was significantly higher in the sample packaged in PP package in ambient storage than the samples in all the other packages where there was no significant difference between them regardless of the storage environment. The interaction between storage time, storage environment and type of package was significant ($P<0.001$) on the change in SFA.

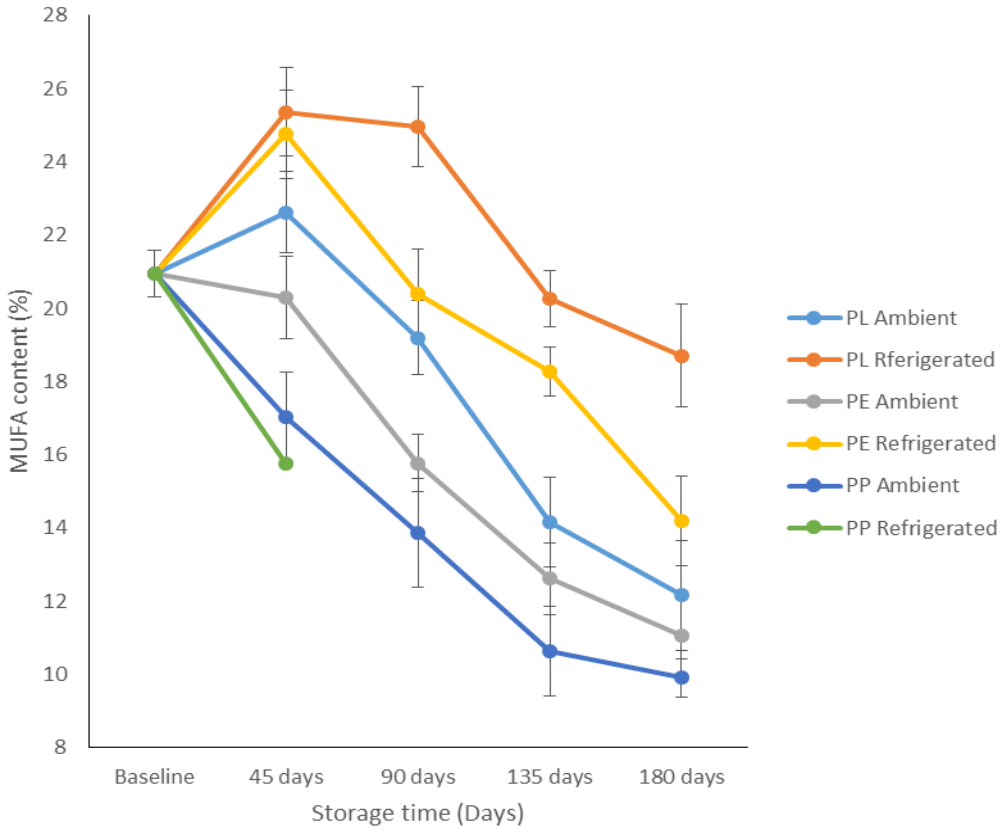


Figure 4.19: Monounsaturated fatty acid (MUFA) content in boiled and solar dried adult house cricket meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Change in the MUFA content of stored semi-processed house cricket meal is as shown in Figure 4.19. The MUFA content seems to have generally decreased during the storage duration. Decrease in MUFA content was attributed to oxidation that was taking place during storage (Bhulaidok et al., 2010). There was a slight increase in the MUFA content during the first 45 days in some of the packages followed by a subsequent decrease. The slight increase in MUFA content was attributed to decrease in the PUFA especially linoleic and linolenic acids. The decrease in MUFA content was higher in ambient storage than in refrigeration. Lipid oxidation rates are directly related to

temperature (Flick et al., 1992) and effect of dissolved oxygen is also increases with increase in temperature (Choe & Min, 2006). In ambient storage the decrease was ranging from; 32.4-49.3% at 90-135 days and 42.0-52.8% at 135-180 days. In Refrigerated storage, the decrease was ranging from 3.3-12.8% at 90-135 days and 10.7-32.3% at 135-180 days of storage. MUFA content in all the sampling stages in both storage environments was decreasing in the order PP<PE<PL. At the end of the storage period, refrigerated samples had significantly higher MUFA content than samples in ambient storage. Refrigerated sample in PL had the highest ($P<0.05$) MUFA content while sample packaged in PP in ambient conditions had the lowest MUFA content although there was no variation in significance among the packages in ambient storage. The interaction effect between storage environment, time and type of package was highly significant ($P<0.001$) on the change in MUFA.

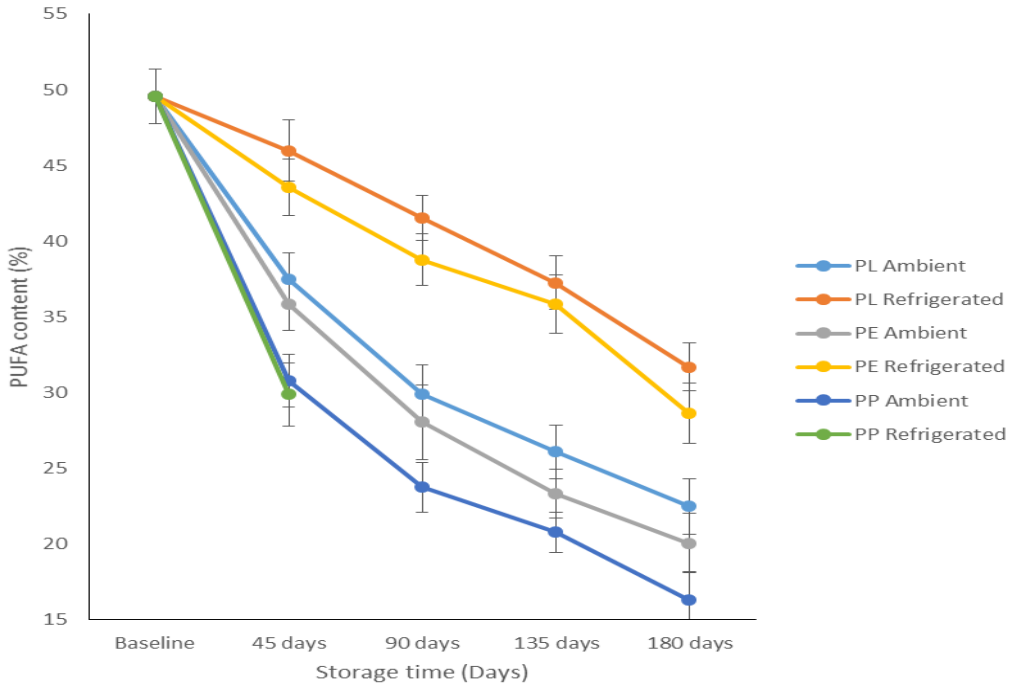


Figure 4.20: Polyunsaturated fatty acid content in boiled and solar dried adult house cricket meal stored for 180 days under different conditions (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

Variation in the PUFA content of stored semi-processed house cricket meal is as shown in Figure 4.20. The PUFA content progressively decreased during the entire storage period in all the packages. Decrease in PUFA content was due to oxidation that was taking place during storage (Bhulaidok et al., 2010). The decrease was significantly higher ($P<0.05$) in refrigerated storage than in ambient. This can be attributed to higher temperatures at ambient storage where auto-oxidation and decomposition of hydroperoxides is higher (Choe & Min, 2006). Effect of dissolved oxygen is also increases with increase in temperature (Choe & Min, 2006). In ambient storage the decrease was ranging from 24.3-37.9% at 0-45 days, 39.6-52.1% at 45-90 days, 47.3-58.1% at 90-135 days and 54.6-67.0% at 135-180 days of storage. In refrigerated storage the decrease was ranging from 7.2-39.7% at 0-45 days, 16.2-21.8% at 45-90 days, 24.8-27.7% at 90-135 days and 36.0-42.2% at 135-180 days of storage. Decrease in PUFA content in all the sampling stages was in the order PP>PE>PL in both storage environments. During the storage; in ambient storage, most drastic decrease in PUFA content was seen at 0-45 days (24.3-37.9%) and 45-90 days (20.2%-22.9%) of storage while in refrigerated storage most drastic decrease was seen at 180 days of storage in both PL (14.9%) and PE (20.1%). At the end of the storage period, refrigerated samples had significantly higher PUFA content than samples in ambient storage. Refrigerated sample in PL had the highest PUFA content although it was not significantly different from the one in PE. In ambient storage, sample in PP package had the lowest ($P<0.05$) PUFA content. The interaction between storage time, environment and type of packaging was highly significant ($P<0.001$) on the change in PUFA content during storage.

During storage highest decrease in the unsaturated fatty (MUFA and PUFA) acids were seen at 0-45 days in ambient storage and at 90-180 days in refrigerated storage in all the packages. In ambient storage conditions drastic decrease in unsaturated fatty acids at the beginning of the experiment indicated that oxidation was highest at this period. Heat treatment (boiling) during processing could have promoted free radical formation (Toci et al., 2013) thus promoting high oxidation rate at the beginning of the experiment.

Drastic changes in refrigerated storage seemed to have been delayed to the period of between 90-180 days of storage. Thus can be attributed to the reduced temperature that resulted in a reduced oxidation rate.

Fatty acid oxidation in the different packages was occurring in the order PP>PE>PL. This can be attributed to differences in the rates of diffusion of both gases and water vapor across the packages. The woven PP package had a higher permeability to both gases and water vapor that could have favored a higher rate of oxidation. Different authors have also noted that polyethylene packages have a higher permeability to gases and water vapor than plastic polypropylene packages as shown in Table (3.2).

4.2.4 Effect of storage time, storage environment and type of package on the colour of adult House cricket meal (*Acheta domesticus*)

Results for color change in semi-processed adult house cricket meal are presented in Table 4.4.

Table 4.4: Color changes in semi-processed adult house cricket stored in PE, PL and PP packages in ambient and refrigerated conditions.

Packaging Material	Storage time (Days)	Hue angle		Chroma		Total color change	
		4^0C	25^0C	4^0C	25^0C	4^0C	25^0C
PE	0	78.4±0.4^{def}	78.4±0.4^{def}	14.5±0.2^{abcd}	14.5±0.2^{abcd}		
	45	82.0±0.4^{abc}	82.5±0.4^{bc}	16.0±0.1^{abc}	16.3±0.5^{c}	2.4±0.9^{ab}	3.0±1.3^{b}
	90	82.1±1.3^{abc}	81.9±0.1^{abc}	14.7±0.5^{abcd}	15.1±0.5^{abcd}	2.1±1.1^{ab}	1.8±0.6^{ab}
	135	83.0±1.6^{c}	81.1±0.5^{abcef}	14.9±0.5^{abcd}	15.9±0.9^{abc}	1.8±0.7^{ab}	1.6±0.7^{ab}
	180	83.3±0.7^{c}	78.7±2.3^{adef}	15.3±0.3^{abc}	14.2±1.1^{abcd}	1.5±0.3^{ab}	1.4±0.4^{ab}
PL	0	78.4±0.4^{def}	78.4±0.4^{def}	14.5±0.2^{abcd}	14.5±0.2^{abcd}		
	45	71.1±1.5^{g}	72.7±0.7^{g}	14.3±0.4^{abcd}	15.4±0.2^{abc}	2.1±0.6^{ab}	2.8±1.0^{b}
	90	77.3±0.3^{d}	79.7±1.5^{abcdef}	14.4±0.6^{abcd}	15.7±0.4^{abc}	2.1±1.4^{ab}	1.8±1.3^{ab}
	135	78.1±0.8^{def}	80.6±1.1^{abcdef}	13.2±0.5^{d}	14.9±0.6^{abcd}	1.9±1.0^{ab}	1.4±0.2^{ab}
	180	80.2±1.3^{abcdef}	77.9±1.7^{de}	14.0±0.4^{ad}	14.4±0.9^{abcd}	1.7±0.2^{ab}	1.2±0.5^{ab}
PP	0	78.4±0.4^{def}	78.4±0.4^{def}	14.5±0.2^{abcd}	14.5±0.2^{abcd}		
	45	78.9±0.1^{abdef}	73.1±1.1^{g}	14.9±0.2^{abcd}	16.1±0.5^{bc}	1.6±0.2^{ab}	4.4±1.1^{b}
	90	Nd	78.9±0.4^{abdef}	Nd	15.8±1.1^{abc}	Nd	3.2±2.5^{b}
	135	Nd	81.7±0.1^{abcf}	Nd	15.8±0.2^{abc}	Nd	2.5±0.5^{ab}
	180	Nd	80.4±0.7^{abcdef}	Nd	15.2±0.6^{abcd}	Nd	1.4±0.9^{ab}

PE= Polyethylene package, PL=Plastic package, PP=Polypropylene package. Nd- Not determined

The hue angle and Chroma values of freshly boiled and solar dried adult house cricket were 78.4 and 14.5 respectively. In ambient storage, there was a significant decrease in hue angle in PL and PP packages for the first 45 days followed by a significant increase at 90 days after which the value did not change significantly. Sample packaged in PE showed a significant increase at the 45^{th} day in hue angle after which it was relatively constant with no further significant ($P>0.05$) changes. The combined effect of storage time, environment of storage and type of package was highly significant ($P<0.001$). The chroma values were relatively constant throughout the entire storage period. Consistently higher values were seen in samples store in ambient conditions than in refrigerated samples but there were no significant differences ($P>0.05$). Combined effect of storage time, environment of storage and type of package was significant ($P<0.001$). Total colour change indicates the magnitude of difference between locations in the CIE L*a*b* color system (Isdell et al., 2003).The total color change seems to have been high at the start of the experiment and then steadily decreased with increase in storage time. The color change was higher in ambient storage although there was no variation in significance. This can be attributed to a higher temperature that accelerates degradation reactions in the sample including oxidation thus leading to a greater color change. Similar results were noted in packaged beef, where samples stored at a higher temperature had a greater degree of color degradation (Isdell et al., 2003) The combined effect of storage time, environment of storage and type of package was not significant ($P>0.05$).

4.3 Water adsorption properties and shelf-life estimation of semi-processed adult house cricket (*Acheta domesticus*) and black soldier fly larvae (*Hermetia illucens)*

4.3.1 Proximate composition of black soldier fly larvae and adult house cricket

Protein, fat, fibre ash and carbohydrate contents are shown in Table 4.5. The composition agrees with previous values reported in the literature for the two insects (Finke, 2002; Tran et al., 2015). The cricket powder contained significantly higher

($p \leq 0.001$) amounts of crude protein, which is comparable to the crude protein content of fresh African palm weevil *Rhynchophorus phoenicus* Fabricius (Coleoptera: Curculionidae) reported to be 66.09% (Ekpo, 2011) and traditionally processed (boiled in salty water for one hour and sun dried) *Hemijana variegate* caterpillar (44.5 %) (Egan et al., 2014). The BSFL powder contained significantly ($p \leq 0.001$) higher levels of fat, fibre, and ash. Fat content of BSFL was comparable to that of fresh African palm weevil (*Rhynchophorus phoenics*) (21.2%) while that of cricket was lower than traditionally processed *Hemijana variegate* caterpillar (Egan et al., 2014). The ash contents were higher than those reported for yellow mealworm (*Tenebrio molitor*) (2.50%) and *Hemijana variegate* caterpillar (2.37%) (Egan et al.,2014). The fibre contents compared well with those recorded for termites (6%) and June beetles (12%) (Siriamornpun and Thammapat, 2008). The available carbohydrate content of both insect meals was higher than that reported for the beetle *Tenebrio molitor* (0.01-3.86%) and the stink bug *Euschistus strennus* (0.01%) (Rumpold & Schluter, 2013) showing that when consumed a higher amount of carbohydrate would be digested and absorbed.

Table 4.5: Proximate composition (g/100g DM) of cricket and BSFL powders

Parameter	Cricket	Black soldier fly larvae
Protein	65.9 ± 4.8^{a}	44.0 ± 0.4^{b}
Fat	12.2 ± 0.2^{a}	25.0 ± 0.5^{b}
Fibre	6.8 ± 0.1^{a}	10.0 ± 0.4^{b}
Ash	4.8 ± 0.2^{a}	9.5 ± 0.0^{b}
Available carbohydrate	10.3 ± 5.0^{a}	11.5 ± 1.5^{a}

Means ± standard deviation (n=3) sharing different letters in the same row are significantly different ($p \leq 0.05$).

4.3.2 Sorption isotherms

Equilibrium moisture content (M_{eq}) of cricket and BSFL powders at different a_W levels of equilibrium relative humidity are shown in Figure (4.21). The two insect meals

exhibited type II sorption isotherms according to Brunauers classification, which is due to monolayer-multilayer sorption mechanism. At low a_W, moisture is adsorbed to active adsorption sites. As the a_W increases more water molecules become associated to the bound moisture covalently creating multilayer that is less strongly bound. Further increase in multilayer sorption at higher a_W levels causes dissolution of soluble low molecular weight constituents causing a sharp increase in equilibrium moisture content at $a_W > 0.8$. Nevertheless, sorption profiles vary depending on the adsorbing substrate (Al-muhtaseb, 2004), and reflect the way in which the water binds the system. Type II isotherms have also been reported for meal worm larvae (*Tenebrio molitor*) (Azzollini et al., 2016) and other high protein foods such as Pirarucu (*Arapaima gigas*) fillet (Martins et al., 2015) and lean beef (Trujillo et al., 2003). Cricket powder was found to adsorb more water at all three temperature regimes, which can be attributed to compositional factors and physicochemical properties of the samples**.** From the proximate composition, BSFL powder contained less protein and more fat, and would therefore have fewer hydrophilic sites as compared to cricket flour. At constant a_W, the M_{eq} decreased with increase in temperature in both substrates, although the change was more evident in the BSFL powder. The decrease in adsorbed moisture with increasing equilibration temperature could be attributed to the excitation of water molecules promoting them to higher energy levels, as such causing them to break away from their binding sites (Al-Mahasneh et al., 2014). Proteins are also known to have more water binding capacities at lower temperatures (Hong et al., 2008). At a constant M_{eq}, a_W of both cricket and BSFL powders was found to increase with increased in temperature, but the BSFL powder was evidently more sensitive to this effect. For instance, at a moisture content of 5g/100g, increasing the equilibration temperature from 25° C to 30° C and 35° C increases the a_W of BSFL powder from 0.49 to 0.57 and 0.67, respectively. A a_W of 0.67 may support mould growth. Moreover, an increase in a_W by 0.1 was shown to cause decrease in shelf-life of a food or feed by a factor of two to three (Labuza, 1984). Thus drying to a

much lower moisture content would be needed to be achieved in warmer storage environments.

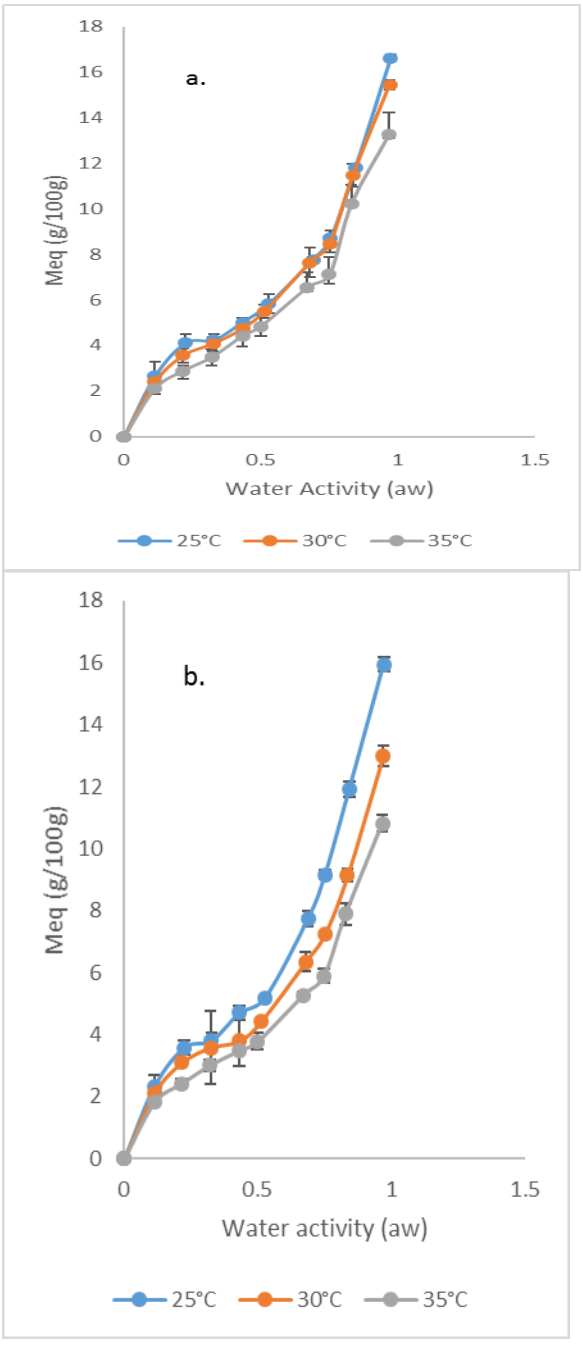


Figure 4.21: Moisture adsorption isotherms for cricket (a) and BSFL (b) at three different temperatures

4.3.4 Fitting of sorption models

Figures 4.22 and 4.23 show the experimental data fitted to GAB (Guggenheim Anderson De Boer), BET (Brannuer Emmet Teller), Oswin, Caurie and Khun models over the theoretically appropriate ranges. The model parameters alongside the measures of fit are presented in Table (4.6). All the models fitted the experimental data well with RMSE of ≤10% but the GAB and BET models provided the best fits judging from the RMSE and R^2 values. The GAB simulation fitted a broader a_W range compared to BET model. In isotherms, the BET model overestimated the sorption at a_W range > 0.43 and could therefore be used to characterize the monolayer sorption whereas the GAB model underestimated the sorption at a_W range >0.75, meaning that the model would be useful in characterizing monolayer and multilayer sorptions of the two substrates except for the cricket powder equilibrated at 25°C. Caurie model fitted the experimental data in the entire a_W range and would be useful in characterizing monolayer, multilayer and free water sorption. The Smith model underestimated sorption at a_W <0.6 whereas the Khun model overestimated sorption at a_W <0.2 and >0.75.

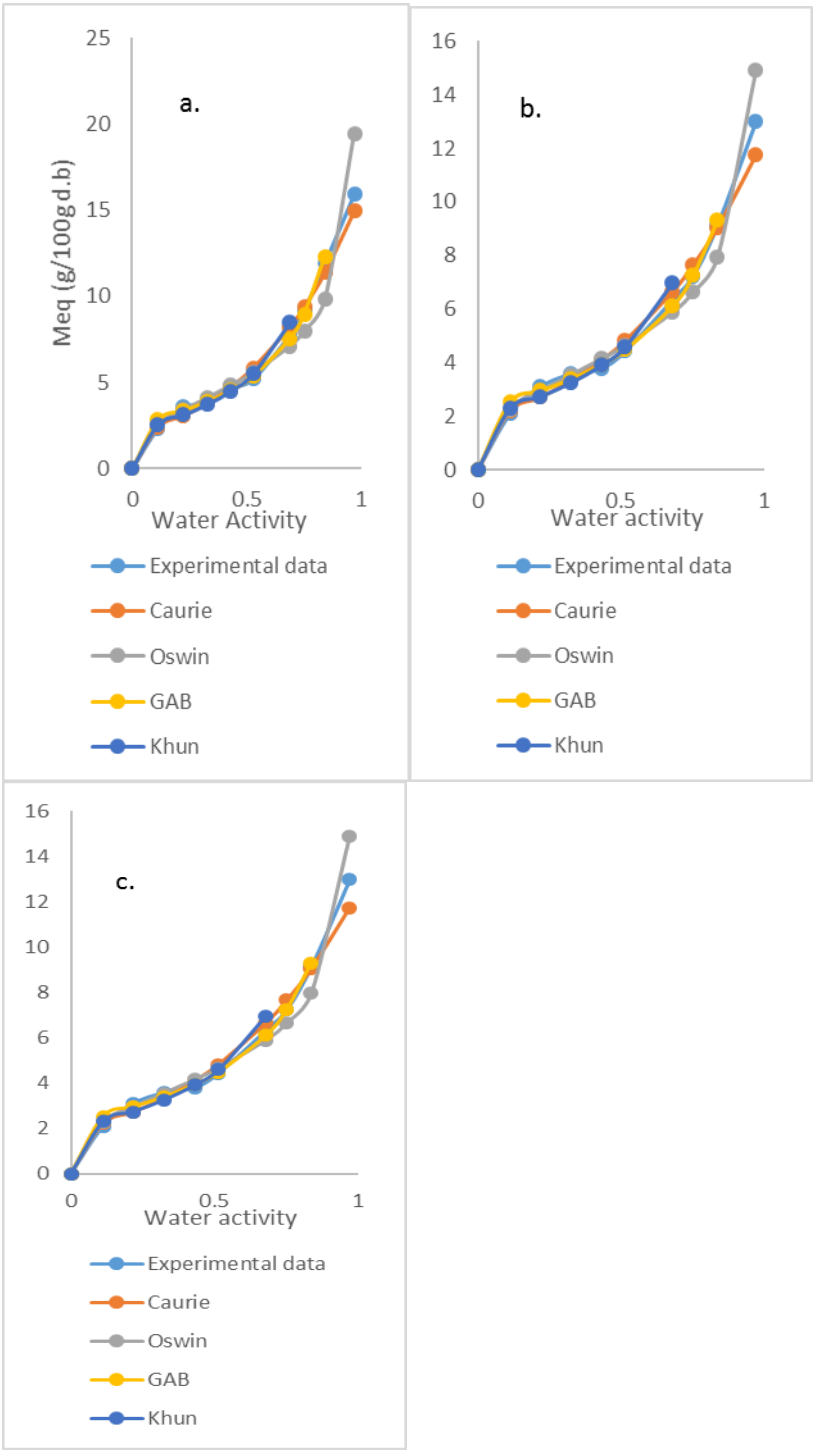


Figure 4. 22: Caurie, Oswin, Smith and GAB fits to experimental data at 25°C (a), 30°C (b) and 35°C (c) for BSFL

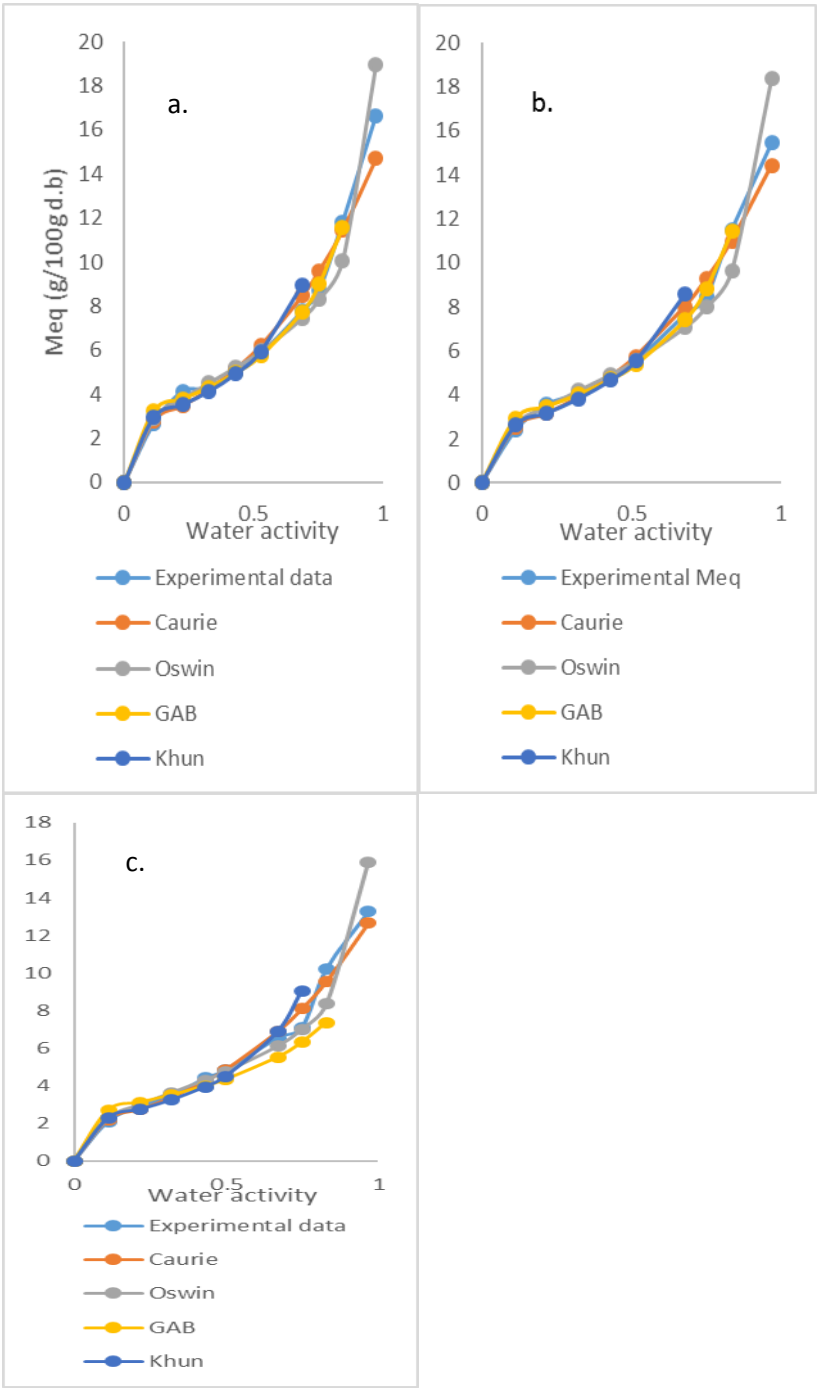


Figure 4.23: Caurie, Oswin, Smith and GAB fits to experimental data at 25°C (a), 30°C (b) and 35°C (c) for cricket

Table 4.6: Model parameters and measures of fit at the various temperatures

Model	Cricket			BSFL		
	25°C	30°C	35°C	25°C	30°C	35°C
GAB (a_w<0.75)						
M_o (g/100g)	3.593	3.556	3.288	2.943	2.852	2.561
C_g	21.463	15.214	13.653	24.948	20.534	18.809
k_g	0.799	0.803	0.784	0.909	0.825	0.783
R^2	0.999	0.997	0.999	0.997	0.997	0.998
RMSE (%)	1.62	1.76	1.78	3.07	2.49	1.69
BET (a_w<0.43)						
M_o (g/100g)	3.017	2.899	2.682	2.833	2.519	2.132
C_b	33.071	23.594	18.334	26.811	24.450	23.998
R^2	0.996	0.997	0.999	0.996	0.995	0.996
RMSE (%)	3.13	2.28	0.88	2.91	2.72	2.52
Oswin (a_w<0.98)						
A	5.643	5.459	4.841	5.537	4.569	3.834
B	0.432	0.443	0.464	0.423	0.437	0.469
R^2	0.998	0.999	0.998	0.997	0.998	0.997
RMSE (%)	6.25	3.45	6.15	4.19	6.387	11.68
Caurie (aw<0.98)						
C_c	1.104	1.099	1.059	1.094	0.999	0.948
M_o	5.111	4.966	4.569	5.026	4.571	4.046
R^2	0.998	0.999	0.998	0.997	0.998	0.997
RMSE (%)	6.23	3.46	6.13	4.17	6.368	11.61
Smith (a_w>0.5)						
A	-1.015	-0.884	-1.216	-0.485	-0.738	-1.201
B	7.495	7.352	6.999	7.001	6.055	5.700
R^2	0.983	0.983	0.979	0.997	0.98	0.973
RMSE (%)	11.77	11.09	13.97	4.96	10.75	15.56
Khun (aw<0.75)						
A	-1.898	-1.929	-1.756	-2.121	-1.635	-1.323
B	2.441	2.228	1.898	1.891	1.862	1.671
R^2	0.980	0.974	0.971	0.990	0.980	0.974
RMSE (%)	4.28	5.50	6.02	3.87	4.91	5.27

C_b, C_g, k_g, C_c, A and B : model constants,; M_o : Monolayer moisture content (g/100g); R^2: coefficient of determination; RMSE: root mean square error.

4.3.5 Properties of sorbed water

The BET, GAB and Caurie models were rated superior as the magnitudes of their parameters have physical meaning (Timmerman, 2003; Rao et al., 2006), unlike those of the Oswin, Khun and Smith model which, at best, only provided mathematical compensation for curve fitting. Theoretically, C_b and C_g constants from the BET and GAB equations relate to the net enthalpies of sorption of the monolayer whereas k_g from GAB model relates to the enthalpy of sorption of multilayer (Mutungi et al., 2011). With increasing equilibration temperature, C_b and C_g decreased in the two substrates, indicating that monolayer binding energies decreased. The parameter k_g also decreased with increasing equilibration temperature particularly for BSLF powder, pointing to a less pronounced multilayer sorption and liquefaction of water molecules in the void spaces of the material (Timmermann, 2003).

The BET, GAB and the Caurie models also estimated the monolayer moisture loading (M_o), that is, the moisture content when all the ionic and polar groups of the adsorbent have been occupied by water molecules (Valdez-Niebla et al.,1993). The M_o is the moisture content for utmost stability of a food material (Igbabul et al., 2013). The BET model predicted lower M_o values 2.1-3.0 g/100g compared to the GAB model 2.6-3.6 g/100 g and caurie model 4.0-5.1 g/100 g. This may be attributed to the small a_W range covered by the BET model. The M_o values, nevertheless, compared well with those reported for dried milled fish (3.24%-5.12%) at 25°C-50°C where the major component, like in dried milled crickets and BSFL, is protein (Sablani et al., 2001). Azzollini et al (2016) estimated GAB M_o of 5 g/100 g for *T. molitor* powder. The BET M_o could be regarded as the tightly bound water. At this very low moisture content, chemical reactions that depend on solvation are expected to be rather slow (Rao et al., 2006). However, deteriorations arising from lipid phase reactions such as oxidative

rancidification may be enhanced. As determined in the proximate composition, about a quarter of dry BSFL powder comprises fat, thus lipid phase deteriorative reactions may be considerable at such low moisture content. Generally, BSFL powder had the lowest M_o. Higher monolayer values of cricket powder can be attributed the higher protein and lower fat content thus providing more active adsorption sites. Furthermore, the M_o of both substrates decreased with increase in equilibration temperature, which correlated well with the decrease monolayer energy constants C_g and C_b predicted by the BET and GAB models, respectively. Similar trends have been reported in numerous foods (Akoy et al., 2013; Al-Mahasneh et al., 2014; Chalermchat & Owasit, 2011; Goneli et al., 2016; Kiranoudis, 1993; Seid & Hensel, 2012).

The Caurie equation further allows evaluation of a number of properties of sorbed water: namely number of binding sites, density of sorbed water, surface area of absorbent and proportion of bound water (Rao et al., 2006). The number of adsorbed monolayers (n_m) may be calculated as the ratio of the Caurie monolayer moisture content to the Caurie constant $\left(M_o/C_c\right)$. Also caurie constant C_c is assumed to be equivalent to the density of adsorbed water in the monolayer, thus enabling estimation of the surface area of adsorbent (A) using the expression: $A = M_o/\left(C_c \times d \times 10^8\right)$, where d is the diameter of water molecule ($d = 3.673\times10^{-10}\,\mathrm{m}$). The per cent bound water is obtained by $M_o \times n_m$. Table (15) presents the computed values for these parameters. The density of sorbed water (C_c) was higher in the cricket powder and decreased with increasing equilibration temperature. Percent bound water, and surface areas of adsorbent were also higher in the cricket powder and decreased with temperature. The decline in bound water with increasing temperature implies a decrease in sorption activity at the low a_W range, and a decrease in surface area reflects a decline in the exposure of charged polar groups and carboxyl groups of peptides that bind water, as proteins generally tend to shrink at higher temperature (Rao et al., 2006). This observation points to the possible adverse

effects on functional properties of the substrates following protracted storage under the warmer conditions.

Table 4.7: Properties of sorbed water according to Caurie equation

Equilibration temperature	Cricket			BSFL		
	n_m	A (m^2/g)	W_b(%)	n_m	A (m^2/g)	W_b(%)
25°C	4.63	126.00	23.65	4.60	125.11	23.10
30°C	4.52	123.01	22.44	4.57	124.50	20.90
35°C	4.31	117.43	19.71	4.27	116.24	17.28

n_m: Number of monolayers; A: area of adsorbent; W_b: bound water.

4.3.6 Isosteric heat of sorption (q_{st})

Adsorption processes emit heat, and q_{st} is a measure of the amount of energy released. Figure 4.24 shows the plots of $\ln a_W$ as a function of $1/T$ for equilibrium moisture contents between 2 – 20 g/100 g. The respective regression parameters are shown. Net isosteric heats of sorption are presented in Figure 4.25. For both substrates, equilibrium moisture contents were those predicted by the Caurie model. Isosteric heats of sorption of the BSLF powder were higher than those of the cricket powder indicating that binding of water in sorption sites was more exothermic in the BSFL than in the cricket powder. In the two substrates, q_{st} was high at low equilibrium moisture content. Below 5 g/100 g moisture content, q_{st} decreased rapidly as more water became adsorbed. The high values at low moisture contents indicate high water binding energy, which is characteristic of monolayer sorption. A transition followed at 5 -20 g/100 g moisture content whereby net isosteric heat of sorption decreased at a decreasing rate. This is explained by the fact that most sites with high water binding energies were already occupied but sorption continued on sites with lower water binding energies (Chalermchat & Owasit, 2011).

Furthermore, declining isosteric heats indicate weakening of water-solid interactions subsequently leading to free moisture (Toujani et al., 2011). At about 20g/100g moisture content, q_{st} approached zero meaning that total isosteric heat of sorption approximated latent heat of vaporization of water. The level of moisture content at which net isosteric heat of sorption approaches zero is often taken to indicate the amount of 'bound' water (Al-Muhtaseb et al., 2004). Thus above 20 g/100 g equilibrium moisture, water existed as free liquefied water. Such water can support profuse microbial and chemical deterioration. A safe storage a_w of 0.7, is generally used for most food /feed products (Ikhu-Ornoregbe & Chen, 2005), which from the sorption isotherms, coincided with moisture contents of 8.0 g/100 g at 25° C in the two insect powders. At 30 and 35°C, the equilibrium moisture contents approximate 6.6 and 5.5 g/100g and 7.9 and 7.1 g/100 g for the BSFL and cricket powder, respectively. These moisture contents situate well within the upper curvature region of the isosteric heat plots.

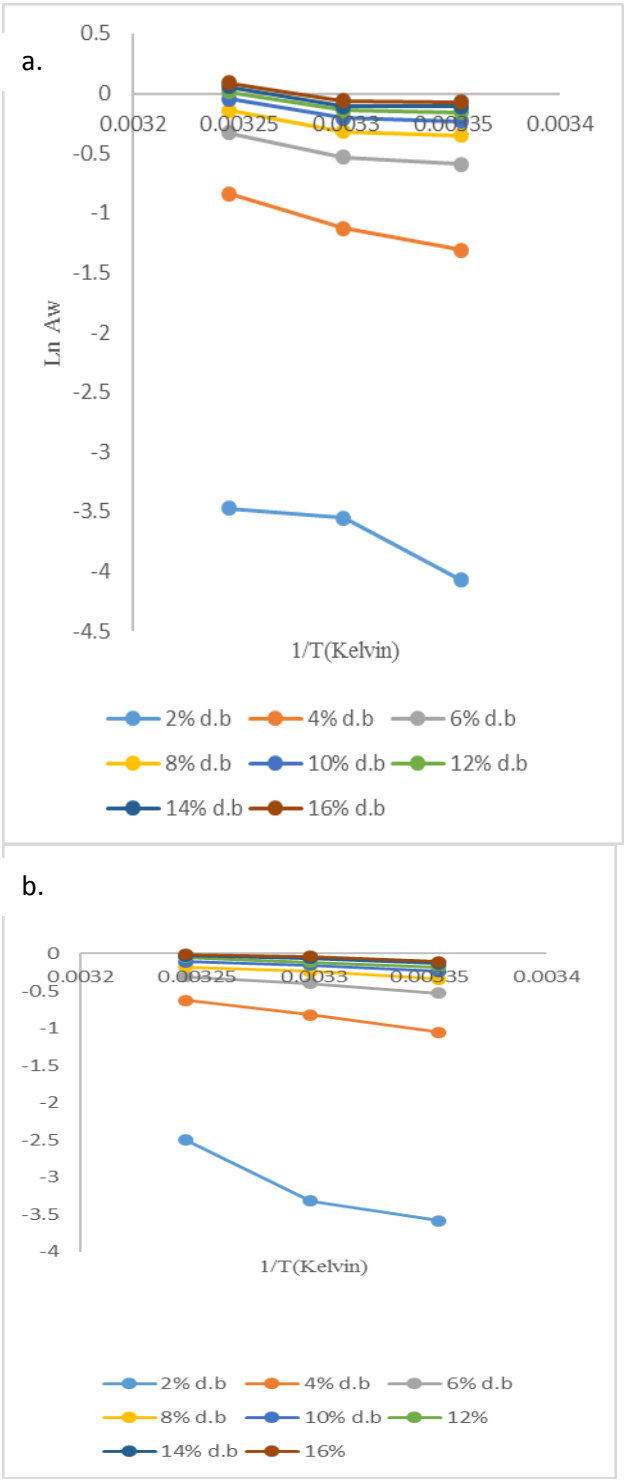


Figure 4.24: Cluasius-Clapeyron-type relationship between water activity and temperature for house

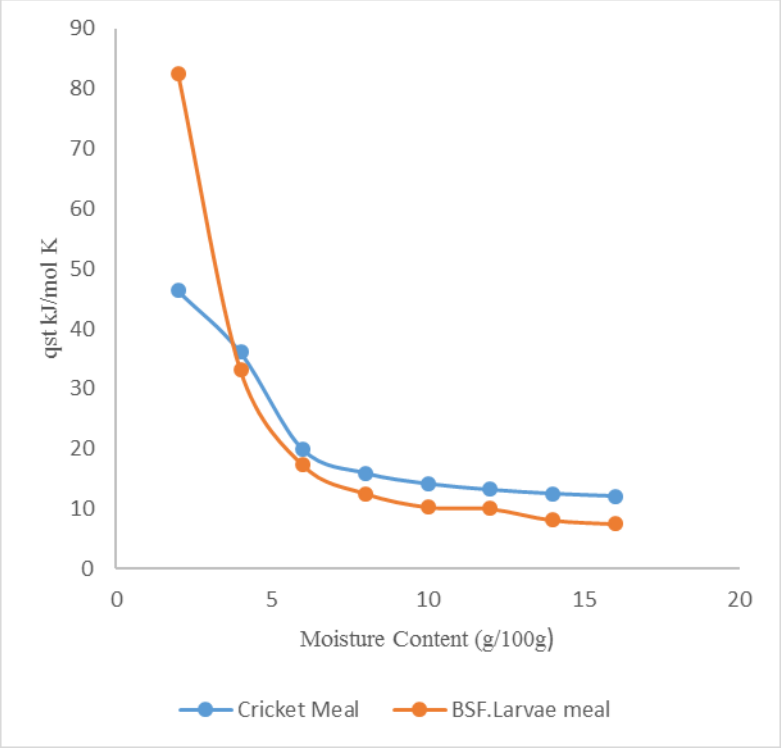


Figure 4.25: Relationship between isosteric heats of sorption and equilibrium moisture contents of cricket and black soldier fly larvae powders

4.3.7 Shelf life estimation of adult house cricket and black soldier fly larvae meals

High ambient relative humidity and temperature in the tropics presents a challenge for storage of dried products. Using the Heiss and Eichner model (Heis & Eichner, 1971) the shelf life of the dried insect powders was predicted in relation to the possible packaging and storage conditions: polyethylene is a common packaging material; ambient temperatures range between 23-35°C; and relative humidity could approach 90%. According to the model, when the critical a_W for a particular system under given storage conditions and the moisture sorption behaviour are known, the potential shelf life of the packaged product in days (t_s) is given by the equation below;

$$t_s = \frac{ln[(x_e - x_i)/(x_e - x_c)]}{k_s\left(A/W_s\right)(P_O/S)}$$

Where X_e is the equilibrium moisture content (g/g d.b) of the product if it is left in contact with the atmosphere outside the package (depends on temperature, relative humidity and on the product adsorption isotherm); X_C is the safe storage moisture content of the product (g/g d.b) i.e. the moisture content corresponding to the safe storage borderline a_W; X_i is the initial moisture content of the product when it is packaged (g/g d.b); K_s is the permeability constant of the package to moisture vapor (kg H_2O µm/m^2/day/Pa); P_o is vapor pressure at storage temperature (Pa); A is the surface area of the package (m^2); W is the weight of the product (dry matter) in the package (Kg); and S is slope of the product isotherm (assumed linear over the range between X_e and X_c). This model was used by other researchers (Gevaudan et al., 1989; Ikhu-Omoregbe & Chen, 2005). A safe storage borderline a_W of 0.7 which is generally used for most food/feed products (Ikhu-Omoregbe & Chen, 2005) was applied. A 90% relative humidity (a_W 0.9) was used as the ambient storage relative humidity, representing the highest ambient air humidity that may be experienced in many tropical areas. From the sorption isotherms, the safe storage moisture content (Xc) of cricket and BSFL powders was 0.0815 and 0.0793 g/g at 25 °C, 0.0796 and 0.0663 g/g at 30 °C and 0.0718 and 0.0571 g/g at 35°C respectively. The estimated shelf lives of the cricket and BSFL powders packaged in 10-Kg polyethylene bags (thickness; 80µm, surface area; 0.1474m^2; water vapour permeability constant 1.55 × 10^{-4} Kg µm/ m^2/ days/Pa (Lima and Cal-Vidal, 1988) are presented in Figure (30). Shelf life increased with increasing initial storage moisture and storage temperature. A shelf life of one year is achieved if the cricket and BSFL powders are dried to approximately 6.5 g/100g and 6g/100g moisture contents respectively and stored at 25°C. However at the same moisture contents, the products will barely store for 3 months at 35°C. At 30°C, the cricket powder will store for one year if storage moisture content is below 5.5% whereas the BSFL powder will require to be dried to moisture content below 4 g/100 g. This estimation of the shelf life focussed on the permeability of the package to water vapour but permeability dynamics to gases that is oxygen and carbon dioxide which may cause oxidation need to be investigated. Another interesting packaging material is

polypropylene in terms of permeability to water vapour and oxygen and may therefore be of interest in combating oxidative deterioration.

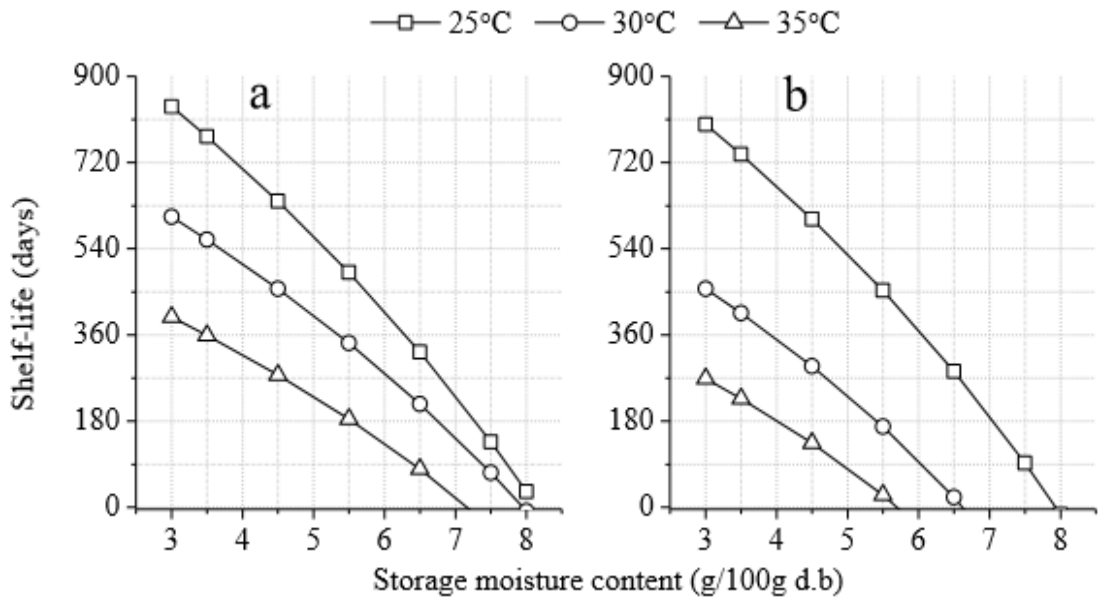


Figure 4.26: Estimated shelf life of cricket (a) and black soldier fly larvae (b) meal packaged in 80µm thick polyethylene bags and stored at different temperatures

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Similar to other animal derived products, insects are nutrient rich and are susceptible to chemical, microbiological and physical deterioration during storage. This research provided an overview of the storage stability of BSFL and adult house cricket stored in different packages under ambient and refrigerated conditions. The results show that both chemical and microbial stability of BSFL and adult cricket meal during storage are significantly ($p<0.05$) affected by the combination of storage environment, storage period and type of packaging. PE, PP and PL packages used in this study maintained the quality attributes of the samples relatively well except the refrigerated woven polypropylene packages that kept the product only for 45 days. The plastic (PL) package outperformed the other two packages in maintaining the quality of the stored product in both storage environments as indicated by the chemical and microbial parameters assessed. Our processed product even at the baseline level had a high microbial count and thus care should be taken while handling, processing and storage to prevent contamination. Based on both chemical and microbial deterioration of the samples, it would be recommended that the samples be stored in plastic packages with a lid in a refrigerated environment for a longer shelf life. If woven polypropylene packages are to be used, it would be advisable to add a layer of polyethylene on the inner side so as to minimize permeability to both air and water vapor and thus prolong the shelf life of the product therein. Both the insect samples exhibited type II sorption isotherms. Chemical composition of the samples influenced their water adsorption capacity with cricket adsorbing a higher content of water due to a higher protein content. Shelf life estimation shows that adult house cricket and black soldier fly larvae meals can last up to one year under ambient conditions if stored at, moisture contents of less than 6.5 and 6.0 g/100 g d.b respectively.

5.2 Recommendations

Good manufacturing practices should be strictly adhered to during handling, processing and storage of the insect products. This will aid in significantly increasing their shelf life and assuring quality protein supply all year round.

Future work regarding moisture adsorption and shelf life of both BSFL and house cricket meals should aim at characterizing the properties and physical stability of the insect-based processed products as influenced by interaction with moisture during processing operations, handling and storage.

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APPENDICES

Appendix 1: Change in fatty acid composition (%) of boiled and solar black soldier fly larvae meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

		Ambient			Refrigerated		
FA	Time (Days)	PL	PE	PP	PL	PE	PP
C12:0	0	21.56±0.92^{i}	21.56±0.92^{i}	21.56±0.92^{i}	21.56±0.92^{i}	21.56±0.92^{i}	21.56±0.92^{i}
	45	24.04±0.85^{ac}	25.03±0.25^{abc}	25.44±1.01^{abc}	22.85±0.68^{ai}	23.09±0.56^{ai}	28.45±1.21^{defh}
	90	28.95±0.45^{def}	29.73±0.29^{deg}	31.83±0.41^{gjk}	24.94±1.21^{abc}	25.98±0.71^{bch}	
	135	29.74±0.25^{deg}	30.43±0.54^{egj}	32.95±0.57^{jkl}	25.52±0.98^{abc}	26.77±0.25^{bfh}	
	180	33.95±0.48^{kl}	34.99±0.78^{l}	37.87±0.75^{m}	27.55±0.86^{bdfh}	29.23±0.57^{defg}	
C14:0	0	2.82±0.27^{f}	2.82±0.27^{f}	2.82±0.27^{f}	2.82±0.27^{f}	2.82±0.27^{f}	2.82±0.27^{f}
	45	3.04±0.30^{abf}	3.05±0.12^{abf}	3.35±0.51^{abcf}	2.91±0.11^{af}	2.99±0.18^{abf}	3.66±0.25^{abcde}
	90	3.99±0.3^{cde}	4.26±0.04^{deg}	4.99±0.11^{gh}	3.32±0.54^{abcf}	3.57±0.17^{abcd}	
	135	4.07±0.02^{cde}	4.47±0.21^{deg}	5.09±0.14^{gh}	3.59±0.17^{abcd}	3.77±0.05^{abcde}	
	180	4.56±0.17^{eg}	4.99±0.22^{gh}	5.75±0.12^{h}	3.89±0.25^{bcde}	4.01±0.22^{cde}	
C16:0	0	12.59±0.82^{a}	12.59±0.82^{a}	12.59±0.82^{a}	12.59±0.82^{a}	12.59±0.82^{a}	12.59±0.82^{a}
	45	13.77±0.21^{ab}	14.15±1.25^{abc}	15.16±1.12^{abc}	12.54±0.62^{a}	12.85±0.18^{a}	14.88±1.21^{abc}
	90	15.84±0.33^{bcg}	18.38±1.33^{efg}	20.38±1.21^{df}	13.25±1.05^{ab}	13.75±0.75^{ab}	
	135	16.86±0.45^{ceg}	19.68±1.14^{def}	22.28±0.95^{d}	13.57±0.42^{ab}	13.97±0.15^{abc}	
	180	19.65±0.23^{def}	21.54±0.55^{d}	26.96±0.57^{h}	14.51±0.64^{abc}	15.21±0.39^{abc}	
C18:0	0	1.53±0.16^{d}	1.53±0.16^{d}	1.53±0.16^{d}	1.53±0.16^{d}	1.53±0.16^{d}	1.53±0.16^{d}
	45	1.62±0.15^{ad}	1.74±0.17^{abd}	2.02±0.25^{abcd}	1.57±0.12^{ad}	1.59±0.21^{ad}	2.11±0.72^{abcd}
	90	2.38±0.44^{abce}	2.53±0.11^{abce}	2.72±0.27^{bcef}	1.96±0.10^{abcd}	2.01±0.04^{abcd}	
	135	2.46±0.52^{abce}	2.69±0.21^{bcef}	2.82±0.35^{cef}	2.01±0.14^{abcd}	2.11±0.16^{abcd}	
	180	3.26±0.33^{efg}	3.62±0.33^{fg}	4.22±0.35^{g}	2.53±0.23^{abce}	2.74±0.13^{bcef}	
C18:1	0	18.17±0.77^{a}	18.17±0.77^{a}	18.17±0.77^{a}	18.17±0.77^{a}	18.17±0.77^{a}	18.17±0.77^{a}
	45	17.71±0.29^{a}	17.69±0.94^{a}	16.7±0.30^{a}	18.15±0.82^{a}	19.01±0.55^{a}	16.15±0.55^{a}
	90	16.25±0.14^{a}	15.55±0.62^{a}	14.14±0.64^{a}	18.98±0.74^{a}	18.05±0.69^{a}	
	135	15.16±0.29^{a}	14.39±0.33^{a}	13.19±0.23^{a}	18.57±0.98^{a}	17.78±0.78^{a}	
	180	13.74±0.12^{a}	12.04±0.45^{a}	11.92±0.19^{a}	17.45±0.21^{a}	16.92±0.25^{a}	
C18:2	0	13.37±0.78^{i}	13.37±0.78^{i}	13.37±0.78^{i}	13.37±0.78^{i}	13.37±0.78^{i}	13.37±0.78^{i}

	45	11.95±0.68^{eghi}	11.51±0.94^{degh}	10.91±0.99^{cdeg}	13.14±0.77^{hi}	12.83±0.99^{ghi}	9.02±0.21^{abcf}
	90	10.24±0.83^{bcdef}	9.59±0.19^{abcdf}	8.67±0.58^{abf}	12.01±0.22^{eghi}	11.61±0.54^{degf}	
	135	9.22±0.17^{abcf}	9.12±0.23^{abcf}	8.14±0.04^{abj}	11.45±0.33^{degh}	10.91±0.64^{cdeg}	
	180	8.15±0.23^{abj}	7.63±0.23^{aj}	6.22±0.42^{j}	10.61±0.37^{cdef}	9.61±0.44^{abcdf}	
C18:3	0	2.55±0.26^{f}	2.55±0.26^{f}	2.55±0.26^{f}	2.55±0.26^{f}	2.55±0.26^{f}	2.55±0.26^{f}
	45	2.00±0.31^{bcf}	1.74±0.41^{abc}	1.67±0.22^{abc}	2.34±0.52^{cf}	2.32±0.11^{cf}	1.52±0.15^{abc}
	90	1.43±0.37^{abcd}	1.11±0.18^{abde}	0.98±0.12^{ade}	1.98±0.41^{bcf}	1.78±0.14^{abc}	
	135	1.25±0.18^{abde}	0.96±0.23^{ade}	0.87±0.11^{ade}	1.78±0.22^{abc}	1.69±0.24^{abc}	
	180	1.01±0.38^{ade}	0.50±0.12^{de}	0.35±0.14^{e}	1.35±0.26^{abd}	1.21±0.22^{abde}	
EPA	0	1.19±0.20^{h}	1.19±0.20^{h}	1.19±0.20^{h}	1.19±0.20^{h}	1.19±0.20^{h}	1.19±0.20^{h}
	45	0.87±0.12^{afgh}	0.89±0.16^{afgh}	0.75±0.25^{abcfg}	1.11±0.16g^{h}	0.98±0.20^{fgh}	0.62±0.02^{abcdefg}
	90	0.54±0.11^{abcdef}	0.51±0.24^{abcdef}	0.41±0.12^{abcde}	0.77±0.12^{acfgh}	0.69±0.16^{abcefg}	
	135	0.41±0.21^{abcde}	0.40±0.04^{abcde}	0.33±0.02^{bcde}	0.75±0.15^{abcfg}	0.61±0.14^{abcdefg}	
	180	0.15±0.01^{d}	0.21±0.01^{de}	0.22±0.01^{bde}	0.65±0.11^{abcdefg}	0.55±0.01^{abcdef}	
DHA	0	2.68±0.31^{k}	2.68±0.31^{k}	2.68±0.31^{k}	2.68±0.31^{k}	2.68±0.31^{k}	2.68±0.31^{k}
	45	2.11±0.15^{cei}	1.99±0.11^{acei}	1.85±0.21^{abce}	2.51±0.15^{ik}	2.31±0.02^{eik}	1.03±0.13^{fgh}
	90	1.63±0.12^{abcdf}	1.51±0.03^{abcdf}	1.73±0.21^{abcde}	1.99±0.19^{acei}	1.82±0.28^{abce}	
	135	1.52±0.03^{abcdf}	1.44±0.13^{abdfh}	1.1±0.10^{dfgh}	1.85±0.11^{abce}	1.74±0.11^{abcde}	
	180	0.85±0.05^{ghj}	0.77±0.19^{gj}	0.25±0.19^{j}	1.41±0.14^{abdfgh}	1.22±0.05^{bdfgh}	

(±) standard deviations from triplicate values. Means sharing a common letter within the same column are not significantly different at 5% confidence level

Appendix 2: Change in fatty acid composition (%) of boiled and solar dried adult house cricket meal during storage for 180 days at different environments (ambient and refrigerated) and packages (polyethylene (PE), polypropylene (PP) and plastic (PL))

		Ambient conditions			Refrigerated conditions		
FA	Time (Days)	PL	PE	PP	PL	PE	PP
C14:0	0	1.77±0.14^{bc}	1.77±0.14^{bc}	1.77±0.14^{bc}	1.77±0.14^{bc}	1.77±0.14^{bc}	1.77±0.14^{bc}
	45	1.68±0.14^{bc}	1.68±0.11^{bc}	2.37±0.21^{c}	1.91±0.12^{bc}	1.44±0.52^{bd}	3.28±0.44^{f}
	90	1.22±0.11^{bde}	1.41±0.15^{bd}	1.53±0.32^{b}	0.60±0.25^{ae}	0.48±0.19^{a}	
	135	0.37±0.17^{a}	0.33±0.20^{a}	0.76±0.12^{ade}	0.19±0.11^{a}	0.22±0.09^{a}	
	180	0.25±0.01^{a}	0.22±0.04^{a}	0.23±0.09^{a}	0.12±0.10^{a}	0.12±0.01^{a}	
C16:0	0	20.57±1.85^{cd}	20.57±1.85^{cd}	20.57±1.85^{cd}	20.57±1.85^{cd}	20.57±1.85^{cd}	20.57±1.85^{cd}
	45	28.25±1.55^{fgh}	32.82±1.21^{hi}	35.82±2.00^{i}	22.13±1.52^{cde}	25.22±1.52^{efg}	30.37±1.54^{gh}
	90	22.23±1.02^{cde}	25.34±1.33^{efg}	27.68±1.54^{fgh}	14.69±1.13^{ab}	18.21±1.23^{bc}	
	135	19.92±1.38^{cd}	20.99±1.21^{cde}	23.68±1.33^{def}	13.77±0.99^{ab}	13.75±1.01^{ab}	
	180	13.30±1.56^{ab}	13.40±1.02^{ab}	17.57±1.22^{bc}	10.78±1.38^{a}	11.19±0.56^{a}	
C18:0	0	4.73±0.59^{ci}	4.73±0.59^{ci}	4.73±0.59^{ci}	4.73±0.59^{ci}	4.73±0.59^{ci}	4.73±0.59^{ci}
	45	5.24±0.22^{cg}	6.74±0.54^{k}	8.17±0.71^{j}	3.84±0.21^{bef}	4.55±0.21^{cfi}	5.69^{g}
	90	5.00±0.41^{cgi}	5.35±0.44^{cg}	8.08±0.32^{j}	3.57±0.72^{beh}	4.26±0.21^{bfi}	
	135	3.01±0.11^{adeh}	3.86±0.10^{beh}	5.32±0.17^{cg}	2.68±0.20^{ad}	2.95±0.10^{adh}	
	180	2.59±0.23^{ad}	3.40±0.30^{bdeh}	3.98±0.30^{bf}	2.23±0.21^{a}	2.35±0.43^{a}	
C18:1	0	20.96±0.64^{dg}	20.96±0.64^{dg}	20.96±0.64^{dg}	20.96±0.64^{dg}	20.96±0.64^{dg}	20.96±0.64^{dg}
	45	22.62±1.11^{gi}	20.29±1.11^{cdg}	17.04±1.22^{ceh}	25.36±1.21^{i}	24.75±1.21^{i}	15.77±1.34^{bef}
	90	19.20±1.02^{cdfg}	15.77±0.79^{bef}	13.85±1.48^{abe}	24.96±1.10^{i}	20.38±1.23^{cdg}	
	135	14.16±1.22^{abe}	12.61±0.99^{abh}	10.63±1.23^{ah}	20.27±0.77^{cdg}	18.27±0.66^{cdf}	
	180	12.15±1.49^{abh}	11.04±1.01^{ah}	9.90±0.52^{h}	18.71±1.41^{cdf}	14.20±1.22^{abe}	
C18:2	0	40.44±1.21^{l}	40.44±1.21^{l}	40.44±1.21^{l}	40.44±1.21^{l}	40.44±1.21^{l}	40.44±1.21^{l}
	45	32.17±1.03^{cde}	31.36±1.02^{cdi}	27.90±1.24^{bci}	38.01±1.53^{kl}	36.28±1.12^{ek}	26.84±1.37^{bh}

							i
	90	26.00±1.25^{bgh}	24.68±1.74^{bfgh}	21.60±1.32^{afg}	34.53±1.02^{dek}	33.16±1.04^{de}	
	135	22.98±1.27^{afgh}	20.96±1.20^{af}	19.25±1.00^{aj}	32.16±1.11^{cde}	31.67±1.22^{cde}	
	180	20.92±1.45^{af}	19.25±1.46^{aj}	15.70±1.63^{j}	27.87±1.24^{bci}	25.70±1.63^{bgh}	
C18:3	0	4.60±0.17^{k}	4.60±0.17^{k}	4.60±0.17^{k}	4.60±0.17^{k}	4.60±0.17^{k}	4.60±0.17^{k}
	45	2.43±0.22^{fgh}	2.05±0.25^{afg}	1.33±0.21^{abcde}	4.19±0.14^{jk}	3.89±0.34^{ij}	1.41±0.28^{acde}
	90	2.13±0.31^{afg}	1.89±0.34^{aeh}	0.99±0.11^{bcd}	3.06±0.22^{hi}	2.83±0.32^{gh}	
	135	1.51±0.25^{ade}	1.31±0.21^{abcde}	0.69±0.11^{bcd}	2.54±0.31^{fgh}	1.93±0.34^{aef}	
	180	0.96±0.16^{bcd}	0.61±0.41^{bc}	0.52±0.21^{b}	1.88±0.11^{aef}	1.15±0.17^{bcde}	
EPA	0	2.72±0.21^{f}	2.72±0.21^{f}	2.72±0.21^{f}	2.72±0.21^{f}	2.72±0.21^{f}	2.72±0.21^{f}
	45	1.90±0.31^{dej}	1.53±0.23^{ade}	1.13±0.13^{abc}	2.35±0.11^{fj}	2.14±0.19^{efj}	1.24±0.41^{abcd}
	90	1.15±0.11^{abc}	1.02±0.15^{abc}	1.05±0.13^{abc}	2.81±0.21^{f}	1.67±0.20^{ade}	
	135	1.08±0.12^{abc}	0.85±0.10^{bci}	0.69±0.20^{bhi}	1.46±0.10^{acd}	1.25±0.11^{abcd}	
	180	0.21±0.12^{ghi}	0.08±0.03^{gh}	0.02±0.01^{g}	1.06±0.13^{abc}	1.00±0.10^{abc}	
DHA	0	1.78±0.21^{i}	1.78±0.21^{i}	1.78±0.21^{i}	1.78±0.21^{i}	1.78±0.21^{i}	1.78±0.21^{i}
	45	1.00±0.14^{cdefg}	0.90±0.22^{bcdefg}	0.43±0.12^{abch}	1.41±0.24^{gi}	1.26±0.21^{fg}	0.39±0.05^{abh}
	90	0.62±0.22^{abcdeh}	0.45±0.21^{abch}	0.12±0.06^{a}	1.14±0.02^{efg}	1.10±0.15^{defg}	
	135	0.52±0.14^{abcdh}	0.22±0.10^{ah}	0.15±0.01^{a}	1.10±0.26^{defg}	0.97±0.24^{bcdefg}	
	180	0.41±0.11^{abch}	0.14±0.04^{a}	0.10±0.02^{a}	0.90±0.09^{bcdefg}	0.79±0.07^{bcdefh}	

(±) standard deviations from triplicate values. Means sharing a common letter within the same column are not significantly different at 5% confidence level