ASSESSING THE QUALITY, SAFETY AND SHELF STABILITY OF REFINED HOUSE CRICKET (*Acheta domesticus*) POWDER AND ITS OIL FRACTION

BY

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DECLARATION

I, Nakamya Leticia, hereby declare that this dissertation is solely my original work and has not been submitted to any academic institution by an individual pursuing a degree award.

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APPROVAL

We confirm that this work was done by candidate under our supervision.

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DEDICATION

Dedicated to my grandmother, Ms. Nalubowa Theresa and my husband, Mr. Kisekka Fred for always having confidence in me and ensuring success in my education and every other area of my life.

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LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Collaboration
B.D	Bulk density
BPW	Buffered peptone water
CAC	Codex Alimenterius Commission
CL/ROOH	Sodium hypochlorite and hydrogen peroxide
DG18	Dichloran Glycerol Agar
FAAS	Flame atomic absorption spectrophotometer
FAO	Food and Agriculture Organization
HDPE	High-density polyethylene
HEA	Hektoen enteric agar
MAMIDECOT	Masaka Microfinance and Development Cooperative Trust Ltd
MDA	Malonaldehyde
OAC	Oil absorption capacity
RVS	Rappaport Vasiliadis broth
SCB	Selenite cysteine broth
T.D	Tapped density
TBA	Thiobarbituiric acid
TPC	Total plate count
UBOS	Uganda Bureau of Statistics
UDHS	Uganda Demographic and Health Survey
UNFPA	United Nations Population Fund
WHC	Water holding capacity
XLD	Xylose lysine deoxycholate agar

ABSTRACT

Incorporation of whole house cricket (*Acheta domesticus*) powder into staple flours such as maize, cassava, millet, and bean has led to an increase in protein levels (> 50%) of its composites. However, the incorporation levels have remained low due to reduced sensory acceptability. The maximum incorporation level is between 5% and 6% as attributes such as aroma and color are affected, causing the flours to become darker and aroma becoming less appealing with higher incorporation levels. To increase levels of incorporation, design expert software was used to generate different house cricket powder refining protocols, that were tested for consumer acceptability. Refined house cricket powder with the highest preference was produced through defatting roasted house cricket powder using petroleum ether and bleaching the powder using two methods. 1. Using a mixture of sodium hypochlorite and hydrogen peroxide; 2. using chloroform. This prompted an investigation into how refinement impacts the quality, safety, and shelf stability of the refined house cricket powder and its oil fraction. To achieve this objective, proximate analysis, functional properties and sensory evaluation of refined house cricket powders and controls were studied using standard and developed methods. The microbial (pour plate and spread plate methods) and chemical (chromatography, mass spectrometry, Kjeldahl) assays were conducted to determine the safety and stability together with sensory stability of the cricket powders and oil. Refined house cricket powder had a higher consumer acceptability than controls. The study also observed improvement in the protein levels of the cricket powder with refinement from 50% to 58.4% ($p<0.05$) in control and in refined powder, respectively. Refined house cricket powders possessed greater oil absorption capacities; solubility (20%) and dispersibility (72%) compared to the controls that had around 11% and 63% ($p<0.05$), respectively. Chemical analysis confirmed the absence of pesticide residues, aflatoxins, and toxic chemicals, except for lead, which was detected in control samples (0.48 and 0.83 mg/kg) and refined cricket powder bleached using chloroform (0.98mg/kg). Cricket powder bleached with chloroform also contained aflatoxin B1 (1.27 pbb). Microbiological assays showed absence of *Escherichia coli, Salmonella* spp, *Listeria spp, Enterobacteriacae,* total coliforms and *Staphylococcus aureus* in the powders. In terms of shelf stability, all the cricket powders remained stable for microbial, chemical and sensory stabilities over the 24-weeks of storage. SV, PV and IV of oil stayed within standard acceptable of 154 mg KOH/g, 8.3 meq O_2/kg and 7.60 g iodine/100g respectively. However, the acid value, moisture and paranisidine value were higher than acceptable limits and the house cricket oil exhibited color changes, becoming darker after 15 weeks of storage. Refining house cricket powder improved sensory acceptability and protein content which increased by 8.4%. Refining did not affect safety and stability of the house cricket powders (met Uganda's edible insect safety standard US 2146:2020). Incorporating refined cricket powder into staple flours may improve protein content, and ongoing investigations should prioritize improving the stability of house cricket oil.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Projections indicate that the global population will reach 8.6 billion by 2030 and 9 billion by 2050, leading to a 70% increase in the demand for animal protein (Bartkiene et al., 2022; Mart et al., 2022; FAO, 2018). By 2050, Sub-Saharan Africa's population is approxmated to reach 2 billion, while East Africa's population is anticipated to surpass 670 million (Wudil et al., 2022; Henchion et al., 2017). Uganda's population is predicated to increase to 55 million people by 2030 and 75 million by 2040 (UNFPA, 2017). As a result, many communities will need alternative environmentally safe high quality protein (Mart et al., 2022).

Uganda, like many other low income countries, continues to face high levels of malnutrition (5 to 37 %) especially among children below 5 years (Adebisi et al., 2019; Mawa & Lawoko, 2018). According to the UBOS and the World Bank, Uganda's dependent population, consisting of children (0-14 years) who make up about 46-48% of the population, and the elderly (65+ years), comprising around 2-3%, primarily depends on staple foods for example millet, cassava, *matooke*, maize, sweet potatoes and beans (UBOS, 2022). These foods have been reported to be inadequate in nutrients such as proteins, lipids, iron, and zinc (Dotto, 2018). There is high prevalence of wasting (3.4%) due to long-term dietary inadequacy of quality protein as indicated among children in the Southwest region of Uganda (Adebisi et al., 2019; Kikafunda et al., 2014).

Many communities in Africa and Asia consume edible insects because of the high protein and fat contents (Djouadi et al., 2022). Out of over 2000 species of edible insects discovered globally, house crickets (*Acheta domesticus*), grasshoppers (*Ruspolia differens*), meal worms (*Tenebrio molitor*), and white ants (*Hodotermes mossambicus*) are the most often consumed (Ordoñez-

Araque et al., 2022). Edible insects for example house crickets contain higher protein content (55% - 70%, dry basis) in contrast to traditional protein foods such as beans (23.5%), soybean (41.1%), beef (44-60%), fish (50-75%), and chicken (50-55%) (Orkusz, 2021).

Because of their elevated levels of protein and fat, house crickets are susceptible to several forms of spoilage (Marzoli et al., 2023; Khetarpaul et al., 2007) including oxidative and microbial deterioration (Nakimbugwe et al., 2020; Ssepuuya et al., 2017) thus affecting shelf stability. House cricket powder is usually incorporated in flours for example maize, cassava and wheat that are refined to improve their sensory acceptability (Khan et al., 2022). Freshly milled wheat flour appears yellow due to carotenoid pigments, therefore chemical bleaching with agents such as hydrogen peroxide is used to whiten it and improve gluten quality faster than natural oxidation (Hasan & Altaee, 2021). Chloroform is used to dissolve and extract lipid-soluble pigments such as carotenoids and xanthophylls from wheat and maize flour, effectively removing the colored compounds (Mohamed et al., 2020). Refining house cricket powder could enhance its shelf stability, increase its protein content, and improve its sensory qualities, while also yielding a by-product (oil) suitable for culinary applications.

1.2 Problem Statement

Incorporating whole house cricket (*Acheta domesticus*) powder into staple flours such as maize and cassava improves their protein content by over 50% accompanied by notable enhancements in lysine and methionine levels (Ssepuuya et al., 2025). The addition also improves mineral composition (iron, zinc, and calcium) of the house cricket powder composites (Widjaja & Surya, 2025). However, this incorporation has led to an inverse correlation between the quantity of house cricket powder used and the product’s sensory acceptability. The higher the amounts of house cricket powder in a recipe, the less appealing the aroma and the darker the product colour. For example, when house cricket powder was used in the production of corn snacks, cookies with higher than 6% inclusion were less acceptable (Ruszkowska, 2022). According to Bartkiene et al. (2022) a substitution rate of 5% gives house cricket powder enriched products acceptable appearance, aroma, texture and taste, however, beyond this level, the sensory acceptability falls. This low incorporation level (5 to 6%) of cricket powder due to reduced sensory quality implies that less contribution to protein, fat and minerals is made by the cricket powder incorporation.

The lipid fraction derived from house cricket powder is abundant in high proportion of unsaturated and saturated fatty acids, along with essential vitamins, rendering it a functional ingredient for food and nutraceutical applications (Pan et al., 2022). However, information regarding the quality and stability of cricket oil remains limited. Incorporating refined house cricket powder into staple flours such as maize and cassava could enhance consumer acceptability of the resulting food products, which may lead to higher incorporation levels. There is limited research on the refinement of cricket powder. Consequently, information is also lacking about the implications of house cricket powder refinement on quality, safety and stability. This research aimed at offering insights regarding the impacts of refining (defatting and bleaching) on quality, safety, sensory acceptability and stability of house cricket powder plus its oil fraction.

1.3 Objectives

1.3.1 General objective

To assess the quality, safety and stability of refined house cricket (*Acheta domesticus*) powder and its oil fraction

1.3.2 Specific objectives

1. To determine the effect of refinement on the nutritional, functional and sensory quality of the cricket powder
2. To ascertain effect of refinement on safety and stability characteristics of the cricket powder
3. To evaluate the quality and stability of oil (byproduct) extracted from refined cricket powder

1.3 Hypotheses

1. There is no significant variation in nutritional, functional and sensory quality of the refined (bleached and defatted) and unrefined cricket powder
2. There is no significant variation in the safety and stability of the refined (defatted and bleached) and unrefined cricket powder
3. Oil extracted from house cricket powder shows no significant difference in quality or stability compared to conventional edible oils

1.5 Justification of the study

Refining house cricket powder may increase acceptability thus increasing its incorporation levels into staple flours hence higher intake of quality protein with minimal negative environmental effects eventually leading to healthy and active individuals.

The food sector will benefit from this study since it will shed light on the refined cricket powder's quality, safety, and shelf stability all of which are crucial for creating formulations of protein-rich cricket powder food products (Bresciani et al., 2022).

House cricket powder refining through defatting may extend shelf life and provide byproducts such as cricket oil that can be used in cooking and baking.

In addition, this study may promote cricket farming in Uganda, lead to development of several products that use house cricket powder as an ingredient and thus may generate income and improve the livelihoods of Ugandans.

CHAPTER TWO: LITERATURE REVIEW

2.1 Food and Nutritional Security

Considering effects of climate change, population growth, and increasing industrialization of developing nations, the primary worldwide concern of the twenty-first century is guaranteeing future food security through eradication of malnutrition (Santpoort, 2020, Kwak, 2019). Food security is achieved when every individual, all the time, acquires nourishing food which meets their nutritional needs for maintaining good health (Akram & Sial, 2019, Hwalla et al., 2016). Over two billion individuals worldwide experience severe to moderate food insecurity including under nutrition, malnutrition, or over nutrition (FAO et al., 2019). In 2024, one in every eleven individuals globally, totaling 733 million, experienced hunger or malnutrition (FAO et al., 2024). Likewise, about 50% of worldwide population is deeply affected by food insecurity, obesity or overweight, or micronutrient deficiencies, highlighting the necessity for a transformation of the current food system (Santpoort, 2020, El Bilali et al., 2019).

Global population projections estimate that the number of people worldwide will reach approximately 9.7 billion by the year 2050 and if present energy and food consumption trends continue, the demand for food will be more than 3.5 times higher than the current requirements (Mart et al., 2022, Kwak, 2019). Even when food is available, it may not be accessible to everyone in need; furthermore, food that is both available and accessible may still lack the essential nutrients required for the body to develop, grow, fight diseases, and fulfill minimum physiological needs (Simelane & Worth, 2020). Recently, the productivity of agricultural crops in many poor nations such as Ethiopia, Haiti, Yemen, and Malawi is nearly stagnant and chronic malnutrition is rampant (FAO, 2021). In the South Asian and Sub-Saharan African regions, undernutrition is the primary cause of death for children below five, accounting for around 45% of all pediatric fatalities worldwide (Gillespie et al., 2015).

Food & nutritional insecurity in many parts of Africa is attributed to several factors including destitution, fast population increase, dependence on rain-fed agriculture, poor infrastructure, extreme weather conditions, plus economic downturns (Muringai et al., 2022). Undernutrition is a significant risk for mortality from infections like pneumonia as well as irreversible stunted growth, cognitive functioning and mental well-being deficiencies, and an increased likelihood of developing obesity and long-term health conditions (Hodge et al., 2015). Africa's undernourished population is gauged to reach 433 million by 2030 (Muringai et al., 2022). In the East African region, 42% among under five years old are vulnerable to stunting, compared to 24% worldwide (Hodge et al., 2015).

Basing on 2024 National Population and Housing Census, around 10.8 million households in Uganda were involved in agriculture either by cultivating land or rearing livestock accounting for approximately 80% of all households nationwide (UBOS, 2024). In Uganda, the majority of food production occurs at the smallholder and subsistence level, relying on rain-fed agriculture and limited use of agricultural inputs (FEWS NET, 2017). The 2022 Uganda Demographic and Health Survey (UDHS) shows chronic malnutrition contributes to over 26% of stunting cases in children under the age of five (UBOS, 2022). This reflects a significant improvement from the 45% stunting rate reported in 1995 (Kikafunda et al., 2014). Despite the country's ongoing economic growth and efforts to achieve food security, childhood stunting remains a critical public health concern.

Micronutrient fortification along with supplementation and fermentation programs have become a focus of national and international health agencies (Motuma, 2016). Bio-fortification has been introduced to overcome the nutritional problem and to make the important micronutrients bio-availability in staple food crops and vegetables (Gupta et al., 2021). Climate Smart Agriculture through the adoption of high-yielding crops, drought-tolerant varieties, farmed fish, and similar measures are tactics that entail creating and encouraging technologies to increase resilience and adapt to climate change and extreme weather occurrences (Atukunda et al., 2021). Utilizing insects as foodstuffs represents one of climate-smart practices that can promote greater food and nutrition security. Because of high efficiency of feed conversion and small rearing space requirements, edible insects offer solution to both current and upcoming food insecurity. According to their species, stage of development, and diet, they offer a significant quantity of calories and protein (Tao & Li, 2018).

2.2 The contribution of edible insects to Africa

Eating insects, or "entomophagy," has long been a practice in different societies (Bartkiene et al., 2022; Huis, 2013). Many countries in Sub-Saharan Africa for example, Ivory Coast, Madagascar, Rwanda, South Africa, Uganda, Cameroon, Tanzania, and Zimbabwe consider insects including grasshoppers, crickets, and mealworms to be traditional delicacies. In these countries, more than 470 insect varieties are consumed (Ssepuuya et al., 2019). The commonest consumed order is *Coleoptera* such as *Tenebrio molitor, Rhynchophorus ferrugineus*, which accounts for 31% of all edible insects. Consumption of insects is widely recognized as the most promising emerging alternative protein source (Nakimbugwe et al., 2020). Edible insects are mainly obtained from their natural habitats and are mostly eaten as eggs, larvae, pupae, adults, or nymphs (Ishara et al., 2023).

Edible insects are characterized by their content of protein (40 - 80%), carbohydrates (1 -10%), and fat (10 - 60% fat) on dry matter (Shah et al., 2022). Edible insects have minimal amounts of carbohydrates, with levels varying between 6.5 to 15.9 milligrams per kilogram in fresh matter

(Kouřimská et al., 2016). Protein part of edible insects is parallel to that found in milk and beef (60-80%) since it has all essential amino acids (Orkusz, 2021). Insect proteins have low quantities of tryptophan or methionine and high levels of lysine and threonine (77 - 98%) (Tuhumury, 2021) implying that they are best consumed as part of a mixed diet to achieve amino acid balance. Their exterior shell contains chitin, which makes up 5 - 20% of their dry mass protein. Studies have shown that chitin lowers blood cholesterol, acts as an anticoagulant, promotes wound healing, and acts as a hemostatic agent for tissue repair (Ignaczak et al., 2021).

2.2.1. Crickets

The cohort *Polyneoptera*, which includes crickets (*Gryllidea*), is a significant group of flying insects that bend their wings to lie flat across their bodies (Kataoka et al., 2022). With over 3364, species divided among 13 sub-families and found across the fauna, the *Gryllidae* are a highly varied family (Wei Yuan et al., 2022). Crickets, including true crickets, camel crickets, and bush crickets such as Mormon crickets, belong to the order *Orthoptera* a subgroup of *Polyneoptera* characterized by auditory communication and combat behaviors (Kataoka et al., 2022; Huis, 2020). In East Africa, people believe that the bush cricket (Ruspolia *differens*) makes a good meal (Huis, 2020). Both nymphal and adult stages of crickets are consumed (Tan et al., 2022). Most common species are *A. domesticus, S. icipe,* and *G. bimaculatus* (Magara et al., 2021).

House crickets are regarded as one of promising insects for consumption because of their rich nutrient content and simplicity of farming (Bresciani et al., 2022; Duffield et al., 2021). East Africa is seeing a sharp increase in the number of cricket farmers, with 378 farmers in Kenya and 140 in Uganda (Tanga et al., 2021). There is an annual production of approximately 32 tons of cricket produced in East Africa by small to medium-sized businesses (Megido et al., 2024).

2.2.2 Importance of crickets

According to Estrada et al., (2021), crickets are utilized in food to enhance nutritional profile of cereal-based flours, bean and tuber flours, animal feed, and honey. Pankiewicz & Sujka (2021) also noted that incorporating cricket powder as an ingredient is more acceptable by different individuals than consuming whole insects due to cultural food taboos. Cricket powder is commonly added to culinary products such crackers, cupcakes, and staple flours to improve their nutritional value and boost consumer approval (Tanga et al., 2021).

In Africa, rice, wheat, millet, sorghum, and maize are the most commonly consumed cereals (Yarnell, 2008). Maize, for instance, has 8% protein and is poor in iron, calcium, and zinc (Acosta-Estrada et al., 2021). Although millet flour has a high nutritional value, it is deficient in lysine and threonine (Jakab et al., 2020). Adding cricket powder to flour improved their protein composition, antioxidant qualities, and total polyphenol content.

2.2.3 Nutrient composition of house crickets

House crickets (*Obunyenyenkule* in Luganda) contain high amounts of lipid 4.30 – 33.44 % and protein 55 – 73 % based on dry matter content (Bartkiene et al., 2022). Compared to regular animal protein sources for example beef and chicken, *Acheta domesticus* has a higher calorie content (Bbosa et al., 2019). This nutritional comparison of house crickets and different food protein sources is displayed below.

Table 2.1: Nutritional comparison (per 100 g dry weight) of house crickets and animal origin protein sources.

Nutrient	House Crickets	Beef	Chicken	Pork	Fish	Milk (liquid)
Protein (g)	55-73	26-28	27-30	25-28	22-25	~3.2
Fat (g)	15-34	15-20	14-18	20-24	10-15	~3.4
Carbohydrates (g)	5-7	0	0	0	0	~5
Fiber (chitin)	5-8	0	0	0	0	0
Iron (mg)	9-12	2.6	1.3	1.0	1.0	0.1
Calcium (mg)	100-150	11	13	10–15	30	120
Zinc (mg)	10-15	4.8	1.0	2.0	0.7	0.4
Energy (kcal)	450-550	~250	~239	~242	~206	~61

Source: (Dunkel & Van Huis, 2024; Bawa et al., 2021).

According to Håkansson (2018), the type of feed that house crickets are fed can influence their nutritional composition. For instance, house crickets fed cassava plant tops contain higher levels of amino acids than those fed conventional chicken feed. The nutritional profile of house crickets comprises amino acids (histidine, leucine, lysine, methionine, threonine, and valine); omega-3 fatty acids (alpha-linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid); omega-6 fatty acids (linoleic acid); dietary fiber; vitamin C; and essential minerals including magnesium, iron, copper, zinc, selenium, manganese, and phosphorus (Table 2.2). These components make house crickets a highly valuable dietary supplement for addressing malnutrition in children (Acosta-Estrada et al., 2021).

Table 2.2: Nutritional Profile of house Crickets (Acheta domesticus)

Nutrient (g / 100 g)	**Zhao et al. (2025)**	**Bbosa et al. (2019)**	**Dada et al. (2018)**	**Hackewitz (2018)**	**FAO (2013)**
Protein	64.4	65.0	68.2	64.4	66.0
Histidine	2.5	2.7	2.8	2.4	1.5
Glutamic Acid	11.2	11.0	11.2	10.9	-
Leucine	7.6	7.9	7.8	6.7	5.9
Lysine	5.9	6.5	6.4	5.1	4.5
Valine	5.7	5.4	5.3	4.8	3.9
Threonine	4.3	3.9	3.7	3.1	2.3
Fat	22.80	15.00	15.70	13.00	14.50
Omega 3 Fatty Acids	0.53	0.55	0.54	0.50	0.52
ALA	1.11	0.22	0.21	0.20	0.21
(DHA)	0.0	0.16	0.16	0.15	0.16
EPA	0.0	0.17	0.17	0.15	0.15
Linoleic Acid	8.4	2.10	2.12	2.00	2.05
Carbohydrates	11.80	12.00	12.20	12.00	12.50
Vitamin C	8.5	2.20	2.15	2.00	2.10
Dietary Fiber	8.70	8.50	8.40	8.00	8.20
Iron	1.07	5.50	5.40	5.00	5.20
Phosphorus	100.30	125.00	124.50	120.00	123.00
Calcium	82.00	80.00	79.50	75.00	78.00
Magnesium	63.00	62.00	62.50	60.00	61.00
Selenium	33.00	32.00	32.50	30.00	31.00
Manganese	1.15	1.10	1.12	1.00	1.05
Zinc	11.20	11.00	10.80	10.00	10.50
Energy	470.00	460.00	465.00	450.00	455.00

ALA: Alpha-Linolenic Acid; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid

2.3 Refining of staple flours and edible insect powders

Refining staple flours for example maize produces a white flour that is more appealing for human consumption by removing bran and germ from the endosperm through wet milling and degermination (Gwirtz & Garcia-casal, 2014).

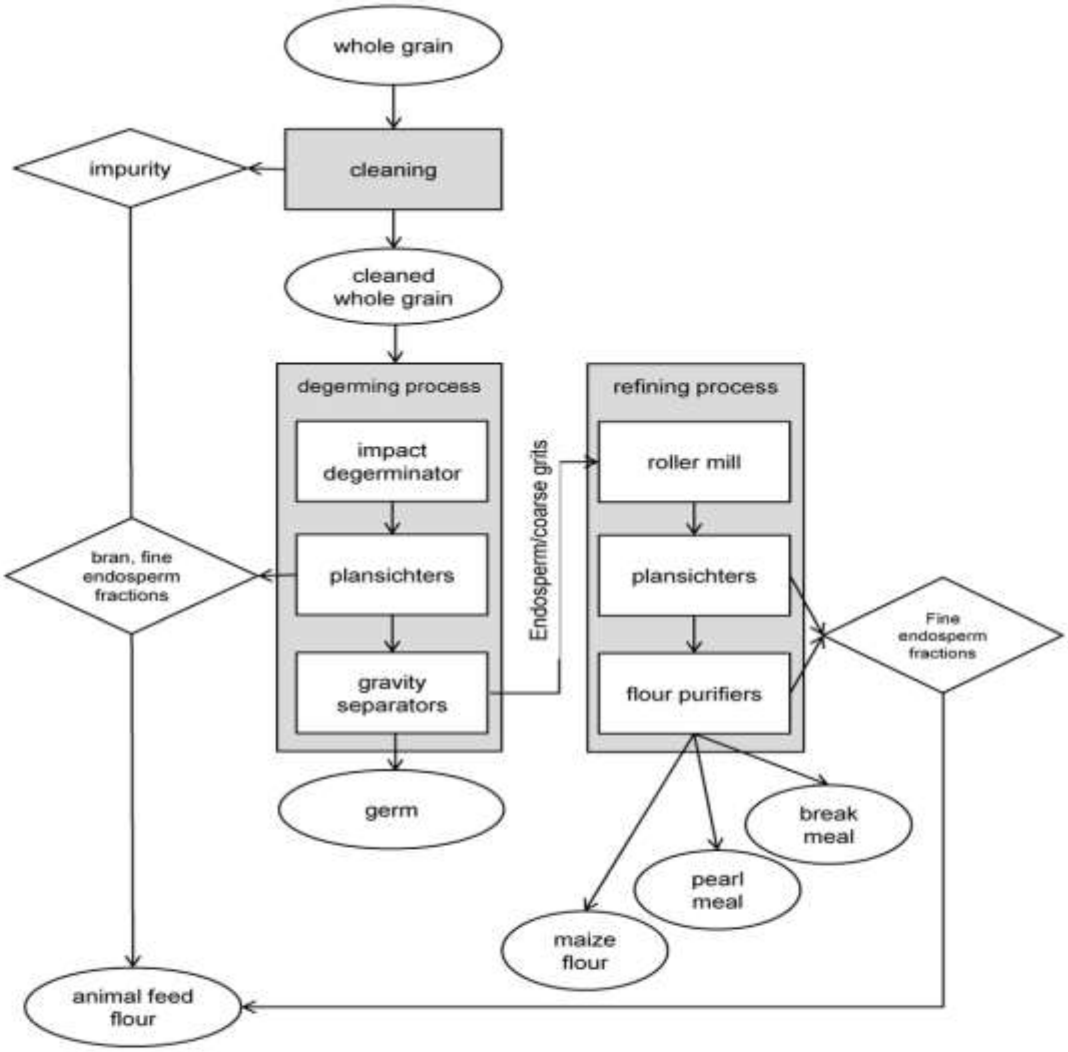


Figure 2.1: Flowchart for refining maize flour (Vanara et al., 2018).

Refined flours are chosen over whole grain flours for the majority of products due to their easy processing and bland taste (Dobhal et al., 2021). Benzoyl peroxide and other oxidizing agents are used by the wheat milling industries for bleaching, to speed up the maturation process, and to improve the dough's stickiness (Nisar et al., 2020), giving it a softer appearance. Conjugated double bonds of carotenoids are broken during flour bleaching, leading to a less complex, colourless system, resulting in flour with the optimal texture and colour for baking (Nisar et al., 2020). White flour has a long shelf life, requires little processing, and makes it a versatile product all of which make it a good choice for storage (Swindler, 2013).

Refining of edible insect powders refers to a series of biochemical, mechanical, or chemical processing steps applied to insect biomass to separate and purify its key nutritional components and to enhance their functionality, safety, and applicability in food, feed, pharmaceutical, or cosmetic formulations (Kim et al., 2022). The core processes include defatting; protein isolation for use in supplements or functional foods; enzymatic hydrolysis to generate bioactive peptides with enhanced solubility and bioavailability (Queiroz et al., 2023); bleaching for colour improvement and chitin removal for purity and texture optimization (Krongdang et al., 2023). Defatting is acknowledged as one of the best methods for raising the amount of protein in food by reducing its lipid content (Min-Soo Jeong, 2021).

2.4 Safety of edible insect powders

Insect powders such as those derived from house crickets, mealworms, or black soldier fly larvae are generally safe when properly processed (Melgar-Lalanne et al., 2019). However, allergenicity, microbiological contamination, heavy metals, anti-nutritional factors, and residual pollutants continue to be of big concern (Ruiz et al., 2025; Mézes, 2018). Products can be ensured safe through rigorous processing and regulatory oversight, especially as insects are introduced as an innovative food (Lähteenmäki-Uutela et al., 2021). Contamination of insect products is affected by the type of insect, the substrate in rearing, processing methods, and the storage environment (Ojha et al., 2021). Insects have the ability to accumulate heavy metals (particularly cadmium, lead, arsenic, and mercury) when grown on contaminated substrates, soil, water, or feed, especially if they are farmed using waste materials, unregulated organic matter, or in open-air environments near industrial or polluted regions. (Ruiz et al., 2025).

Insect microbiological contamination is harmful, but if they are handled, prepared, and kept correctly, they can be safe (Ignaczak & Kowalska, 2021). For instance, studies on microbiological contamination of *Tenebrio molitor* and *Acheta domesticus* and their powders have revealed the presence of *Enterobacteriaceae* (Messina et al., 2019). Proteins, particularly tropomyosin, arginine kinase, and hemocyanin, are cross-reactive with shellfish allergens and people with crustacean, mite, or shellfish allergies are most vulnerable (Marchi et al., 2021). Terrestrial insects such mealworms, caterpillars, wasps, and grasshoppers constitute the source of most documented cases of insect allergies (Zhao et al., 2021).

Heat treatment is one of the most effective method for destroying microbial contaminants such as *Salmonella* and *E. coli*. Drying at 121°C for 15 to 20 minutes can achieve complete microbial inactivation without significant nutrient loss (Van Huis et al., 2022*)*. Lipid removal through defatting with petroleum ether removes lipophilic contaminants including pesticides and polycyclic aromatic hydrocarbons *(*Poma et al., 2019*)*.

2.5 Shelf life of food flours and insect powders

Shelf life denotes the period during which a product remains in specific conditions without changing its efficacy, safety, or quality (Zhou et al., 2025). Shelf stability refers to the ability of a product to remain safe, nutritious, and sensory-acceptable (taste, smell, colour, texture, and appearance) over a specified period under defined storage conditions, without the need for refrigeration or other preservation interventions (Awulachew, 2021). Moisture content is central for the determination of shelf life for flours, i.e., the less moisture in flour, the greater is its storage stability (Martelli-Tosi et al., 2017). Moisture is a key element in preventing grain infection and infestation. In general, grain infestation is restricted by moisture content of 14% or below, especially for certain fungal species for example *Aspergillus*, *Fusarium*, and *Penicillium* (Nasir et al., 2003). Yeasts and moulds cannot develop at lower moisture levels however, they can grow at 14% moisture or higher (Alp & Bulantekin, 2021). Improper sealing of staple flours and insect powders may allow increased air circulation and moisture infiltration, leading to changes in their characteristics over time.

The shelf life of cricket powder depends on several factors, including raw material quality, manufacturing processes, packaging, and storage conditions (Kamau et al., 2017). Owing to their seasonal availability, edible insects have not received much recognition from researchers worldwide for long-term preservation (Ssepuuya et al., 2017). During preservation, edible insects are susceptible to microbiological, chemical, and physical alterations. Extrinsic (humidity, temperature) and intrinsic parameters (water activity, nutrient content) parameters have a considerable impact on the aforementioned changes. According to Wanjiku (2018), microbial growth and oxidation causes off flavors, rancidity, and changes in appearance which are the main causes of edible insect deterioration. Unpleasant odors and flavors could arise from the generation of free fatty acids due to hydrolytic rancidity (Shahidi & Hossain, 2022). Free fatty acids undergo auto-oxidation, resulting in undesirable flavors and aromas (Ssepuuya et al., 2017). The concern can be solved by maintaining ideal temperature (≤ 20°C), humidity (≤ 60% RH) and increase ventilation to limit microbial growth that promotes lipolysis (Qiu et al., 2022). Additionally, refining techniques such as defatting insect powders can enhance shelf stability through fat removal (Fornari et al., 2023). Routine quality evaluations, including sensory assessments, microbial testing, and chemical analyses, help track freshness and safety, ensuring that cricket powder consistently meets acceptable quality standards over time (Akullo et al., 2018).

2.6 Fatty acid profile of house cricket oil

House crickets are rich in lipids (10-25% dry weight), which can be extracted with the help of organic solvent for example hexane and petroleum ether into edible oils for culinary functions or bio-based industrial inputs (Sogame et al., 2025). The chemical properties, fatty acid content, and antioxidant determine quality, nutritional value, and stability of cricket oil (Pan et al., 2022). House cricket oil is distinguished by a high proportion of unsaturated fatty acids, predominantly linoleic, oleic, and palmitic acids (Murugu et al., 2024). Ajdini et al (2025) confirmed that *Acheta domesticus* oil contains linoleic (35-45%), oleic (25-35%), palmitic (15-20%) and stearic (5-10%) acids. House cricket oil contains endogenous antioxidants for example vitamin E and phenolic compounds that improve oxidative stability (Mart et al., 2022).

2.7 Sensory evaluation

This is important in the food industry for tasks such as developing new products, modifying recipes, and evaluating existing ones (Świąder & Marczewska, 2021). Furthermore, it significantly affects upholding quality standards and enabling successful product marketing (Sudirjo, 2023). The integration of sensory and consumer testing throughout the product development stage frequently facilitates the economical production and distribution of goods that satisfy customers, thereby diminishing the probability of product flaws (Meiselman et al., 2022). Qualitative descriptive analysis and acceptability testing are some of the techniques used in sensory evaluation. According to Mian & Masood (2017), an acceptability test evaluates a product's palatability, taste, appearance, and general customer satisfaction. To find out how well a product satisfies target customers' sensory preferences through likes and dislikes, a group of target consumers are asked to provide feedback. According to Ruiz-Capillas & Herrero (2021), acceptance tests are essential for making sure a product lives up to customer expectations, satisfies market demands, and upholds high quality standards.

One method of sensory evaluation used to explain a product's sensory attributes is qualitative descriptive analysis (Leighton et al., 2010). Trained panelists assess and describe the different sensory qualities, such as overall perception, flavor, texture, and visual attractiveness. In qualitative descriptive analysis, line scaling is normally a utilized method for assessing the intensity or quantity of sensory qualities (Rane et al., 2023). Following the presentation of a number of reference samples, each of which represents a distinct degree of the sensory attribute being evaluated, panelists are asked to assess the samples on a continuous line or scale based on the perceived intensity of the characteristic (Marques et al., 2022). This method allows one to compare the sensory attribute's amplitude or strength in relation to the samples (Estanqueiro et al., 2016).

Additionally, research has demonstrated that the appearance of a product can influence how its flavor, aroma, and taste are perceived. Colour also affects ratings of attribute intensity, liking, and readiness to eat. (Jeesan & Seo, 2020). The color, flavor development of thermally treated food is mostly attributed to products of the Maillard process. The olfactory note of Maillard reaction products is distinctive and can have a favorable or negative impact on a product's sensory acceptance (Świąder & Marczewska, 2021).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sample acquisition and preparation

Freshly killed *A. domesticus* (20 kg) were purchased from Masaka Microfinance and Development Cooperative Trust Ltd (MAMIDECOT), the organization responsible for cricket growers in greater Masaka region. After being brought to Kyambogo University Food Science laboratory, the crickets were strained to remove any unwanted material and cleaned four times with cold, clean running water to do away with dirt, polythene, and wood fragments. After that, the crickets were roasted for 2 hours at 150°C in an electric oven (Memmert GmbH Co KG, UM400); others were oven dried for 17 hours at 75°C (Dobermann et al., 2019). Crickets were cooled to ambient temperature before finely grinding into powder using a laboratory-scale mill. The obtained cricket powder was labeled, sealed, and kept at room temperature in a cabinet after being placed into sterile zip-lock high-density polyethylene (HDPE) bags.

3.2 Optimization of refining protocol

3.2.1 Defatting cricket powder

Defatting was achieved by 24 hour soaking of house cricket powder in petroleum ether (60℃ boiling point) at a ratio of 3:1 (solvent to powder). The mixture was decanted after 24 hours to separate the flour and solvent. The slurry was emptied into a pan and left to dry overnight at 65℃ in an oven (WTB binder, Tuttlingen, Germany). Sterile zip-lock bags containing defatted house cricket powder were sealed and kept at room temperature in a cabinet (25°C) before bleaching. The mixture of oil and petroleum ether was separated by removing the solvent using a rotary evaporator (Neo-Tech SA 4041, Milmort/BELGIQUE) at 60 °C for 30 minutes (Choi et al., 2017).

To eliminate any remaining moisture and solvent, the obtained oil after evaporation was dried for an hour at 60°C in an electric oven (Memmert GmbH Co KG, UM400). The oil was then weighed to obtain its percentage in the cricket powder and calculated based on the following expression;

Fat % (on dry basis) = $\frac{weight\ of\ flask+lipid-weight\ of\ empty\ flask}{weight\ of\ the\ sample*(1-moisture\ content)} * 100$……………..………...(1)

The obtained oil was stored in the freezer (control), on shelf one without foil and the other covered with foil after which the chemical and sensory quality plus stability (15 weeks) was determined.

3.2.2 Bleaching cricket powder

Defatted house cricket powder was bleached using two methods that were most preferred after the color ranking consumer acceptability testing. For the first method, a mixture of sodium hypochlorite solution and hydrogen peroxide was used. The mixture containing house cricket powder and reagents was dried in an electric oven (Memmert GmbH Co KG, UM400) by heating it at 65 °C while shaking for 25 minutes (Calkin, 1969). The mixture was cooled and the resultant powder placed in sterile zip seal bags, labeled and kept at ambient temperature in a cabinet before analysis.

The second technique involved soaking the cricket powder in chloroform (Sigma-Aldrich, GJ Chemical; 61°C boiling point) for 24 hours. After 24 hours, the mixture was separated by decanting and the slurry was dried overnight in an oven (WTB binder, Tuttlingen, Germany) at 65°C. The bleached cricket powder was cooled to room temperature, then packed into sterile zip seal bags, securely sealed, appropriately labeled, and maintained at room temperature in a cabinet.

3.2.3 Consumer acceptability screening and testing

Design expert (version 13) software was used to generate 24 combinations of different refining (defatting and bleaching) levels for the sensory screening. The combinations were given three letter blind codes to avoid bias during sensory acceptability evaluation of their appearance, colour, aroma and overall acceptability. Fifty untrained panelists, who were staff and students at Kyambogo University, evaluated the sensory attributes on a nine-point scale to assess hedonic preferences (Sharif et al., 2017); where 1 represented "dislike extremely," 2 "dislike very much," 3 "dislike moderately," 4 "dislike slightly," 5 "neither like nor dislike," 6 "like slightly," 7 "like moderately," 8 "like very much," and 9 "like extremely.

Before the evaluation, the panelists were requested to provide informed consent by reviewing and signing the consent form. The meanings of the descriptions on the sensory ballot were also explained to the panelists to enable them carry out the sensory evaluation accurately.

After the sensory acceptability exercise, the results of the combinations with the highest desirability were obtained using design expert software. Using the same procedure, the screened combinations were further ranked for colour using a scale of 1 to 4, where 1 = most bright colour and 4 = least bright colour. The refining procedures corresponding to the most preferred combinations were used to produce the refined house cricket powder products that were studied further to determine their quality, safety and shelf stability.

3.3 Proximate analysis

Proximate analysis is a method used to determine approximate amounts of macro nutrients in a food or feed sample. It provides an estimate of the major constituents such as moisture, crude protein, crude fat, ash, fiber and carbohydrates that make up the sample often expressed as percentages (Echebiri et al., 2022).

3.3.1 Determination of moisture content

About 3 grams of house cricket powder was weighed in triplicate using an analytical balance. The sample was transferred into cooled, pre-weighed porcelain crucibles that had been previously heated to 105 °C and allowed to cool to room temperature in a desiccator. Crucibles were placed inside a free convection air oven (model 65SF, Fisher Scientific USA) for drying at 105 °C. Samples were cooled in a desiccator and then precisely weighed to the nearest 0.001 g. The moisture content (W) was computed as a percentage relative to the weight of the product, based on the original sample weight, using equation 2;

$$W = \frac{M_1 - (M_2 - M_0)}{M_1} \times 100 \quad \text{……………………………………………………(2)}$$

Where; M_1 = the weight of the test portion (g); M_0 = weight of crucible (g); M_2 = weight of crucible and sample post-drying (g).

3.3.2 Determination of crude protein

Crude protein was determined according to AOAC method number 920.87 (AOAC, 2012). About 0.5 g of the house cricket powder was weighed into a digestion flask to which 1 g (a tablet) of the Kjeldahl catalyst (copper and potassium sulfate), after which 15 mL of 98% sulphuric acid was added. The flask containing sample was transferred to the digestion apparatus (Kjeltec System HT 2, Foss tecator, Hoganäs, Sweden) and the contents digested at 400 °C for 3 h until the mixture turned pale green with no further bubbling/ fume evolution. The digest was cooled and diluted using 50 mL of distilled water in a distillation flask. 3 drops of the mixed indicator (methyl red and methylene blue) and 50 mL of sodium hydroxide solution (50%) was gently added. The digest was distilled and the ammonia gas liberated was captured in a 0.025M boric acid (2%) solution. After for 10 minutes distillation, the ammonia in the boric acid solution was titrated using standard

0.05M sulphuric acid solution. The volume (A mL) of the acid required for complete reaction was recorded. Similarly, a blank was prepared and titrated, and the volume (B mL) of sulphuric acid required to reach the end-point was also recorded. Using normality (N) of sulphuric acid, total nitrogen was determined as shown in equation 3. Crude protein was obtained by multiplying the nitrogen content with the conversion factor of 6.25 using equation 4;

$$\text{Total nitrogen} = \frac{(A-B)\times N \times 14}{10\times W} \qquad \text{(3)}$$

$$\text{\% Protein} = \text{total nitrogen} \times 6.25 \qquad \text{(4)}$$

Where, A = Volume of the standard sulphuric acid utilized in the sample titration; B = Volume of the standard sulphuric acid used in the blank titration; N = Normality of the acid in four decimal places; W = weight of the sample used in the determination

3.3.3 Crude fat determination

Approximately 3 g of well ground homogeneous dried house cricket powder was placed into an extraction thimble whose bottom was fitted with a small piece of cotton wool to prevent sticking of the sample. Each of the extraction cups of known weight, 50 mL of hexane was added. Fat in the samples was placed in the thimbles and extracted by refluxing the solvent through the samples for 15 hours and collected in the cups. The cups were then dried in an Isotemp oven, (model 65SF, Fisher Scientific USA,) for 30 minutes at 105°C to evaporate off any moisture traces. After drying, the cups were left to cool in a desiccator and re-weighed. Crude fat was computed as per equation 5.

$$\text{Crude Fat (\%)} = \frac{W_2-W_1}{W} \times 100 \qquad \text{(5)}$$

Where; W_2 = Weight of dish with fat in grams; W_1 = Weight of dish without the fat in grams; W = Weight of material in grams taken for test.

3.3.4 Determination of ash content

Approximately 1 g of house cricket powder, in triplicate, was placed into cool pre-weighed and pre-conditioned porcelain crucibles (105°C for 2 hours). Sample was incinerated in a muffle furnace (Fisher Scientific Isotemp Muffle oven, model 186A, Fisher Scientist Co. USA) at 550°C for a duration of 5 hours to guarantee total burning of organic matter. Following incineration, the crucibles were allowed to cool to ambient temperature inside a desiccator and were then re-weighed. The ash content was computed using equation 6;

$$\text{Ash content (\%)} = \frac{M_2 - M_0}{M_1 - M_0} \times 100 \quad \text{……} \quad (6)$$

Where M_0 = Weight of empty crucible (g); M_1 = Weight of the sample and crucible before incineration (g); M_2 = Weight of the ash and crucible after incineration (g)

3.3.5 Determination of crude fiber

About 0.5 g of house cricket powder was measured into fiber crucibles that were subsequently positioned beneath Dosi- fiber columns (J.P Selecta, South Africa,). About 120 mL of dilute sulphuric acid was added to the sample through the columns to hydrolyse the organic compounds. 1-octanol anti-foaming agent (3 drops) was added and solution heated up to boiling. After boiling, the heating rate was reduced and the solution further heated for 30 minutes to allow for acid hydrolysis. The columns were subsequently washed using distilled water in order to remove residual sulphuric acid. 120 mL potassium hydroxide was added for alkaline hydrolysis at 100°C for 30 minutes. The contents of the crucibles were subsequently dried in a forced air convection oven (Fisher Scientific Isotemp oven, model 65SF, Fisher Scientific Co. USA) at 105°C for 45 minutes to evaporate the moisture and cooled in a desiccator before being weighed. The samples were subsequently incinerated in the muffle furnace at 550°C for 3 hours, cooled in a desiccator and weighed again. Crude fiber content in the samples was subsequently computed following equation 7.

Crude Fiber (%) = $\frac{A_2 - A_1}{A_0} \times 100$ ……………………………………………………………...(7)

A_o = weight of sample; A_1 = Weight of crucible plus residue after incineration; A_2 = Weight of crucible plus residue before incineration

3.3.6 Determination of carbohydrate content

The carbohydrate content was calculated according to the difference AOAC number 986.25 method (AOAC, 2012). Total of all measured proximate constituents (crude fat, crude protein, crude fiber, ash and moisture) was deducted from 100 % equation 8;

$\% Carbohydrate = 100 - (\% moisture + ash + fat + protein + fiber)$………. (8)

3.4 Evaluation of functional properties of the house cricket powders

3.4.1 Water Holding Capacity

Water holding capacity (WHC) was measured using procedure reported by Mesías & Morales (2017). A pre-weighed centrifuge tube was filled with 2 g of the powder and 15 mL of water was added. Mixture was vortexed for 1 minute and allowed to stand at ambient temperature for 30 minutes. It was then centrifuged (Hettich Zentrifugen D-78532 Tuttlingen) for 15 minutes at 1400g. Any non-absorbed water was carefully emptied after centrifugation, and the tube's weight was recorded. The formula was used to calculate the mass increase per gram of sample to establish the water holding capacity as follows;

$WHC = \frac{W_{2-}w_1}{W_2} \times 100$ ……………………….……...……….…………………...……………… (9)

Where, W_2 = weight of tube with sample plus retained water; W_1= weight of tube with sample

3.4.2. Oil Absorption Capacity (OAC)

Castilho, Fontanari, and Batistuti (2010) methodological steps guided the assessment of oil absorption capacities. Approximately 2.0 g of house cricket powder sample was placed in a 15 mL centrifuge tube, followed by the addition of 10 mL of soybean oil, after which the mixture was subjected to centrifugation (Hettich Zentrifugen D-78532 Tuttlingen) for two minutes at 1,600g. The non-absorbed oil was drained and the remaining contents of tube were weighed. OAC of the sample was calculated using equation 10;

$OAC = \frac{W_{2-}w_1}{W_2} X100$………………………………….……...……………………………(10)

Where W_2 = weight of tube with sample plus oil retained; W_1= weight of tube with sample

3.4.3 Swelling capacity and solubility

Swelling capacity was assessed following procedure described by Kaur, Oberoi, Sogi, and Gill (2011). In this method, about 0.25 g of house cricket powder was combined with 5 mL of distilled water in a 15 mL centrifuge tube. Mixture was heated at 90°C for 30 minutes with continuous agitation in a shaking water bath (Gupta Pvt Ltd, Ambala Cantt, India). After heating, the sample was cooled to room temperature using a stream of cold water and then centrifuged (Hettich Zentrifugen D7200, Tuttlingen, Germany) for 10 minutes at 3200g. Clear supernatant was carefully transferred into a pre-weighed aluminum dish and dried at 110°C for 24 hours. Sample was cooled in a desiccator and weighed to determine the solubility using Equation 11;

$\% \, solubility = \frac{weight\ of\ soluble\ starch}{weight\ of\ sample} \times 100$ ……………………...…………………..……..(11)

To calculate the swelling power, the weight of the wet sediment in the centrifuge tube was recorded. After determining the sediment weight, swelling power was computed according to equation 12.

$$swelling\ capacity = \frac{\text{sediment weight}}{\text{dry sample weight}} \qquad (12)$$

3.4.4 Bulk density (B.D) and Tapped density (T.D)

The bulk density was determined according to Mandge, Sharma, and Dar (2014). Without shaking, 15 g of the house cricket powder was weighed and put in a 100 mL clean measuring cylinder. After leveling the powder with a leveling rod, the measuring cylinder was used to determine the sample's bulk volume. After 50 consistent even taps on a level surface with the measuring cylinder, the powder was leveled again with a spatula the tapped volume read. Bulk and tapped densities (in g/ml) were determined using equations 13 and 14.

$$\text{B.D} = \frac{Weight\ of\ flour}{Volume\ of\ flour\ before\ tapping} \qquad (13)$$

$$\text{T.D} = \frac{Weight\ of\ flour}{Volume\ of\ flour\ after\ tapping} \qquad (14)$$

3.4.5 Dispersibility

Dispersibility is the ability of a substance, typically a powder to break apart and evenly distribute within a liquid. Dispersibility was determined according to Olapade's (2014) method. Roughly10 g of house cricket powder was mixed into 100 milliliters of distilled water until fully dissolved. After shaking gently for 5 minutes, mixture was left to stand for about 3 hours. Dispersibility was calculated using equation 15.

$$\%Dispersibility = 100 - volume\ of\ settled\ particles \qquad (15)$$

3.5 Microbial safety of house cricket powder

3.5.1 *Escherichia coli*

E.coli was enumerated following procedure described in ISO 16649-2 (2001). To 10 g of the house cricket powder, 90 mL of sterile buffered peptone water was added. The suspension was homogenised using a stomacher for 2 minutes, and there after used to make further serial dilutions. To 1 mL of each of the selected dilution in triplicate, about 15 mL of molten tryptone-bile-glucuronide (TBX) medium, maintained at temperatures between 44 and 47°C, was added. The inoculum was gently mixed with the medium and allowed to solidify on a cool, levelled surface. Once fully solidified, the prepared plates were inverted and incubated at 44°C for 24 hours. Control tests were done using *Escherichia coli* as positive control and *Enterococcus faecalis* as the negative control. After the 24 hours of incubation, *Escherichia coli* colonies demonstrating β-glucuronidase activity in each dish containing up to 150 blue colonies was examined.

The quantity of microorganisms, indicated as log colony forming units of test sample, a weighted average derived from two consecutive dilutions, was calculated following equation 16.

$$N = \frac{\Sigma C}{V \times [\, n_1 + (0.1 \times n_2)]d} \quad \text{……………………………………………………………..…………..……(16)}$$

Where;

ΣC = the total of the colonies enumerated from the two examined petri dishes from two successive dilutions, wherein at least one dish contains a minutes of 10 colonies; n_1 = number of dishes retained at the first dilution; n_2 = quantity of dishes inspected during the second dilution; v = amount of the inoculant placed in each dish, in millilitres; d = dilution corresponding to the first dilution examined.

3.5.2 *Enterobacteriaceae (*cfu/g)

This was enumerated according to method number 21528 -2 (ISO, 2017). Initially, 10 g of house cricket powder (Kiiru et al., 2024) was weighed into a sterile stomacher bag to which 90 mL of BPW was added to make 10^{-1} dilution from which subsequent dilutions were obtained. After each dilution, the contents were vortex-mixed (232 Fisher Scientific, USA) for 10 s. Duplicate sterile petri dishes were inoculated each with 1 mL of the target dilution. To each petri dish, about 15 mL of violet red bile glucose (VRBG) agar, maintained at a temperature range of 44 – 47°C was poured into each petri dish containing the inoculum. The medium and inoculum were gently mixed and allowed to solidify on a cool horizontal surface. Once fully solidified, an overlay of approximately 5 mL of VRBG agar was added to create semi-anaerobic conditions, let to solidify, inverted, put in an incubator at 37°C for 24 hours. Pink colonies of *Enterobacteriaceae* were examined after 24 hours of incubation. Control tests were also carried out using *Klebsiella aerogenesas* positive control and *Staphylococcus aureus* as the negative control. Five colonies from the positive and negative control samples were randomly chosen from each dish for sub-culturing onto pre-dried nutrient agar (NA) by streaking for the biochemical confirmation tests. These plates were incubated at 37°C for a duration of 24 hours. For biochemical tests, the oxidase reaction (Chavan et al., 2022) was positive if the oxidase disc did not exhibit a change in colour to dark blue-purple from white within 10 seconds. The fermentation test was performed (Britton et al., 2021) on oxidase-negative samples and the reaction was regarded positive when the yellow colour emerged across the entire content of the tube. The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated following the equation 16.

3.5.3 *Staphylococcus aureus* (cfu/g)

This was enumerated following to method number 6888-1 (ISO, 2003). Initial suspension was prepared by measuring a quantity of cricket powder (10g) into a sterile stomacher bag, subsequently; 90 mL of buffered peptone water (BPW) was introduced. The initial suspension was then homogenised using a stomacher for 2 minutes. Further dilutions were prepared up to 10^{-3} by pipetting 1mL of previous dilution into 9 mL BPW successively. After each dilution, the contents were vortex-mixed (232 Fisher Scientific, USA) for 10 seconds. Using a sterile pipette, 0.1 mL of the initial suspension (10^{-1} dilution) was transferred, to both of the two pre prepared sterile Baird-Parker agar (15 mL per petridish) plates. The process was reiterated for subsequent decimal dilutions. The inoculum was carefully spread rapidly and evenly across the surface of the agar plates, trying not to touch the sides of the dish, using a sterile spreader. The plates were allowed to air dry with their lids secured for approximately 15 minutes at room temperature (20^{o}C) in the laboratory, after which they were inverted and then incubated for 48 h in the incubator maintained 37°C. Control tests were carried out using Staphylococcus aureus as the positive control and Escherichia coli as the negative control. A colony of growth on the control culture slope was touched and inoculated into 9 mL BPW, this is 10^{-1} dilution, 10^{-2} dilution was prepared, followed by gentle mixing and a spread plate of 1 loopful of the 10^{-2} dilution was carried out on Baird-Parker agar and was incubated alongside the sample. The same was repeated for the negative control and uninoculated buffered peptone water. After 48 hours of incubation, coagulase-positive *Staphylococcus aureus* on each dish containing a maximum of 300 colonies, including 150 colonies of the typical type (colonies that are black or grey, glossy, and raised with a clear area around them) and/or unusual colonies (shiny black colonies lacking a clear zone) at two successive dilutions were examined and counts taken using a colony counter.

The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated following the equation 16.

3.5.4 Total coliforms (cfu/g)

This was enumerated based on ISO 4832:2006. House cricket powder (10.00 g) was measured to which 90 mL (9 x mass of sample weighed) of the diluent, that is sterile buffered peptone water was added. This initial suspension was homogenised using a stomacher for 2 minutes. Following each dilution, the contents were thoroughly vortex-mixed for a duration of 10 seconds. Using a sterile pipette, 1 mL of the initial suspension (10-1 dilution) was transferred to two sterile petri dishes, creating duplicate plates. Subsequently, a series of successive dilutions of factor 10 up to 10^{-5} were prepared by transferring 1 mL samples into 9 mL of buffered peptone water, and then these samples were plated. Around 15 mL of molten Crystal violet neutral red bile lactose agar (VRBL), maintained at a temperature of 47°C, was poured into each Petri dish. The inoculum and the medium were carefully mixed by gently rotating the Petri dishes, and the mixture was left to solidify by placing the Petri dishes on a cool, horizontal surface. Once the plates had completely solidified, they were inverted and placed in the incubator at 37 ± 1^{o}C for 24 hours. Control tests were also carried out using *Klebisella aerogenes* as positive control and *Staphylococcus aureus* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was 10^{-1} dilution, 10^{-2} dilution was also prepared, mixed gently and a pour plate of a loopful of 10^{-2} dilution carried out on Crystal violet neutral red bile lactose agar and incubated alongside the sample. The same procedure was repeated for the negative control. Pour plates of pipetted 1ml of BPW, approximately 1 mL of un pipetted BPW and Crystal violet neutral red bile lactose agar were also prepared to check for sterility of the reagents and apparatus used. After 24 hours of incubation, the Petri dishes containing with up to 150 colonies of purplish red/pink colonies with a diameter of at least 0.5 mm (sometimes surrounded by a reddish zone of precipitated bile) were selected. The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 16.

3.5.5 *Salmonella* species

The detection of suspected *Salmonella typhimurium* followed the method number 967.25 (AOAC, 2012). Approximately 25 g of house cricket powder was placed aseptically into a stomacher bag, and sterile lactose broth diluent was added (225 mL), a quantity equal to 9 times the measured weight of the sample (sample weight x 9 mL). The mixture was homogenized for 2 minutes. The stomacher bag was sealed and then incubated at 35°C for 24 hours for pre-enrichment. After incubation, the mixture was gently shaken, and 1 mL of the incubated solution was transferred to 9 mL of Selenite cysteine broth (SCB), while 0.1 mL was transferred to 10 mL of Rappaport Vasiliadis broth (RVS), both separately. The SCB was incubated at 35°C, and the RVS was incubated at 42°C both for 24 hours for selective enrichment. After incubation, the contents of the tubes were vortexed, and a loopful from each tube was streaked onto three sterile selective media: Bismuth sulphite agar (BSA), Hektoen enteric agar (HEA), and Xylose desoxycholate agar (XLD) in petri dishes. The plates were then incubated at 35°C for 24 hours, while BSA plates were examined after 48 hours.

Control tests were carried out using *Salmonella typhimurium* as positive control and *Escherichia coli* as negative control where a colony of growth on the control culture slope was inoculated into 9 mL of lactose broth and then treated as the sample.

3.5.6 *Listeria* species

This was detected following the method number ISO 11290-1 (2017). Approximately 25 g of house cricket powder was placed aseptically into a stomacher bag, and sterile one broth *Listeria* medium was added (225 mL), a quantity equal to 9 times the measured weight of the sample (sample weight x 9 mL). The stomacher bag was sealed and incubated the (initial suspension) at 30±1°C for 24±2 hours for primary enrichment. After 24 hours, the primary enrichment mixture was gently shaken. A loopful was transferred using a sterile inoculating loop by streaking the surface of previously prepared sterile brilliance *Listeria* agar plates, to obtain well-separated colonies there after incubated at 37°C for 24 hours. Plates were examined for presumptive blue/green colonies of *L.monocytogenes*. Presumptive *L. monocytogenes* colonies on brilliance *Listeria* agar (BLA) were considered as blue colonies with/ without halo zone.

Presumptive colonies of *L. monocytogenes* were done by use of either the Oxoid Biochemical Identification System (OBIS) mono test kit or biochemical tests which included gram staining (microscopy), catalase test, oxidase test, haemolysis on blood agar test, Carbohydrate utilization (*Listeria monocytogenes* gives a positive reaction on L-Rhamnose and a negative reaction on D-Xylose) and motility test.

3.5.7 Total plate count (cfu/g)

This was determined following ISO 4833:2013-1 method. Approximately 10 g was measured to which 90 mL (9 x mass of sample weighed) of the diluent, that is sterile buffered peptone water was added. This initial suspension was homogenized using a stomacher for 2 minutes. After each dilution, the contents were vortex-mixed for 10 s. Duplicate sterile petri dishes were each inoculated, by means of a sterile pipette with 1 mL of the initial suspension (10^{-1} dilution). Further, successive pf factor 10 up to 10^{-7} were prepared by pipetting 1 mL samples into 9 mL buffered peptone water and then plated. Approximately 15 mL of sterile plate count agar maintained at a temperature of 44 to 47°C in a water bath was poured into individual Petri dishes. The inoculum was then gently mixed with the agar by swirling the petri dishes, and the medium was allowed to solidify by placing the dishes on a cool horizontal surface. Once solidified, the prepared plates were inverted and placed inside an incubator (Memmert by Schwabach FRG, Germany, model D-91126) set at a temperature of 30 ± 1 °C for a duration of 72 ± 3 h. Following the specified incubation period, plates containing colonies up to a maximum of 300 were carefully examined, and counts were recorded using a colony counter. The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 16.

3.5.8 Yeasts and moulds (cfu/g)

This was determined following procedures in ISO 21527 - 2 method. 10 g of house cricket powder was weighed accurately to which 90 mL of the diluent (buffered peptone water), was added. This initial suspension was homogenized using a stomacher for 2 minutes. After each dilution, the contents were vortex-mixed for 10 s. Onto one Dichloran 18 % glycerol agar (DG 18) plate, using a sterile pipette, 0.1 mL of the initial suspension (10^{-1} dilution) was inoculated. Further 10-fold serial dilutions up to 10^{-4} were prepared by pipetting 1 mL samples into 9 mL buffered peptone water and 0.1 mL plated on DG18 for each dilution using a sterile pipette each time. For all dilutions duplicate plates were prepared. Using a sterile spreader, the liquid was evenly spread across the agar's surface until full absorption into the medium occurred. The plates were then positioned upright, with lids facing upward, and placed in the incubator for aerobic incubation. (Memmert by Schwabach FRG, Germany, model D-91126) set at 25 ± 1 ˚C for 5 days. Control tests were carried out using yeasts – *Candida albicans*, moulds – *Aspergillus flavus* as positive control and *Escherichia coli* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was 10^{-1} dilution, 10^{-2} dilution was also prepared, mixed gently and a spread plate of a loopful of 10^{-2} dilution carried out on DG 18 and incubated alongside the sample. The same procedure was repeated for the negative control. Spread plates of pipetted 0.1 mL of Buffered Peptone Water, approximately 1 mL of un pipetted Buffered Peptone Water and DG 18 were prepared to check for sterility of the reagents and apparatus used. After 5 days of incubation, the dishes containing up to 150 colonies were selected and counted The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 16.

3.6 Chemical safety of the house cricket powder

3.6.1 Pesticide residues

This was determined according to the multi residue method. The samples were characterized using Gas Chromatography Mass Spectrometry together with Liquid Chromatography Mass Spectrometry (GC-MS and LC-MS). Acetyl acetate extraction was used in a multiple pesticide residue analysis approach to show their presence or absence in the cricket powder samples. After weighing the sample (5 g) into a centrifuge tube, 10 ml of MilliQ water, 10 ml of ethyl acetate, and 1% acetic acid were added, and the mixture was vortexed. After adding10 g of sodium sulphate, the mixture was violently shaken for ten seconds, ultra-sonicated for 25 minutes, and thereafter centrifuged for 3 minutes at 3200 g (Zukryandry et al., 2021). The supernatant (acetyl acetate extract) was filtered into a test tube and a portion (0.5g sample/ml) subjected to GC- and LC-MS/MS analysis on Zorbax Plus c18 column (1.8 µm particle size, 2.1x150 mm, Agilent Technologies Santa Clara, California, USA) as the stationery phase. Mobile phases consisted of:

Phase A, composed of water comprising 0.1% formic acid, 5 mM ammonium formate, and 2% methanoland Phase B, composed of methanol containing 0.1% formic acid, 5 mM ammonium formate, and 2% water. Column temperature: 35ºC, injection volume 5 µL, and rate of flow: 0.3 ml/minutes. A series of known pesticide concentrations (calibration standards) were prepared and run through the analytical method to create a calibration curve. Internal standards were added to the samples to monitor the extraction and analytical process and ensure accuracy. The concentration of each pesticide in the sample was quantified using mass spectrometric data, based on the corresponding calibration curve.

3.6.2Total aflatoxins

This was determined following the procedures outlined in ISO method number 16050 (2003). Approximately 20 g of the cricket powder was weighed into a 100 mL amber glass conical flask. To the mixture was added 3 g of sodium chloride and 50 mL of a mixture of methanol and a mixture (30:20). The resulting mixture was shaken in a homogenizer at a speed of 200 g for 1 h. After shaking, the suspension was let to settle and then filtered to obtain the first filtrate using Whatman No. 1 filter paper. The filtrate (50 mL) was collected in a conical flask and 10 mL of the filtrate was transferred to a beaker to which 30 mL of phosphate buffer solution was added. Using 0.1 mol/L sodium hydroxide (NaOH), the pH of the solution was adjusted to 7.4. The next step involved passing the sample through an immune-affinity column to acquire the second filtrate, followed by a rinse of the column with 20 mL of ultrapure water. Using 1.5 mL of HPLC grade water-free methanol, the total aflatoxins were subsequently extracted.

A high-performance liquid chromatography (HPLC) system was employed to separate and quantify aflatoxins. The setup consisted of a binary pump, auto sampler, fluorescence detector, reverse phase column C18 (Hypersil ODS, 4.6 × 100 mm, 5 µm), and a column heating chamber.

A consistent mixture of water and acetonitrile (75 mL: 25 mL), with a rate of flow set at 1.0 mL/minutes was as the mobile phase. The fluorescence detector's setup involved an excitation wavelength at 360 nm and an emission wavelength at 423 nm. The retention time for the elution of each specific aflatoxin was recorded. Standard samples of aflatoxin-B1, aflatoxin-G1, aflatoxin-B2, and aflatoxin-G2 were added to blank flour samples at three different concentration levels to validate the reliability of the method, taking into consideration the background contamination levels of the cricket powders.

The mass (m_t) of test sample within the portion of the second filtrate subjected to the immunoaffinity column was calculated using equation 17.

$$m_t = m_0 \times \frac{v_2 \times v_4}{v_1 \times v_3} \quad \text{……………………………………………………………………..(17)}$$

Where; m_0 = mass of test portion in grams; V_1 = total volume of first filtrate in millilitres; V2 = volume of the diluted fraction from the initial filtrate, measured in millilitres; V_3 = total volume of the second filtrate, in millilitres; V_4 = volume of the second filtrate portion in millilitres.

The weight proportion of each aflatoxin, represented as, w_i was calculated in micrograms per kilogram of the sample using equation 18.

$$w_i = \frac{v_5 \times m_i}{v_6 \times m_t} \quad \text{…………………………………………………………...………………..(18)}$$

Where;

V_5 = volume of the eluate, in microliters; V_6 = volume of the refined and injected sample extract, measured in microliters; m_i = weight of each aflatoxin i contained within the injected volume, corresponding to the peak area or peak height obtained from the calibration curve, measured in nanograms; m_t = weight of test sample in grams within the portion of the second filtrate collected for processing through the immunoaffinity column (V_4).

3.6.3 Heavy metals

This was determined following AOAC Official Method 2015.01 and US EPA 200.2 and 200.7. About 1 g of fine sample was weighed into a PTFE vessel. 3 ml HCl + 9 ml. HNO3 was added. The mixture was digested in microwave for 30 minutes using the optimized program. The PTFE vessel was cooled; the contents were quantitatively transferred and made up to 50 ml in a volumetric flask with deionized water. Reagent Blank (RB) was prepared at the same time and under the same conditions. About 10 g of sample was weighed into a clean dry silica wash/ crucible, 1.5ml of 15% magnesium acetate was added, heated on a hot plate to completely char the sample, and placed the crucible/silica wash in a muffle furnace maintained at 45℃ and allowed to ash for 4 hrs. After removal from the muffle furnace, the crucible was cooled until ambient temperature in a desiccator. 1 mL of deionized water added, 1 mL of concentrated hydrochloric acid was subsequently added, the resulting mixture was slowly heated to dissolve all the minerals and bring it to near dryness. Another 5 mL each of water and 1 mL hydrochloric acid was added, warmed to dissolve, transferred quantitatively into a 50 mL volumetric flask using deionized water and made up to volume. The sample was diluted to get the mineral into the linear concentration range of instrument using deionized water.

By reference to the calibration graph, for each metal, the concentrations corresponding to the absorbances of the test Portion and of the blank as were determined according to equation 19.

$$Conc. Element, mg/kg \quad = \quad [(Cs\ - Cb)\ x\ V\ x\ D.F]\ /M \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (19)$$

Where;

Cs = Concentration (mg/L) of element in the sample solution, Cb = Concentration (mg/L) of element in the blank solution, M = weight of the sample taken in gram, D.F. = Dilution factor, if any, V =Final Volume made up in m

3.7 Chemical stability of the cricket powder

3.7.1 Acid value

This was analyzed following AOAC Method 940.28 (AOAC, 2012). Approximately 0.5 g of sample was dissolved in 25 mL of neutralized absolute ethanol and allowed to stand for around 30 minutes with occasional shaking. The mixture was then carefully decanted to obtain the alcoholic layer, into which phenolphthalein indicator was added in three drops. To titrate this solution, 0.05 M sodium hydroxide was used. Free fatty acid content was quantified as oleic acid, and the acid value was calculated in milligrams of potassium hydroxide (KOH) per gram of sample using Equation 20.

$$FFA = (ml\ NaOH \times NaOH\ normality \times 28.2)/(weight\ of\ the\ sample)$$

$$Acid\ value\ (mg\ KOH/g) = FFA \times 1.99 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(20)$$

Where 28.2 = constant from RFM of oleic acid; 1.99 = conversion factor from % oleic acid to mg KOH/g of sample.

3.7.2 Peroxide Value (PV)

PV determination was conducted in accordance with method number Cd 8-53 as stated by American Oil Chemists' Society (AOCS, 2003). Exactly 2.5 g house cricket powder was added to 25 mL of a glacial acetic acid–chloroform solution in 3:2 ratio (v/v) and left to stand for roughly 30 minutes with periodic agitation. 0.5 mL of saturated potassium iodide (KI) solution was added. Mixture was allowed to stand for an additional 10 minutes with intermittent stirring, after which

30 mL of distilled water was incorporated. Titration was conducted with 0.1 M sodium thiosulfate ($Na_2S_2O_3$) until the solution exhibited a pale yellow colour. At this stage, 0.5 mL of starch indicator was introduced, and titration continued until the blue color disappeared. A blank determination was carried out under the same experimental conditions. The peroxide value was computed using Equation 21.

$$Peroxide\ value\ = \frac{(S-B) \times M}{W} \quad \text{………………………………………………..…………}(21)$$

Where S = titre of sample (mL); B = titre of blank (mL); M = molarity of the thiosulfate solution; W = weight of sample (g).

3.7.3 Thiobarbituric acid

This value was determined using the TBARS (thiobarbituric acid reactive substances) assay, with spectrophotometric analysis conducted at a wavelength of 532–535 nm. A precisely weighed 0.2 g of house cricket powder was transferred into a test tube, followed by addition of three drops of an antioxidant solution. The solvent was removed using rotary evaporation under reduced pressure at a water bath temperature maintained between 35 to 40°C. Subsequently, 3 mL of TBA reagent and 17 mL of trichloroacetic acid solution were added to the tube. A stream of nitrogen gas was passed through the tube to displace oxygen, and the tube was immediately sealed with a stopper. 100°C boiling water bath was used to heat sample for a duration of 30 minutes to facilitate color development and subsequently cooled to ambient temperature using running water. Approximately 5 mL of chloroform was introduced into the mixture, and contents were briefly mixed using a vortex mixer. The colored solution (15 mL) was put into a glass tube, then centrifuged at 3,000 g for 10 minutes. If aqueous phase remained turbid, centrifugation was repeated at 10,000 rpm for an additional 10 minutes. An aliquot of the clarified aqueous phase was used for measurement of absorbance at 532 nm. Under the same conditions, a blank test was executed. TBA value calculated using Equation 22.

TBA no. (mg malonaldehyde/kg fat)

$$TBA = (Ab. x\ F\ x\ 0.2\ )/\ W \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(22)}$$

Where; Ab = absorbance at 532 nm, W = weight of fat in volume of extract (g), F = factor = 46.

3.8 Chemical quality parameters of cricket oil

The chemical quality of the cricket oil which included saponification value, iodine value, paranisidine value.

3.8.1 Saponification value

Each sample, in triplicate, was accurately weighed to 5 g and transferred into individual 250 mL flasks. Alcoholic potassium hydroxide (KOH) solution, measuring 50 mL, was introduced into each flask. Additionally, two blank samples were prepared containing only 50 mL of alcoholic KOH. To prevent bumping during boiling, three boiling beads were introduced into both sample and blank flasks. Each flask was then fitted with a reflux condenser (BOROSIL Scientific, GRAHAM, DRIP TIP, I/C JOINT) and gently heated in a water bath until the contents formed a homogeneous solution. After the boiling process, flasks were taken from the condenser, cooled to ambient temperature, and 1 mL phenolphthalein indicator was added. Hydrochloric acid (0.5 N) was used to titrate until pink coloration disappeared, indicating neutralization. This procedure was repeated for all sample and blank preparations. Saponification value was subsequently calculated following Equation 23.

Saponification value (mg KOH/g)

$$= \frac{(\mathrm{Bk}-\mathrm{S})\mathrm{x\ Nx\ 56.1}}{\mathrm{M}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(23)$$

Where;

Bk = Volume of titrant (mL) for blank, S = Volume of titrant (mL) for sample, N = Normality of HCl, M = Sample mass (g)

3.8.2 Iodine value (IV)

In triplicate, 0.5 g of house cricket powder was accurately measured out then put in 500 mL glass-stoppered conical flask. Approximately 10 mL of chloroform was mixed with sample and blank flasks to dissolve oil, thereafter addition of 25 mL of Wij's iodine solution. Flasks were stored in the dark for 30 minutes, with gentle shaking performed every 10 minutes. After the incubation period, 20 milliters of potassium iodide solution was introduced into flasks, and the contents were vigorously shaken. Subsequently, 100 milliters of water (boiled and cooled) was added to rinse any residual iodine from the stoppers. The liberated iodine in both the sample and blank solutions was titrated with a standard sodium thiosulfate solution, with continuous shaking, until the yellow coloration disappeared. After that, 2 mL of starch indicator was added, and titration was continued until the blue color was completely discharged. Titrant volume used was noted, and IV was calculated using Equation 24.

Iodine value (IV) g/ 100g of sample

$$= \frac{(\mathrm{X}-\mathrm{G})\ \mathrm{x\ N\ x\ 126.9}}{\mathrm{N}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(24)$$

Where; X = Volume of titrant (blank), G = Volume of titrant (ml) for the sample, N = Normality of HCl, N = Sample mass (g)

3.8.3 Paranisidine value

In triplicate, 1 g of the prepared house cricket powder was weighed directly into a 25 ml volumetric flask. The sample was initially dissolved in 5 mL of isooctane, and volume was adjusted to the calibration mark using the same solvent. To prepare an un-reacted test solution, 1 ml of the test solution was put into a test tube. 1 ml of glacial acetic acid was added, stoppered the tube and shaken well. The test tube was kept in the dark at (20-26°C) for 8 minutes. Within a further 2 minutes, the solutions was placed into a clean, dry spectrometer cell. Giving a reaction time of 10 minutes, the procedure followed specified in part below. A reacted test solution was prepared; 5 ml of isooctane was put in a test tube. And 1 ml of anisidine reagent added, the tube stoppered and shaken well. The test tube was kept in the dark at (20-26°C) for 8 minutes. Within a further 2 minutes, the solutions was placed into a clean, dry spectrometer cell. For spectrometric measurement, the zero absorption of the spectrometer was adjusted with isooctane at 350 nm. The absorbances of (i) the un reacted solution (A), (ii) the reacted solution (B) and that of the blank (C) were measured and the anisidine expressed according to equation 25.

$$\text{Anisidine value} = \frac{100\ \text{QV}}{\text{m}}\ [1.2\ (\text{B} - \text{A})] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(25)$$

Where;

V = volume of rest sample dissolved (V = 25 ml), m = the mass of the test portion (g), Q = the sample content of the measured solution on which the anisidine value is expressed in g/mm, (Q = 0.01 g/ml), B = absorbance of the reacted solution, C = the absorbance of the blank, A = absorbance of the unreacted solution

3.9 Sensory stability of cricket powder and its oil fraction

Prior to sensory shelf life testing, qualitative descriptive analysis was conducted using a methodology by Navarro et al. (2021) to generate descriptors for aroma and colour to be monitored during shelf life testing. At Kyambogo University, the sensory characterization of the cricket powder was done in a room with sufficient natural light. Three steps made up the method, i.e., hiring, preliminary screening, and, with the use of reference sources, developing descriptive language and terminology. Four triangle exams were part of the pre-selection process for hiring candidates. Then, based on a qualification score of 75% of the tests, twelve panelists were chosen. After that, the panelists received training on how to come up with descriptive terms and then form an agreement around them. Assessors were presented with the product and asked to come up with descriptors for color and aroma by consensus. Assessors completed a one-month training program to become familiar with the developed terminology during the familiarization phase. After the instruction, the assessors performed a preliminary evaluation to confirm their competence.

The approach outlined by Ojo & Adeola (2017) was modified and used to determine the sensory shelf life. A low density polyethylene (LDPE) film (16 cm × 19 cm) containing 100g of the of the refined cricket powder sample was sealed, placed on a shelf at ambient temperature, and monitored for a period of six months. Twelve trained panelists assessed the color and aroma of samples after every three weeks. Based on the predetermined descriptors, a 15 cm line scale was utilized to determine the extent of the changes in the sample's color and aroma.

3.10 Qualitative descriptive analysis of the cricket powder and oil

A qualitative descriptive analysis was conducted by 12 trained panelists who developed sensory descriptors for house cricket powders and oil, as presented in table 3.1.

Table 3.1: Colour and aroma profile of cricket powder and oil

House cricket powder/ oil	Colour	Aroma
Protocol 3	Mukene powder	Eucalyptus leaves
Protocol 4	Pumpkin powder	Peppermint sweets
Control 1 (dried)	Cooked banana leaves	Faint fishy
Control 2 (roasted)	Coriander powder	Faint fishy
Cricket oil	Dark brown	Nutty-earthy

The panelists identified clear distinctions in the colour and aroma characteristics that corresponded with each refining method. In protocol 3, the house cricket powder sample resembled *mukene* powder in appearance and emitted an aroma similar to eucalyptus leaves. Protocol 4 on the other hand had visual similarities to pumpkin powder and an aroma reminiscent of peppermint sweets, indicating a sweeter, more menthol-like odour. For control 1, the dried cricket powder resembled cooked banana leaves in color, with a faint fishy aroma, suggesting preservation of the original insectaceous characteristics in the unrefined house cricket powder (Ssepuuya et al., 2025). Control 2, the roasted house cricket powder was visually comparable to coriander powder having faint fishy aroma of insectaceous characteristics similar to that in control 1. House cricket oil was described as having a dark brown color and a nutty-earthy aroma, indicative of lipid oxidation and Maillard reaction byproducts (Shahidi et al., 2022).

3.11 Statistical Evaluation

SPSS software, version 22, was used for statistical analyses; every measurement of the study being carried out three times. The proximate composition and chemical quality data were analyzed using one-way ANOVA, and Tukey's Honestly Significant Difference test was used to compare the means at 95% confidence level. Wilcoxon signed-rank test was used to evaluate rank distributions for the study of color ranking data without making the assumption that the color features of the refined house cricket powders were normally distributed. Additionally, non-parametric data specifically, functional characteristics that deviated from the expected properties of normal distribution was analyzed with Kruskal-Wallis test, with a 95% significance threshold.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Establishment of cricket powder refinement protocol

From sensory screening of the 24 formulations generated using Deign Expert software, 6-cricket powder refining protocols were obtained as shown in table 4.1.

Table 4.1: Sensory attribute scores for house cricket powder refining protocols

Protocol	Sensory attribute scores				
	Aroma	**Colour brightness**	**Appearance**	**Overall acceptability**	**Desirability**
1	6.191	6.245	5.702	5.953	0.568
2	6.191	6.245	5.702	5.953	0.568
3	6.191	6.245	5.702	5.953	0.568
4	6.191	6.245	5.702	5.953	0.568
5	6.191	6.245	5.702	5.953	0.568
6	6.191	6.245	5.702	5.953	0.568

Protocols indicate different cricket powder refining formulations generated by design expert software.

Protocols with the highest desirability (0.568) included the six obtained by either drying or roasting, defatting by petroleum ether and bleaching. The details of the protocols have been withheld until the associated intellectual property assessments are completed. After sensory screening, together with the panel we reached a consensus to further screen the six protocols using color through a ranking test using a consumer panel. The ranks were between 1 (most bight) and 4 (least bright). This is because the samples had a similar aroma and therefore colour was the sensory acceptability deciding factor.

Table 4.2: Colour ranking of the optimized refining methods (defatting and bleaching)

Protocol	Mean rank	50th Median
1	3.04 ± 1.03^{a}	3.00
2	1.76 ± 0.96^{b}	1.00
3	1.52 ± 0.51^{d}	2.00
4	1.48 ± 0.51^{d}	1.00
5	3.10 ± 0.95^{ac}	3.00
6	2.10 ± 0.91^{bc}	2.00

Every value is the mean of fifty calculations. Means having matching superscripts are significantly similar ($p < 0.05$).

According to findings, cricket powder refining protocol 4 gave the lowest rank (1.48) followed by the cricket powder formulation in protocol 3 (1.52) and the highest colour rank number was protocol 5 (3.10). The low rank indicates that refining protocol 4 was perceived as having the brightest colour and considered more desirable compared to other protocols. The cricket powder from protocol 3 was ranked slightly higher, with a rank of 1.52, meaning its colour was less bright compared to protocol 4. Both protocols (3 and 4) had the same processing and defatting methods but different bleaching methods, implying that the bleaching method in protocol 4 was more effective at leading to a brighter colour. Hydrogen peroxide, one of the bleaching agents in protocol 4 is an oxidizing agent capable of reacting with a broad spectrum of colored organic compounds, by breaking down pigments into colourless compounds (Moreira et al., 2013). Chloroform on the other hand works by dissolving and extracting pigments and other coloured compounds, leading to a noticeable whitening of the material (Saati et al., 2011).

Control 1 **Protocol 3**

Control 2 **Protocol 4**

Figure 4.1: Images of house-cricket powder refined by different protocols.

4.2. Sensory Quality of refined house cricket powder

After the color-ranking test, sensory acceptability was assessed for the two most acceptable protocols in terms of colour; i.e. protocol 3 and 4, and the non-refined controls (either roasted or dried) as presented in Table 4.3. The house cricket powders showed variations in the sensory acceptability of the evaluated attributes with cricket powder from protocol 4 being the most preferred for appearance, colour and overall acceptability compared to other samples.

Table 4.3: Sensory acceptability of refined cricket powder

House cricket powder	Appearance	Colour	Aroma	Overall acceptability
Protocol 3	5.81±2.01^{b}	6.41±1.64^{b}	6.06±1.99^{a}	6.06±1.84^{b}
Protocol 4	8.09±0.86^{a}	8.25±0.76^{a}	6.19±1.91^{a}	7.34±1.62^{a}
Control 1 (dried)	6.41±1.48^{b}	6.65±1.33^{b}	6.19±1.80^{a}	6.56±1.61^{ab}
Control 2 (roasted)	6.60±1.48^{b}	6.69±1.40^{b}	6.28±2.13^{a}	6.38±1.96^{ab}

Each value is the mean of forty measurements. Means bearing different superscripts are significantly different ($p > 0.05$).

All cricket powder samples received mean sensory scores above the indifferent score of 5, indicating that all products were acceptable to the panelists. This suggests that both the control and refined house cricket powders had satisfactory sensory quality. The aroma scores of the control and refined house cricket powders were significantly ($p > 0.05$) the same, implying that processing methods used did not cause noticeable changes in aroma perception among the samples. This agrees with Shahrai et al. (2021), who also reported minimal sensory differences in aroma across various insect powder treatments. Similarly, no significant differences ($p > 0.05$) were identified in appearance and color between the refined protocols and the control samples. Nonetheless, protocol 3 recorded slightly lower mean scores for both attributes, suggesting that it was somewhat less visually appealing than the controls. In contrast, cricket powder from protocol 4 achieved significantly higher scores ($p < 0.05$) for appearance and color than both protocol 3 and the control samples. These results are consistent with the ranking test, which identified protocol 4 as the most preferred sample in terms of color. Although all powders were deemed acceptable, significant differences, ($p < 0.05$) were noticed in their overall acceptability. Protocol 4 attained the highest mean overall acceptability score (7.34), followed by control 1 (6.56), while protocol 3 and control 2 recorded slightly lower but still acceptable scores. The enhanced color and appearance of protocol 4 likely contributed to its superior overall acceptability. Overall, these findings indicate that while all the refined and control cricket powders maintained acceptable sensory quality, improvements in visual attributes particularly color and appearance can enhance consumer preference. Therefore, refining processes similar to those used in protocol 4 may be beneficial in optimizing the sensory appeal of house cricket powder without compromising its acceptability.

4.3 Proximate composition of the refined cricket powders

As in Table 4.4, moisture, crude protein, fat, ash, crude fiber, and carbohydrate contents of house cricket powder processed following refining protocols 3 and 4 were analyzed.

Table 4.4: Nutritional composition of the refined house cricket powder

House Cricket powder	Crude protein	Crude fat	Ash	Moisture	Crude fiber	Carbohydrates
Protocol 3	58.93 ± 5.38^a	4.50 ± 0.44^d	3.08 ± 0.01^b	2.46 ± 0.14^b	7.72 ± 0.39^a	23.31 ± 5.81^a
Protocol 4	55.72 ± 3.04^a	9.60 ± 0.64^c	3.77 ± 0.02^a	8.09 ± 0.07^a	4.57 ± 0.27^d	18.34 ± 2.21^a
Dried (control 1)	51.35 ± 1.34^a	17.69 ± 0.63^b	2.48 ± 0.18^c	2.65 ± 0.11^b	4.78 ± 0.18^c	21.05 ± 1.72^a
Roasted (control 2)	50.49 ± 1.73^a	21.39 ± 0.09^a	3.22 ± 0.06^b	1.83 ± 0.25^c	5.44 ± 0.11^b	17.63 ± 1.62^a

Every value is a mean of triplicate separate examinations. Means in a column that have varying superscripts differ significantly ($p > 0.05$).

4.3.1 Moisture

Overall, moisture level of cricket powders meets the allowable limit of 5-8% for edible insects, as specified by the Uganda standard US 2146: 2020. Moisture content ranged from 1.83% to 8.09%, with control 2 (roasted cricket powder) showing the lowest moisture content (1.83%) and refined cricket powder from protocol 4 having the highest moisture content at 8.09% (Table 4.4). The elevated moisture content in protocol 4 might result from the hydrogen peroxide (H_2O_2) releasing oxygen during the bleaching process, allowing moisture from the peroxide solution to be absorbed into the powder. Additionally, the peroxide treatment could have made the powder more porous or alter its surface properties, leading to increased moisture absorption from the environment. This outcome aligns with findings of the report by Martelli-Tosi et al. (2017) where moisture content of the soybean protein isolate increased with treatment of hydrogen peroxide because oxidation disrupted the protein-protein interactions, creating a more porous or expanded structure that held additional moisture.

4.3.2 Crude protein

Crude protein composition of the house cricket powders did not differ in a way that was statistically significant ($p > 0.05$). However, both refined cricket powders exhibited higher protein levels in comparison to controls. The rise in protein content of the refined house cricket powders can be attributed to the defatting during refinement which increased the proportion of protein as that of fat reduced. This increase contributes approximately 26 % to the recommended dietary allowance of 0.83 g per kg bodyweight per day for an adult (FAO, 2011). This finding is in agreement with Min-Soo Jeong (2021)who reported that defatting is an effective technique for enhancing protein content and lowering lipid content. Mart et al. (2022) additionally observed increase in protein from 61.39% for whole cricket powder to 68.48% for defatted cricket powder.

4.3.3 Crude fat

A significant difference ($p < 0.05$) was observed in the fat content of house cricket powders, with refined samples showing lower fat levels than the control samples. Protocol 3 cricket powder had the lowest fat content (4.50%) followed by protocol 4 (9.60%), control 1 (17.69%) and the control 2 (21.39%) that had the highest. The low fat content in refined cricket powders can be attributed to the defatting that was part of the refining procedure. This observation aligns with the results of Cunha et al. (2025), who stated that the lipid content of the house cricket protein extract was reduced due to defatting, versus the control.

The high level of fat in the roasted cricket powder of control 2 may be attributed to the significant moisture loss that occurs during roasting compared to oven drying. In contrast to findings that show a clear reduction in fat content following defatting, Pellerin & Doyen (2024) reported that ethanol-based defatting did not consistently lower lipid levels in *Acheta domesticus* protein extracts, suggesting that solvent type and extraction conditions can influence lipid retention and recovery efficiency. Similarly, Lin et al. (2023) observed that certain drying methods**,** such as freeze-drying, led to residual lipid concentrations comparable to full-fat controls**,** indicating that processing conditions can counteract expected lipid removal.

4.3.4 Ash content

House cricket powder ash levels varied significantly ($p < 0.05$). Nonetheless, ash content did not differ notably between protocol 3 and control 2 (roasted cricket powder). Protocol 4 exhibited the highest ash content at 3.77%, while control 1 (dried) had the lowest at 2.48%. Ash content represents the inorganic mineral content left after combustion and hence the removal of moisture leads to concentration of minerals. Roasting in protocol 3, 4 and control 2 involves high temperatures (150°C) that effectively evaporate higher amounts of moisture from the crickets compared to drying (75°C) resulting in a greater amount of ash than dried crickets. The outcomes are consistent with the conclusions of Lu et al. (2024) who stated that the edible insects' (*Acheta domesticus, Tenebrio molitor, and Henicus whellani*) mineral content (Zn, Ca, and Fe) prepared by roasting increased compared to those prepared by oven drying and boiling.

4.3.5 Fiber content

Results varied significantly ($p < 0.05$) in fiber among house cricket powder samples. Fiber concentration varied between 4.57% and 7.72%, with Protocol 3 having the highest fiber levels and Protocol 4 having the lowest. These outcomes are similar to those of Gantner et al. (2024) who reported house crickets having fiber content of 7.8%.

4.3.6 Carbohydrates content

This ranged from 17.63% to 23.31%, without significant variations observed ($p > 0.05$). Control 2 recorded the lowest carbohydrate content, while protocol 3 exhibited the highest. The absence of significant variation indicates that the processing techniques applied did not markedly influence carbohydrate composition of final products. Outcomes align with publication of Khatun et al. (2021) that similarly described no notable differences ($p > 0.05$) were observed in carbohydrate content between *Acheta domesticus* samples subjected to different processing methods.

4.4 Functional properties of refined house cricket powder

Results for the functional characteristics of refined cricket powders are reported in Table 4.5 which include swelling power, water holding capacity, oil absorption capacity, solubility, bulk density, tapped density, and dispersibility.

Table 4.5: Functional attributes of the refined house cricket powder

House Cricket powder	WHC (g/100 g)	OAC (g/100)	SP (g/g)	Solubility (%)	BD (g/ml)	TD (g/ml)	Dispersibility (%)
Protocol 3	179.52±4.78^a	141.00±3.47^a	4.11±0.14^a	12.05±0.90^a	0.43±0.01^a	0.62±0.03^a	64.00±0.00^b
Protocol 4	133.12±9.02^a	123.84±1.96^b	3.34±0.12^d	20.95±0.49^a	0.40±0.03^b	0.60±0.00^b	72.67±1.15^a
Control 1	167.51±18.97^a	111.26±3.33^c	3.58±0.32^c	11.54±1.80^a	0.36±0.01^c	0.55±0.12^c	63.33±2.31^c
Control 2	186.57±0.60^a	105.29±1.63^d	3.90±0.18^b	11.09±0.81^a	0.33±0.12^d	0.52±0.02^d	61.67±0.58^d

Every value is the average of three tests. Mean values along the column sharing identical superscripts do not vary significantly ($p > 0.05$). WHC: Water Holding Capacity; OAC: Oil Absorption Capacity; BD: Bulk Density; TD: Tapped Density; SP: Swelling Power.

4.4.1 Water holding capacity

A food product's Water Holding Capacity (WHC) is its potential to absorb water against gravitational forces, even when exposed to mechanical and thermal processes such as heating, grinding, or pressing (Gebrehiwot et al., 2018). Water holding capacity values ranged from 133.12g/100g to 186.57g/100g. The refined house cricket powder from Protocol 4 exhibited the lowest water holding capacity, while Control 2 (roasted) had the highest. However, the differences in water holding capacity of cricket powders were not significant ($p < 0.05$).

The low water holding capacity in Protocol 4 may have resulted from the refining process altering the protein structure, which decreases its ability to retain water. This protocol likely resulted in a product with reduced dietary fiber, which contribute to water retention as well. Additionally, the refinement process might have produced smaller or uniform particles, which could have a lower surface area for water absorption. Powders with low WHC, such as the refined Protocol 4, are less prone to caking and moisture absorption during storage, potentially improving shelf stability (Hasmadi, 2021). The WHC aligns with those published by Aider et al. (2024) where bleaching by hydrogen peroxide resulted in a lower water retention capacity of wheat bran. The high WHC of roasted cricket powder (186.57 g/100 g), maybe due to its high protein content as proteins are known for having strong interaction with water. These proteins are capable of forming hydrogen bonds with water molecules effectively retaining moisture. Roasting reduces the moisture content of the crickets before grinding them into powder, which concentrates the protein content and boosts their water-holding capacity. High WHC powders, such as roasted house cricket powder in Control 2, may be more hygroscopic, requiring airtight and low-humidity storage conditions to prevent clumping or microbial growth (Hasmadi, 2021). These observations concur with those published by Gantner et al. (2024) who reported a high water holding capacity measured at 2.53 g of water per gram due to high protein content in the house cricket powder sample.

4.4.2 Oil Absorption Capacity

A food product's Oil Absorption Capacity (OAC) indicates its capacity to retain oil or fat, which is crucial for enhancing flavor, mouthfeel, and texture of food (Wang et al., 2020). Statistically detectable difference ($p < 0.05$) was noticed in house cricket powders' OAC, ranging from 105.29 to 141.00 g/100 g. Control 2 (roasted) had the lowest value (105.29 g/100 g), while protocol 3 (chloroform bleach) has the highest. The high temperatures involved in roasting might have caused variations in the protein structure potentially leading to reduced oil-binding capacity (Lee et al., 2019). Defatted cricket powders in protocol 3 and 4 have higher oil absorption capacities (OAC) compared to the controls because the structure of cricket powder is changed when fat is removed thus increasing the availability of binding sites for oil.

Cricket powders with lower oil absorption capacity (OAC), such as roasted samples, tend to contain less surface-exposed lipid and are therefore less susceptible to oxidative rancidity, contributing to improved shelf life under ambient storage conditions (Lee et al., 2019; Zielińska & Pankiewicz, 2020). In contrast, high-OAC powders retain more lipophilic compounds, making them more prone to lipid oxidation and rancidity development during storage. Consequently, such powders require low-temperature, oxygen-reduced packaging conditions such as vacuum sealing to minimize oxidative degradation and preserve functional properties (Cruz-l et al., 2024). Conversely, lower-OAC powders, such as roasted cricket powder, are more suitable for dry formulations for example flours, protein bars, and instant mixes, where excessive oil absorption can negatively impact flowability, compaction, and product uniformity (Zielińska & Pankiewicz, 2020; Yi et al., 2013). Outcomes are comparable to those of Cruz-l et al. (2024) who concluded that increase in oil holding capacity of defatted *Acheta domesticus* that were used as food additives.

4.4.3 Swelling power

Swelling Power (SP) of a food product indicates its potential to absorb moisture and expand when heated in the presence of water (Chiodetti et al., 2024). Swelling power ranged from 3.34 to 4.11 g/g with protocol 4 (CL/ROOH bleach) 3.34 g/g exhibiting the lowest and protocol 3 (chloroform bleach) 4.11 g/g exhibiting the highest value. The findings revealed that the swelling power showed significant variation ($p < 0.05$) among the cricket powders. Chloroform bleach in protocol 3 might have created more favorable conditions for swelling, such as by modifying the protein or polysaccharide structure to enhance water absorption. In contrast, CL/ROOH bleach might have caused changes that reduce swelling capacity, possibly by affecting the powder's porosity or surface properties (Klunklin et al., 2023). Powders with higher swelling power demonstrate greater hydration potential, making them suitable for thickened or gel-like applications such as sauces, soups, or emulsified products, where water retention enhances viscosity and stability (Zielińska & Pankiewicz, 2020). Conversely, lower-swelling power powders (e.g., Protocol 4) are preferable for dry blends and bakery formulations, where limited swelling helps maintain desirable texture, dough consistency, and flowability (Tan et al., 2022). Powders with higher swelling power often show greater water affinity and hygroscopicity, which may increase susceptibility to moisture uptake and caking during storage, thus requiring airtight, low-humidity packaging (Ibrahim et al., 2025). On the other hand, lower-swelling power powders are typically more stable under ambient conditions, making them easier to handle and store for extended periods.

4.4.4 Solubility

No statistical notable variation ($p > 0.05$) existed in the solubility values among the cricket powder samples. Solubility ranged from 11.09% to 20.95% with control 2 (roasted) having the lowest and protocol 4 (CL/ROOH) having the highest. Different treatments can alter the protein structure in ways that affect solubility (Vihinen, 2020). Roasting, for example, may denature proteins or cause them to cross-link, which can reduce solubility by making the proteins less accessible to solvents (Li et al., 2023). In contrast, CL/ROOH treatment might enhance protein solubility or reduce protein aggregation, thereby improving solubility (Rahban et al., 2023).

In Protocol 3, the use of chloroform, which results in a solubility of 12.05%, might have denatured proteins and altered their structure, leading to a reduction in solubility. This solubility is comparable to the European standard for protein solubility, which ranges from 12% to 23% between pH 3 and 9, as reported by Noyens et al. (2021). Denatured proteins may aggregate or form insoluble complexes, which are less likely to dissolve in solvents (Vihinen, 2020). Findings are in alignment with those published by Magara et al. (2021), who obtained solubility of cricket powder between 4% to 20% with the use of organic solvents like chloroform and methanol.

4.4.5 Bulk density and tapped density

The mass per unit volume of a food product, including both the solid component and the air spaces between particles, is known as its bulk density (B.D). Tapped density measures how much the powder settles or compacts under vibration or tapping. No significant variation ($p > 0.05$) was found in both bulk and tapped densities among the cricket powder samples. B.D of the samples was between 0.33 and 0.43 g/ml, while the tapped density values were between 0.52 and 0.62 g/ml. Among the treatments, Protocol 3 (chloroform bleach) exhibited the highest values for both bulk and tapped density. This was followed by protocol 4 (CL/ROOH), then control 1 and control 2. High bulk density may be due to decreased particle size or greater compaction of the powder (Suhag et al., 2024). The chloroform bleaching in protocol 3 might have altered the physical structure of the powder, possibly causing particles to pack more closely together. A high tapped density in protocol 3 suggests that the powder has a greater tendency to pack down more tightly.

4.4.5 Dispersibility

Dispersibility refers to the ability of a substance, such as a powder, to spread evenly throughout a medium, usually a liquid, without forming clumps or aggregates (Wahyu et al., 2021). A statistical notable difference ($p < 0.05$) was identified between the cricket powders' dispersibility values. The dispersibility ranged from 61.67% to 72.67%. Control 2 (roasted) had the lowest and protocol 4 (CL/ROOH) had the highest. The high dispersibility in protocol 4 cricket powder (CL/ROOH) suggests that the CL/ROOH treatment effectively improved the powder’s ability to disperse in the liquid medium. The treatment might have modified the powder’s properties in a way that reduces aggregation, enhanced particle uniformity, or otherwise facilitates better dispersion (Wahyu et al., 2021). Higher dispersibility is highly advantageous for instant formulations, such as protein drinks, soups, and bakery batters, where rapid and uniform hydration is desirable (Zielińska & Pankiewicz, 2020; Tan et al., 2022). Powders with lower dispersibility, like roasted samples, may be better suited for dry-mix applications (e.g., energy bars or snacks) where minimal interaction with water maintains structural integrity. Regarding storage stability, highly dispersible powders often possess greater surface area and exposure to moisture, making them more hygroscopic and susceptible to caking or microbial growth if not properly packaged (Ibrahim et al., 2025). Therefore, such powders benefit from airtight and low-humidity storage conditions, while low-dispersibility powders remain more stable under ambient storage.

4.5 Safety and stability of the cricket powder

4.5.1 Microbial safety and quality of the cricket powder

All the microbial results of the different parameters i.e.; Yeasts and molds, Total plate count (TPC), *Salmonella* species, *Listeria* species, *Staphylococcus aureus*, *Enterobacteriaceae, Escherichia coli,* and total coliforms were within the acceptable ranges as displayed in table 4.6 below.

Table 4.6: Microbial safety and quality (log cfu/g) of the selected house cricket powder protocols

Microbial parameter (log cfu/g)	Protocol 3	Protocol 4	Control 1	Control 2	Maximum limits	Reference standard
Total plate count	4.28 ± 0.06^{a}	<10	4.13 ± 0.07^{b}	3.88 ± 0.08^{c}	5	ISO 4833
Yeast and moulds	<10	<10	<10	<10	3	ISO 21527-2
Escherichia coli	<10	<10	<10	<10	Absent	ISO 16649-2
Total coliforms	<10	<10	<10	<10		ISO 4832
Staphylococcus aureus	<10	<10	<10	<10	Absent	ISO 6888-1
Enterobacteriaceae	<10	<10	<10	<10		ISO 21528 -2
Salmonella species (/25g)	N.D	N.D	N.D	N.D	Absent	967.25 (AOAC, 2012)
Listeria species (/25g)	N.D	N.D	N.D	N.D	Absent	ISO 11290-1

N.D: Not detected. Every value is the average of duplicate analysis. Means along each row having different superscripts are significantly different ($p < 0.05$).

Mean total plate count (TPC) values of the house cricket powders varied significantly among the different processing protocols ($p < 0.05$), indicating that processing methods had a notable influence on microbial load. The <10 cfu/g result obtained for Protocol 4 (CL/ROOH) suggests that the microbial count was below the detectable limit of the test method, implying excellent microbiological quality with minimal viable microorganisms present. This low count may be attributed to the effectiveness of CL/ROOH to reduce microbial contamination by disrupting microbial cell membranes and denature proteins, thereby lowering viable counts in food matrices (Osimani et al., 2018; Vandeweyer et al., 2017). In contrast, Protocol 3 (chloroform bleach) recorded the highest TPC at 4.28 log cfu/g, which, although significantly higher than other treatments, still falls within the acceptable safety limit prescribed by the Uganda Standard US 2146:2020- Edible Insects Specification, which sets a maximum TPC of 5.00 log cfu/g for edible insect powders. The observed differences in TPC among the treatments could be due to variations in processing intensity, residual moisture content, and exposure to microbial recontamination during handling or drying.

All of the cricket powders counts were less than 10 cfu/g for yeast and moulds, total coliforms, *Staphylococcus aureus, Escherichia coli,* and *Enterobacteriaceae. Salmonella* species (/25 g) and *Listeria* species (/25 g) were not detected in all the cricket powder samples. These results comply with the Codex Alimenterius Commission (CAC, 2010) limits and the Uganda standard US 2146:2020 for edible insects specifications that require absence of *Enterobacteriaceae, Salmonella typhimurium*, *Listeria monocytogenes* and *Eschechia coli* and *Staphylococcus aureus.*

4.5.2 Chemical safety of the refined cricket powder

The refined cricket powders and controls were analyzed for chemical safety (heavy metals, aflatoxins and pesticide residues) and findings are presented in the upcoming Tables 4.7, 4.8 and 4.9 below.

4.5.2.1 Heavy metal composition of the cricket powders

Heavy metal composition of the house cricket powders including lead, arsenic, cadmium, and mercury was determined.

Table 4.7: Heavy metal composition (mg/kg) of the house cricket powders

Metal	Protocol 3	Protocol 4	Control 1	Control 2
Lead	0.98 ± 0.01^a	< 0.05 LOD	0.48 ± 0.01^c	0.83 ± 0.01^b
Arsenic	< 0.05 LOD	< 0.05 LOD	< 0.05 LOD	< 0.05 LOD
Cadmium	< 0.02 LOD	< 0.02 LOD	< 0.02 LOD	< 0.02 LOD
Mercury	< 0.1 LOQ	< 0.1 LOQ	< 0.1 LOQ	< 0.1 LOQ

Each value is the average of three separate determinations. Significant variations ($p < 0.05$) exist between mean values in each row that has different superscripts. Limit of quantification (LOQ); Limit of detection (LOD).

The variation in lead content among the cricket powder samples was significant ($p > 0.05$). Protocol 4 (CL/ROOH) had the lowest lead content, with values below 0.05 mg/kg, while protocol 3 (chloroform bleach) showed the highest lead content at 0.98 mg/kg. The CL/ROOH bleaching process, involving oxidative and chlorinated reagents, likely promoted conversion of inorganic lead species into more volatile or soluble forms, such as lead chloride ($PbCl_2$) and lead oxide (PbO), which can partially volatilize or leach out during subsequent drying at elevated temperatures (Okorie et al., 2018; ATSDR, 2020). These volatile lead compounds have low thermal stability, decomposing or evaporating at relatively moderate processing temperatures (above 500 °C), thus reducing the residual lead content in the final product (Phipps & Livingston, 1995). In protocol 3, chloroform being a lipid-soluble solvent, may have co-extracted these lipid-bound lead compounds and redeposited or concentrated them during drying. Arsenic, cadmium and mercury were all below detection limit and quantification limit, respectively. These findings are in agreement with the limits of the Codex Alimenterius Commission (CAC, 2010) and the Uganda standard US 2146:2020 for edible insects specifications that require absence of arsenic, cadmium and mercury.

4.5.2.2 Pesticides residues in refined powders and controls

The results show that all pesticide levels were less than the 0.01 mg/kg detection threshold as shown in Table 4.8.

Table 4.8: Pesticide residues of house cricket powders and controls

Pesticide residue (mg/kg)	Protocol 3	Protocol 4	Control 1	Control 2
Methacrifos	< 0.01	< 0.01	< 0.01	< 0.01
a-BHC	< 0.01	< 0.01	< 0.01	< 0.01
4-4-DDE	< 0.01	< 0.01	< 0.01	< 0.01
Fludioxonil	< 0.01	< 0.01	< 0.01	< 0.01
Endrin	< 0.01	< 0.01	< 0.01	< 0.01
Endosulfan 11	< 0.01	< 0.01	< 0.01	< 0.01
Chlorothiophose	< 0.01	< 0.01	< 0.01	< 0.01
4-4-DDD	< 0.01	< 0.01	< 0.01	< 0.01
Endrin aldehyde	< 0.01	< 0.01	< 0.01	< 0.01
Cypermethrin 2,3	< 0.01	< 0.01	< 0.01	< 0.01
Flucythrinate 2,3	< 0.01	< 0.01	< 0.01	< 0.01
Fenvalinate 2	< 0.01	< 0.01	< 0.01	< 0.01
Fluvalinate2	< 0.01	< 0.01	< 0.01	< 0.01
Deltamethrin	< 0.01	< 0.01	< 0.01	< 0.01
Propoxur	< 0.01	< 0.01	< 0.01	< 0.01
Tetrachlor(m)Xylene	< 0.01	< 0.01	< 0.01	< 0.01
Sulfotep	< 0.01	< 0.01	< 0.01	< 0.01
Atrazine	< 0.01	< 0.01	< 0.01	< 0.01
Malathion	< 0.01	< 0.01	< 0.01	< 0.01

Every value is an average of triplicate determinations. Protocol 3: chloroform bleach; Protocol 4: sodium hypochlorite and hydrogen peroxide bleach; Control 1: dried cricket powder; Control 2: roasted cricket powder.

The pesticide residues in all the house cricket powders were found to be below 0.01 mg/kg. This low level suggests that house crickets were fed by cricket farmers on feed (such as grains, food waste and cassava leaves) that was free from pesticide residues (Tanga et al., 2021). Findings agree with a publication by Kolakowski et al. (2021), who also revealed that house crickets and other edible insects had no detectable pesticide levels (< 0.01 mg/kg).

4.5.2.3 Aflatoxin levels in refined powders and controls

The aflatoxin levels were below the detectable level of 0.025 ppb except for protocol 3 that showed 1.27 ppb as shown in Table 4.9.

Table 4.9: Aflatoxin levels (ppb) in the house cricket powders

Aflatoxin	Protocol 3	Protocol 4	Control 1	Control 2
B1	1.27±0.003	<0.025	<0.025	<0.025
B2	<0.025	<0.025	<0.025	<0.025
G1	<0.025	<0.025	<0.025	<0.025
G2	<0.025	<0.025	<0.025	<0.025

Every value is a mean of triplicate determinations. PPB: parts per billion; Protocol 3: chloroform bleach; Protocol 4: sodium hypochlorite and hydrogen peroxide bleach; Control 1: dried cricket powder; Control 2: roasted cricket powder.

Protocol 3 where chloroform was used to bleach, shows a concentration of 1.27 ppb (parts per billion) of aflatoxin B1, while all other cricket powders had < 0.025 parts per billion (pbb). Aflatoxin B1 is a highly potent mycotoxin (which are secondary metabolites of yeast and mold) produced by *Aspergillus* molds and is known for its carcinogenic effect (Benkerroum, 2020). Aflatoxin occurrence may have resulted from feed-derived contamination, inadequate equipment sanitation, environmental exposure, or solvent-induced concentration during processing (Csaba et al., 2019). Bleaching with chloroform might have facilitated the extraction or concentration of aflatoxins during processing (Csaba et al., 2019), leading to the higher reported levels observed in the samples treated with this protocol. The contamination levels in Protocol 3 were however below the maximum allowable limit for aflatoxin B1 specified in the Uganda standard US 2146:2020, which stipulates that aflatoxin B1 must not exceed 5 µg/kg in edible insects. Aflatoxins B2, G1, and G2 were all detected at levels below 0.025 ppb in every cricket powder samples, confirming that all samples are considered safe for human consumption.

4.6 Shelf life

4.6.1 Moisture content

Moisture level is crucial in edible powder's shelf life; the less moisture in the powder, the greater its storage stability (Martelli-Tosi et al., 2017). The moisture content of the cricket powders increased steadily from week 1 to week 6, with the lowest initial value of 2% observed in control 2 at week 0 and the highest value in protocol 4 at week 6 (Figure 4.2).

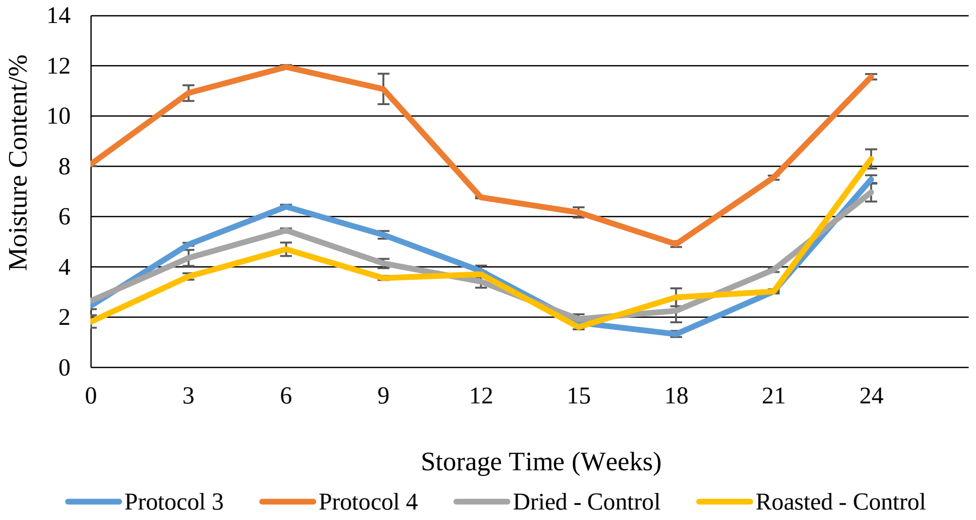


Figure 4.2: Changes in the moisture content of cricket powders with time

From weeks 6 to 15, the moisture content gradually decreased, with control 2 reaching the lowest level of 1% and protocol 4 the highest at 6%. Following week 15, the moisture content gradually increased for all cricket powders up to week 24. The gradual increase in moisture content from week 1 to week 6, with protocol 4 showing the highest value indicate that the powders might have absorbed moisture from the water vapor with in the sealed atmosphere. The lower moisture content in control 2 at the start might suggest better initial lower inherent moisture absorption of the roasted cricket powder. The reduction in moisture content between week 6 and week 15 suggests that moisture might have been lost through evaporation because of the permeability of ziplock bags (Odiko et al., 2017). The subsequent increase in moisture content for all cricket powders up to week 24 could be attributed to reabsorption of moisture from the environment or changes in ambient humidity and potential degradation of the ziplock bags over time (Vinayak et al., 2022). The results align with the conclusions of Drăghici & Marcu (2023), who observed that *A. domesticus* powder is hygroscopic. Given the hygroscopic nature of cricket powders, packaging materials with low water vapor transmission rates are recommended. High-barrier multilayer films such as aluminum foil laminates, metalized polyethylene terephthalate (MET-PET), or polypropylene/polyethylene (PP/PE) composites would be suitable to minimize moisture exchange (Vinayak et al., 2022). Storage should be conducted under cool, dry conditions, ideally at a relative humidity below 50% and temperature below 25°C, to preserve powder stability and prevent microbial or oxidative deterioration (Odiko et al., 2017). For long-term storage, vacuum sealing can also help to minimize both oxygen and moisture ingress, increase shelf life and maintaining product quality (Drăghici & Marcu, 2023).

4.6.2 Cricket powder microbial shelf life

A food product’s shelf life is duration during which a product remains safe for consumption and maintains its desired sensory qualities such as aroma, colour, taste, texture, and appearance as well as its nutritional value (Khan & Rahman, 2021). During this time, the food should be free from spoilage or deterioration when stored under specified conditions. Beyond this period, the food may begin to lose quality, become unsafe to eat, or pose health risks. To ensure consumer safety of the house cricket powders, the microbial shelf stability (total plate count, yeasts and moulds) was determined over a 24 weeks period.

4.6.2.1 Total plate count (TPC)

Figure 4.3 illustrates an overall slight decline in total plate count of house cricket powders over 24-week storage period at room temperature except for protocol 4, which shows a gradual rise between week 12 and 24.

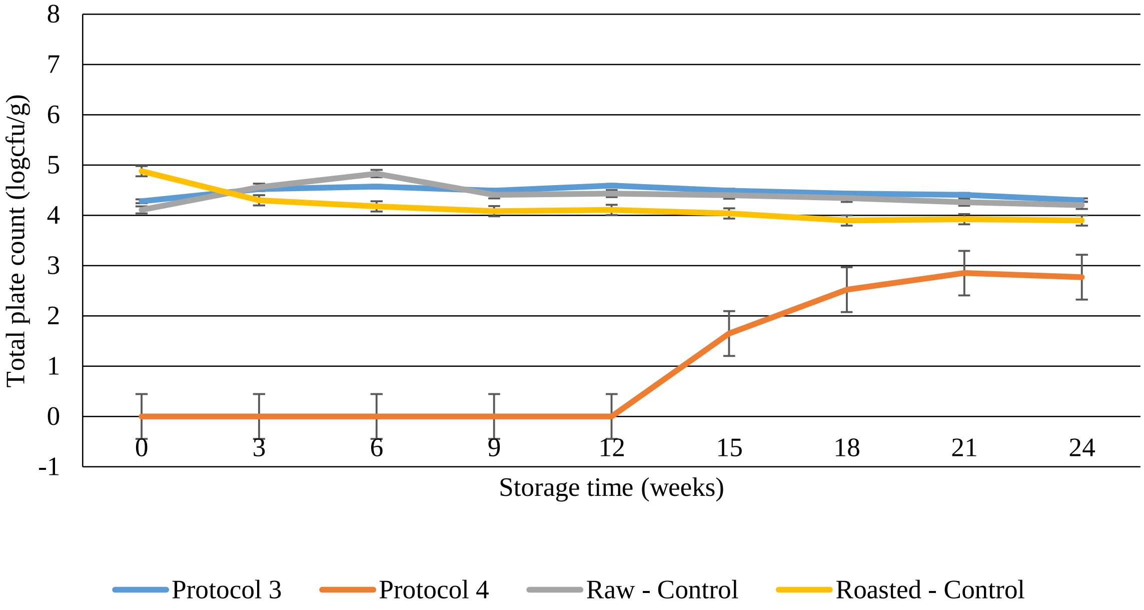


Figure 4.3: Changes in TPC counts of the cricket powders

The highest TPC recorded was 4.8 log cfu/g for control 2 (roasted) at 0 weeks, while the lowest was 0 (<10) cfu/g for protocol 4 (CL/ROOH) at the same time point. Outcomes differ from those of Ssepuuya et al. (2017), who reported a maximum total plate count of 3.2 log cfu/g for processed edible grasshoppers.

At 24 weeks the TPC was constant and highest result was observed in protocol 3 with 4.2 log cfu/g, while lowest was 2.8 log cfu/g in protocol 4. At week 24, the microbial counts were below the maximum of 10^5 cfu/g recommended for edible insects, as specified by the East African Standard EAS 1186: 2023 for insects that are edible.

There is a notable decline in the TPC of the control powders, with control 1 dropping by 73.5% and control 2 decreasing by 89.5%. This reduction in TPC suggests a decrease in the overall microbial population within the cricket powders over the observed period. The low moisture content observed in this study, combined with the use of tight packaging (zip lock bags), may have contributed to the decline in total plate counts by limiting the moisture and air available for microbial growth (Amankwah et al., 2009). These findings have positive implications for product stability and safety, as a decrease in total plate count suggests a lower risk of microbial spoilage, potentially extending the product's shelf life.

4.6.2.2 Yeast and moulds

Findings in Figure 4.4 indicate a gradual decline in the yeast and moulds counts of the refined cricket powders in protocol 3, while the counts for the controls remained generally constant.

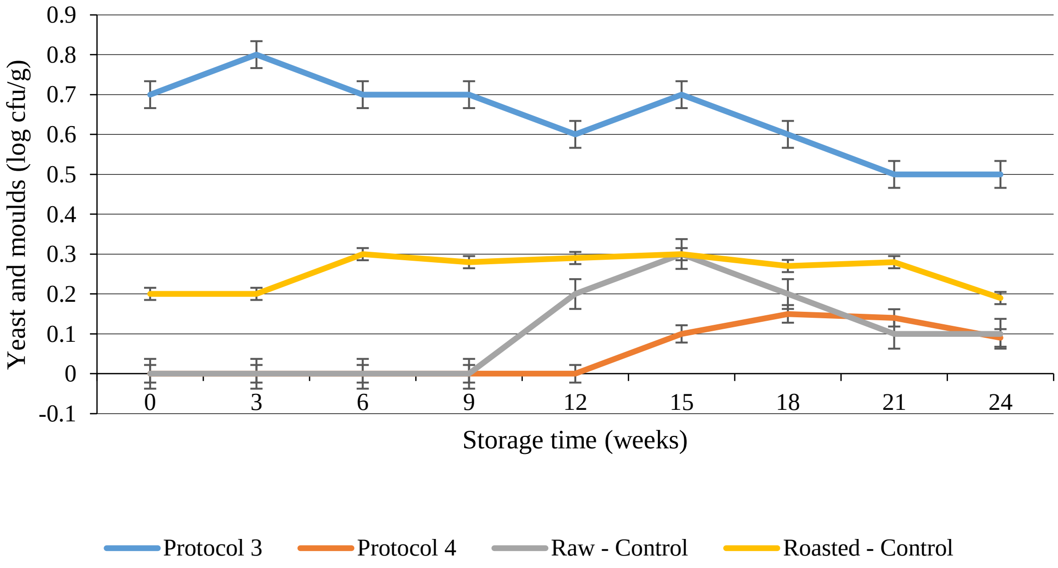


Figure 4.4: Changes in yeasts and moulds counts of cricket powders with time

Protocol 3 cricket powder had the highest yeast & moulds count at 0.8 log cfu/g by week 3 after which there was a gradual decline of up to 0.5 log cfu/g by week 24. Refined house cricket powder in protocol 4 had constant < 10 cfu/g by week 12 and a slight increase upto 0.1 log cfu/g by 24 weeks of storage. These findings comply with the specifications outlined in the East African Standard EAS 1186:2023 for edible insects, which sets a maximum allowable limit of 10^3 cfu/g. These low yeast and mould counts may be attributed to the low moisture content observed in this study, coupled with the use of tightly sealed packaging (zip lock bags) which restricted the moisture and air available for microbial growth. Results align with Ramashia et al., (2020) who noted that dried edible insects (mopane worms, termites and stink bugs) with moisture content below 10% did not support yeast and moulds growth.

4.6.3 Cricket powder chemical shelf life

4.6.3.1 Acid value

Results show that the acid values of the cricket powders were constant with control 1 exhibiting the highest AV level of 30 mg KOH/g at week 0 and 44 mg KOH/g at week 24 (Figure 4.5). Protocol 3 had the lowest acid value of 15 mg KOH/g at 0 weeks and 18 mg KOH/g at 24 weeks.

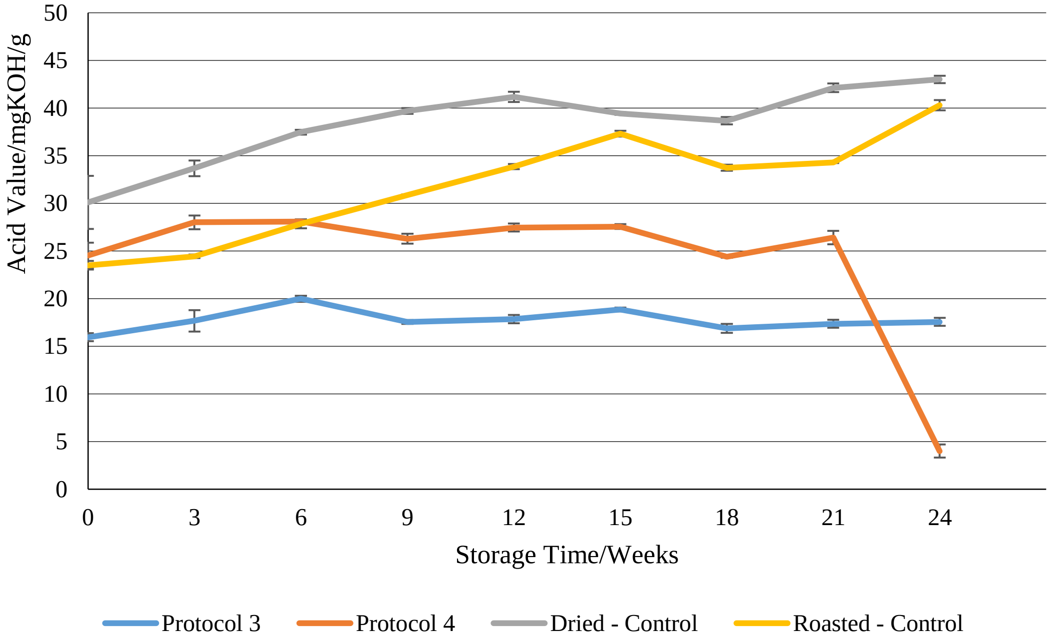


Figure 4.5: Changes in the acid value of cricket powders with time

High acid values generally indicate increased levels of free fatty acids, which could suggest degradation by fat hydrolysis over time (Medeiros Vicentini-Polette et al., 2021). Increase in acid value over time suggests that the cricket powder may be deteriorating as it ages, likely due to exposure to air, moisture, or temperature fluctuations leading to lipid breakdown (Umebara et al., 2024). Refined cricket powder in Protocol 4 had a generally constant AV level below 30 mg KOH/g up to week 21 and below 10 mg KOH/g at 24 weeks of storage. Despite these variations, all acid values for the cricket powders over 24 weeks of storage remained below the regulatory threshold of 100 mg KOH/g for free fatty acid content specified by the Uganda standard US 2146:2020.

4.6.3.2 Peroxide value

The findings indicate that peroxide values of house cricket powders generally decreased over time, with exception of protocol 3, which showed a gradual increase from 10 to 17 meq O_2/kg between week 0 and week 12 before following the same trend (Fig 4.6).

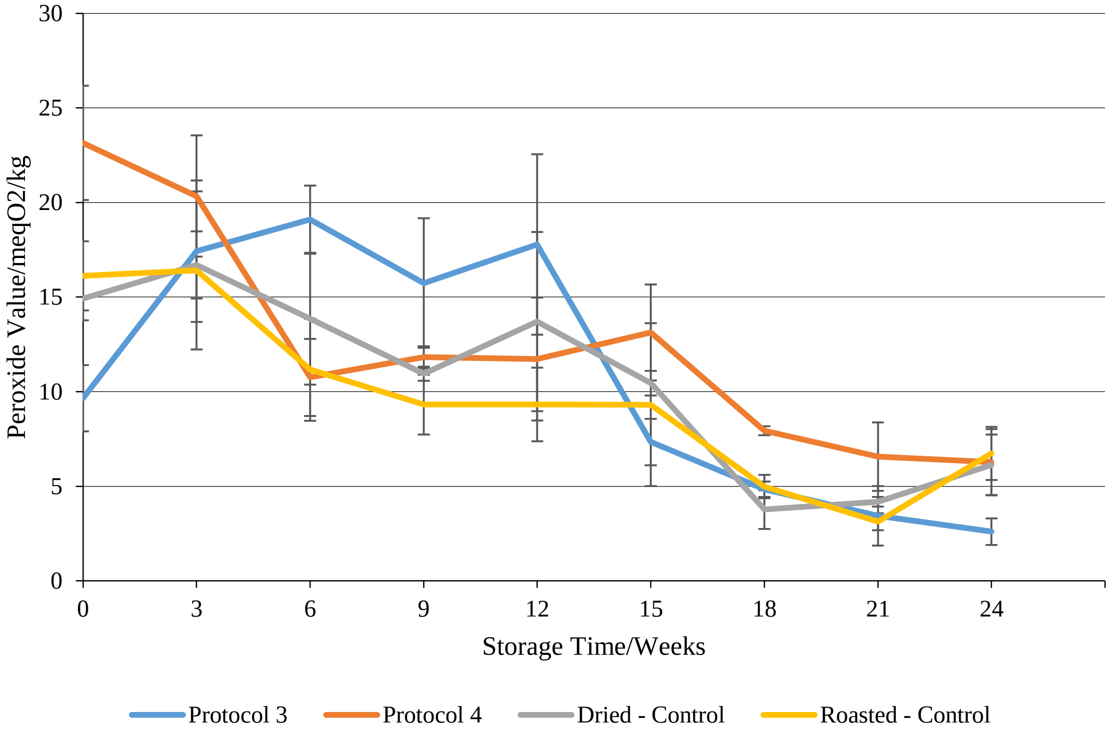


Figure 4.6: Changes in peroxide value of cricket powders with time

The refined cricket powder from protocol 4 had the highest peroxide value of 23 meq O_2/kg at week 0 but decreased to 7 meq O_2/kg over 24 weeks of storage. The controls exhibited peroxide values ranging from 16 meq O_2/kg at week 0 to 7 meq O_2/kg /kg at week 24. Increase in peroxide value of protocol 3 for the first 12 weeks indicates that the fatty acids in the cricket powder may have undergone oxidation. This could be due to oven drying of the cricket powder after the bleaching. This is comparable to the results of (Marzoli et al., 2023) who showed that oven drying enhanced lipid oxidation of unrefined *A. domesticus.*

Peroxide value quantifies the amount of hydro peroxides present, that are primary oxidation products formed when fats and oils undergo oxidative degradation (Miyazawa, 2021). The elevated peroxide value at week 0 in protocol 4 suggests that the residual peroxide from the bleach could have influenced the high levels. This reduction could mean that the initial oxidation products are decomposing during storage. The overall decline in peroxide values over 24 weeks points to a trend of decreasing oxidation, potentially due to the natural breakdown of peroxide (Musakhanian et al., 2022).

4.6.3.3 Thiobarbituric acid value

TBA is the measure of extent of oxidative deterioration, especially in lipid-containing substances such as oils and fats, by quantifying the presence of malonaldehyde (MDA), a breakdown product of fatty acids when they undergo oxidation (Domínguez et al., 2019).

All samples showed a gradual increase in TBA values over the 24 weeks, reflecting the steady progression of secondary oxidation as the samples aged.

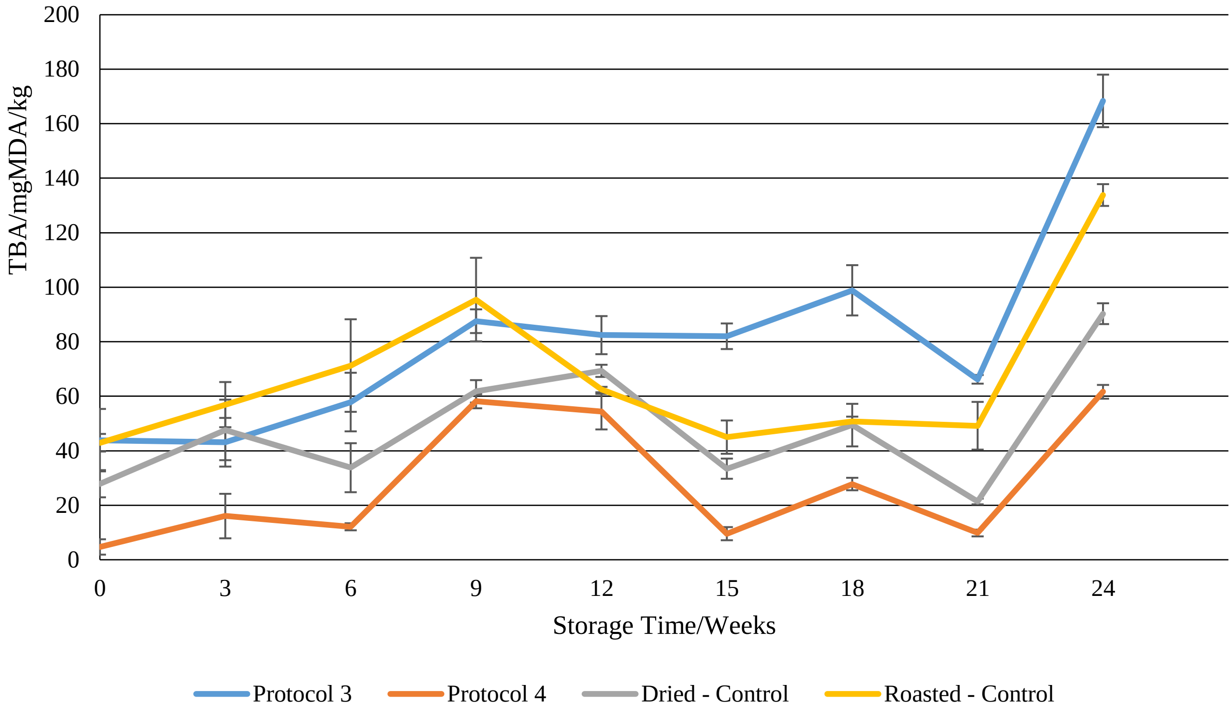


Figure 4.7: Changes in the TBA value of cricket powders with time

The TBA value of the cricket powders generally increased over time, with Protocol 4 (CL/ROOH) showing the lowest values, starting at 3 mg MDA/kg in week 0 and reaching 60 mg MDA/kg by 24 weeks of storage. Protocol 4 (CL/ROOH), with the lowest initial TBA value, indicates it was highly efficient in inhibiting lipid oxidation at the start of the storage period (Aguilar Diaz De Leon & Borges, 2020). Between weeks 9 and 15, there was a slight decline in the TBA values of all the cricket powders. This drop could be attributed to the formation of intermediate compounds (hexanal, alkanals, alkenals) during oxidation, which may have interacted and reduced the detectable MDA (malondialdehyde) levels. Additionally, natural antioxidants in the cricket powders may have become more active or effective during this mid-storage period, temporarily slowing down the oxidation process (Vlahova-Vangelova et al., 2023).

Protocol 3 (chloroform bleach) had the highest TBA value, starting at 42 mgMDA/kg level in week 0 and rising to 170 mg MDA/kg level after 24 weeks of storage. The high initial TBA value in protocol 3 suggests that the use of chloroform bleach may have had an oxidizing effect, accelerating the breakdown of unsaturated fats before storage even started (Dewage et al., 2021). Control 2 exhibited a moderately higher TBA value, rising from 40 mg MDA/kg at the start (week 0) to 140 mg MDA/kg by the 24th week, whereas Control 1 began at 22 mg malondialdehyde per kilogram and reached 90 mg malondialdehyde per kilogram by the end of the storage duration. Roasting temperatures in control 2 might have caused the higher TBA value compared the drying temperatures in control 1 (Arab et al., 2022).

4.6.4 Sensory shelf life of the cricket powder

4.6.4.1 Changes in colour

Findings in figure 4.8 show a minor deviation in colour perception from 1 at week 0 to 2.3 at week 24 of storage with exception of protocol 3 cricket powder which shows a decline from 2.9 at week 0 and 1.7 at week 24.

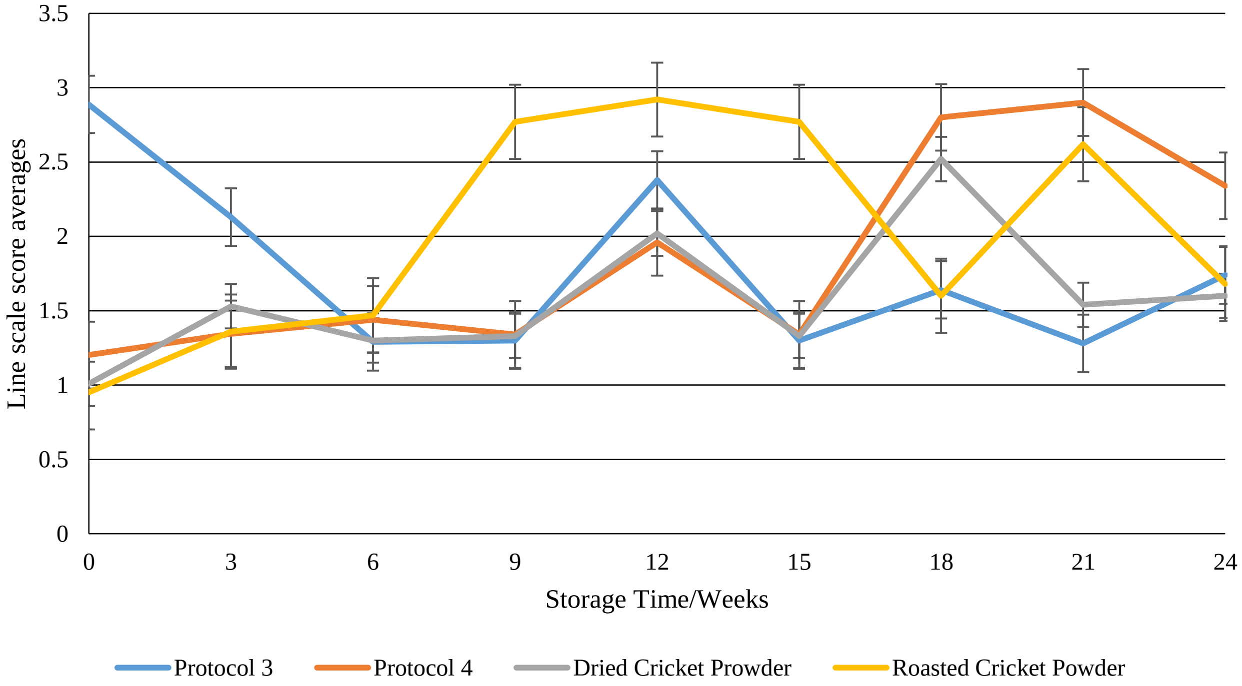


Figure 4.8: Changes in colour of cricket powders with time

For most cricket powders, the color perception in comparison with the reference increased slightly steadily, this suggests that the visual appearance of the powders became more darker over time, which might be due to oxidative chemical changes for example lipid oxidation (Tanga et al., 2021). As fats degrade, oxidation by-products such as aldehydes and ketones may cause color changes. Additionally, breakdown of pigments may alter the appearance over time. The gradual color change suggests that most treatments (except Protocol 3) allowed for slower, more progressive changes in the cricket powder's visual characteristics during storage.

4.6.4.2 Changes in aroma

Findings show a gradual deviation in the aroma from 1 on week 0 to 3.7 during week 24 of storage with exception of protocol 3 whose aroma decreased in comparison to the reference sample from 4 at week 0 to 3 at week 24 (Fig 4.9).

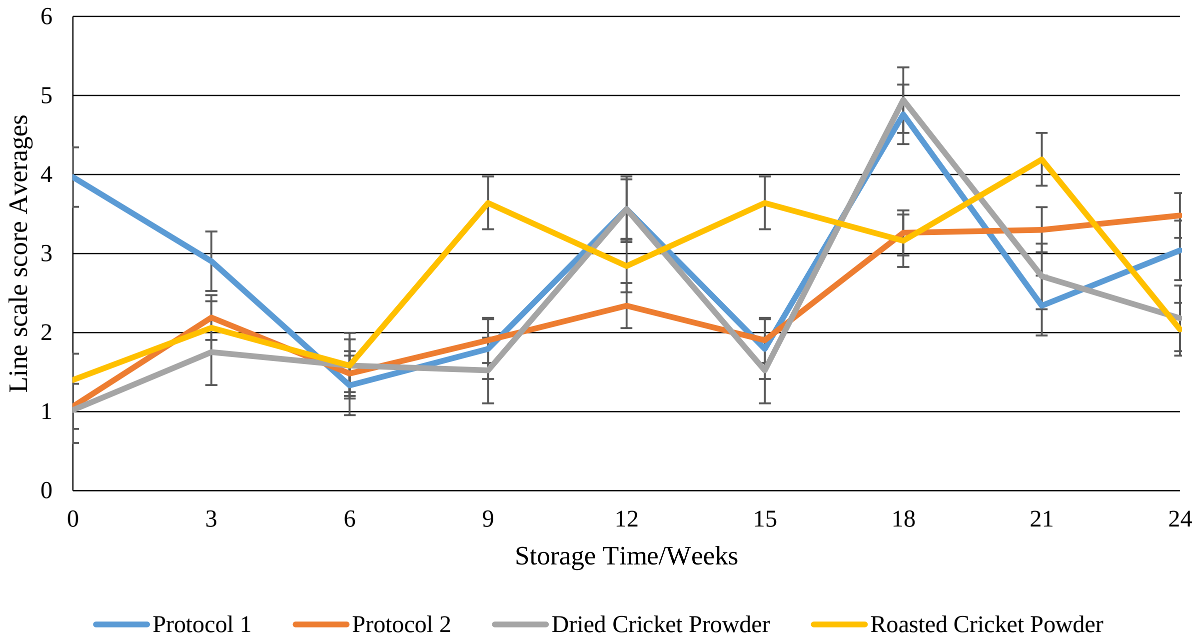


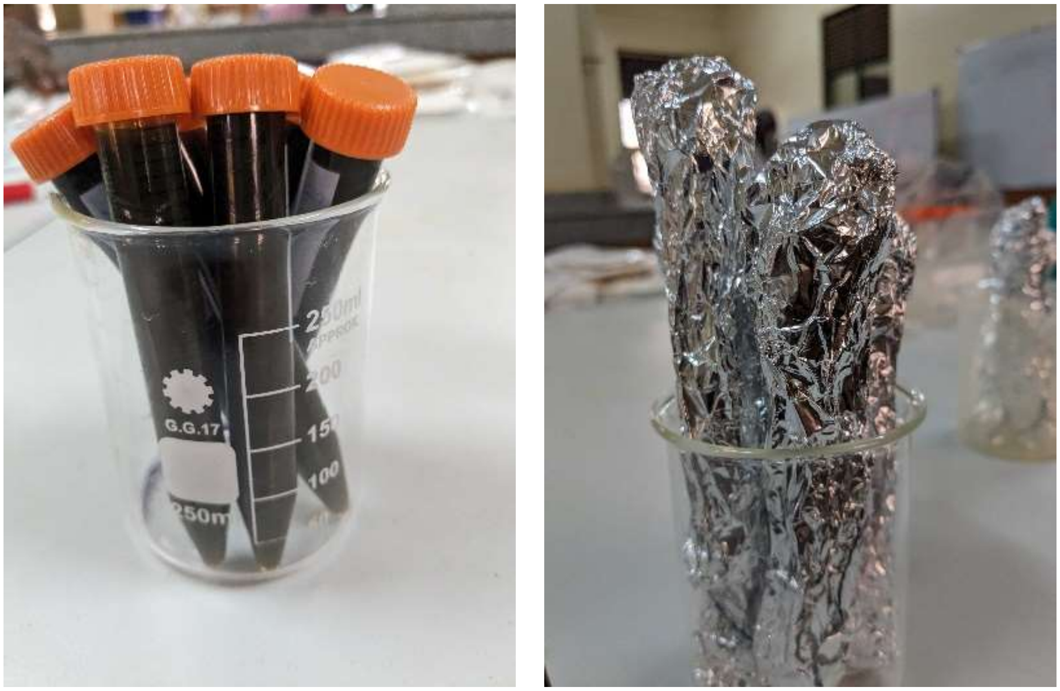
Figure 4.9: Changes in aroma of cricket powders with time

The aroma scores for all samples, as measured on a line scale average, fluctuated modestly throughout the 24-week storage period, with values generally ranging between 1.0 and 4.7. At week 0, protocol 3 started with highest aroma score (~4), while the other samples began with lower scores (~1–2), indicating fresher or more pronounced aroma characteristics at the beginning. Between weeks 3–6**,** aroma scores declined slightly for most treatments, reaching a low point around week 6. This may indicate early stages of aroma degradation or equilibration of volatiles in the storage environment. For weeks 9–15**,** there was a mild increase in aroma scores for roasted cricket powder, peaking at around week 9, suggesting that roasting might have contributed to more intensifying aromatic compounds. By week 18, all protocols had a notable increase in aroma scores, especially Protocol 3, which peaked around 4.7. This spike might be due to compound transformation during mid-storage. Between weeks 21–24**,** the aroma scores slightly declined across all samples (Akullo et al., 2023).

4.7 Quality and stability of oil (byproduct) extracted from house cricket powder

4.7.1 Quality of the cricket oil

The oil yield obtained from defatting house cricket powder was between 14% to 16%; the quality parameters of the cricket oil i.e.; moisture, paranisidine, thiobarbituric acid, peroxide, iodine, SV, and AV varied significantly ($p > 0.05$).



Cricket oil without foil

Cricket with foil

Figure 4.10: Images of cricket oil obtained by defatting roasted house cricket powder

Table 4.10: Chemical quality of the house cricket oil

Quality parameter	**Mean value**	**CODEX STAN 19-1999, Rev. 2**
Saponification value (mg KOH/g)	154.31±2.45^{a}	184 - 200 mg KOH/g
Iodine value (g iodine/100g)	7.60±0.62^{de}	10-150 g iodine/100g
Peroxide value (meq O_2/kg)	8.33±1.38^{d}	15 meq O_2/kg
Acid value (mg KOH/g)	45.33±0.17^{b}	4.0 mg KOH/g
Thiobarbituric Acid (meq of MDA/g)	17.94±0.49^{c}	
Moisture (%)	9.38±0.12^{d}	0.2% m/m
Paranisidine	5.09±0.12^{e}	<10

Every value is a mean of triplicate determinations. Mean value with different alphabet letter as a superscript in the column are significantly different from one another ($p > 0.05$).

As shown in Table 4.10, the quality attributes of cricket oil exhibited significant variation ($p < 0.05$). However, means for moisture content (9.38%) and peroxide value (8.33 meq O_2/kg) are substantially ($p > 0.05$) different. Notably, moisture content of 9.38% exceeds the recommended maximum specification of 0.2% set by the Uganda standard US EAS 321:2018.

A saponification value (SV) of 154.31 mg KOH/g stipulates that cricket oil has a variety of fatty acids with differing chain lengths (Gautam & Bhandari, 2023). Typically, higher saponification values are associated with oils that have shorter fatty acid chains, while lower values correspond to oils with longer chains (Ivanova et al., 2022). For reference, saponification values for different oils generally fall between 150 and 200 mg KOH/g (US ISO 3657:2013).

According to Geng et al. (2023), iodine value (IV) determines extent of unsaturation of oils, reflecting quantity of iodine (g) taken up by 100 g of oil. IV of 7.60 g iodine/100 g for cricket oil is considerably lower compared to many common vegetable oils, such as olive oil (80–90 g iodine/100 g) and sunflower oil (110–130 g iodine/100 g), as reported by Ebong & Christopher (2019). This low IV indicates that cricket oil may contain a high proportion of saturated fats. Oils with higher saturation (lower iodine values) tend to be more solid or semi-solid at room temperature, which aligns with the characteristics of cricket oil.

The results show that the cricket oil's acid value (AV) of 45.33 mg KOH/g substantially exceeds that of the set maximum limit of 4.0 mg KOH/g recommended by Uganda standard US EAS 321:2018 for virgin oils and fats. A high acid value might have been caused by the oil experiencing considerable degradation or breakdown of triglycerides, which is often attributed to inadequate handling, improper storage conditions (MacArthur et al., 2021). However, peroxide value of 8.33 meq O2/kg complies with the Uganda standard US EAS 321:2018, which sets maximum limit of 15 meq O2/kg for virgin oils and fats.

A thiobarbituric acid value of 17.94 meq of MDA/g in cricket oil suggests a significant level of lipid oxidation, indicating the presence of oxidation byproducts and some degree of fat degradation (Aguilar Diaz De Leon & Borges, 2020). The PV and TBA results demonstrate that cricket oil has already undergone measurable oxidation and is highly prone to further deterioration. Therefore, it should be packaged in high-barrier, light-protective, oxygen-impermeable materials and stored under cool, oxygen-limited, and dark conditions to maintain its chemical quality and sensory acceptability (Aguilar Diaz De Leon & Borges, 2020; Alsufiani & Ashour, 2021; Kochhar & Henry, 2020). Paranisidine value quantifies aldehydes, specifically secondary oxidation products for example 2-alkenals, which form during fat and oil breakdown (Alsufiani & Ashour, 2021). A p-Anisidine value of 5.09 suggests that the oil's shelf life may be reduced if not properly stored (e.g., in a cool, dark place), as these secondary oxidation products can develop, causing off-flavors and odors commonly described as rancid or stale. These outcomes agree with Rad et al. (2018), who showed that p-Anisidine values in fresh plus mixed frying oils ranged from 3.98 - 4.79 and 5.12 - 5.70**,** respectively, indicating acceptable oil quality.

4.7.2 Stability of the cricket oil

4.7.2.1 Chemical shelf life of cricket oil

4.7.2.1.1 Acid value

There was a decrease in acid value of cricket oil, from 49 mg KOH/g on week 0 to 44 mg KOH/g at week 3. However, the acid values are generally stable. The initial decline could indicate a reduction in free fatty acids, possibly due to low moisture, oxygen, light and inactivated lipase enzymes that initially decreased the acid value before stabilization.

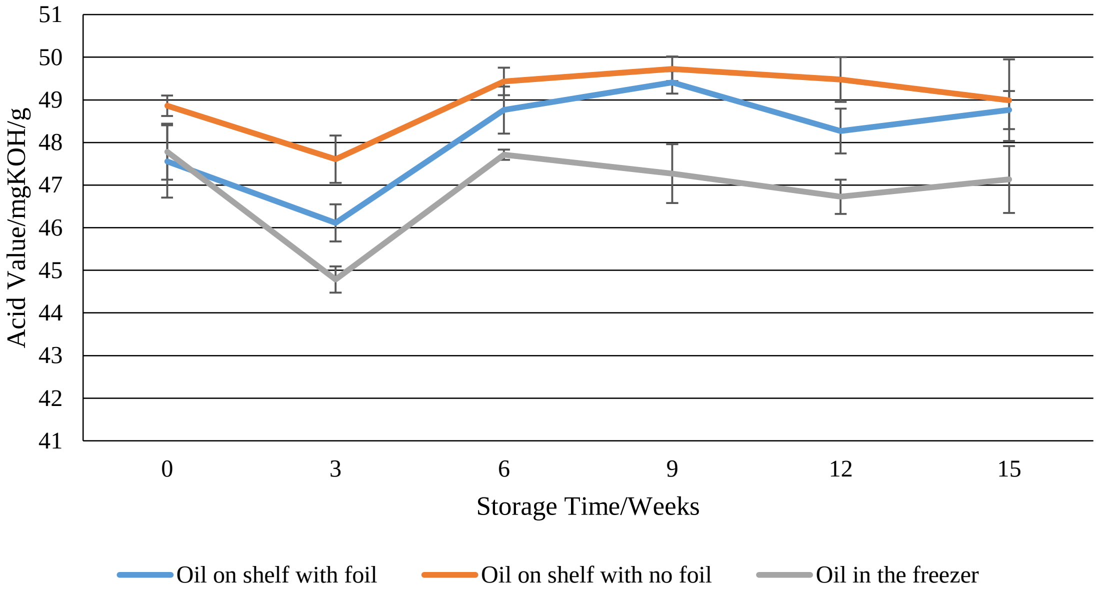


Figure 4.11: Changes in acid value of cricket oil with time

Among the samples, cricket oil stored on the shelf without foil had the highest acid value, followed by cricket oil stored on the shelf with foil, while cricket oil stored in the freezer had the lowest acid value. The acid value of cricket oil stored on the shelf without foil ranged from 47.5 mg KOH/g at week 3 to 49.5 mg KOH/g at week 9, then slightly declined to 49 mg KOH/g after 15 weeks of storage. This suggests that exposure to light and air, without the protective foil, may accelerate acid formation fatty acids due to hydrolysis (Umebara et al., 2024). The cricket oil stored in the freezer had the lowest acid value suggesting that low temperatures significantly slow down the hydrolysis reactions, preserving the oil's quality over time (Catherine Bukola et al., 2015).

4.7.2.1.2 Peroxide value

The findings reveal a decline in the peroxide value (PV) of cricket oil from week 0 to week 3 (Fig 4.12). At week 0, cricket oil stored on the shelf without foil had highest peroxide value of 59 meq O_2/kg, while the oil stored with foil and the oil in the freezer both had a PV of 30 meq O_2/kg.

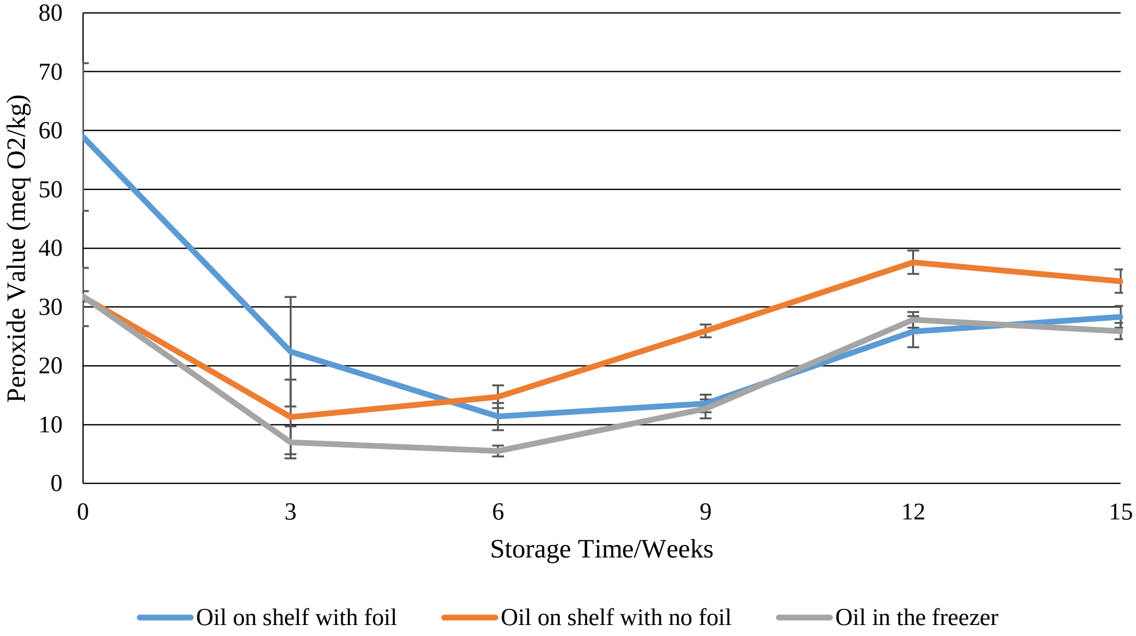


Figure 4.12: Changes in peroxide value of cricket oil with time

This initial decrease likely resulted from the breakdown of primary oxidation products (hydroperoxides), that are what the PV measures (Miyazawa, 2021). This suggests that the higher initial oxidation in the oil stored without foil was due to exposure to light and air (Liu et al., 2019).

By week 3, the PV had decreased to 22 meq O_2/kg for oil on the shelf without foil, 10 meq O_2/kg for oil on the shelf with foil, and 7 milliequvalents of oxygen per kilogram for oil in the freezer. Between week 3 and week 12, the PV gradually increased, stabilizing by week 15. At week 12, the oil stored on the shelf without foil had the highest PV at 39 meq O_2/kg, which then slightly decreased to 35 meq O_2/kg by week 15 (Miyazawa, 2021).

4.7.2.1.3 Thiobarbituric acid (TBA) value

Results shown in Figure 4:13 indicate a gradual increase in malonaldehyde (MDA) levels from week 0 to 15 weeks of storage, rising from 17 mg MDA/kg to 59 mg MDA/kg for cricket oil stored on the shelf with foil (Reitznerová et al., 2017).

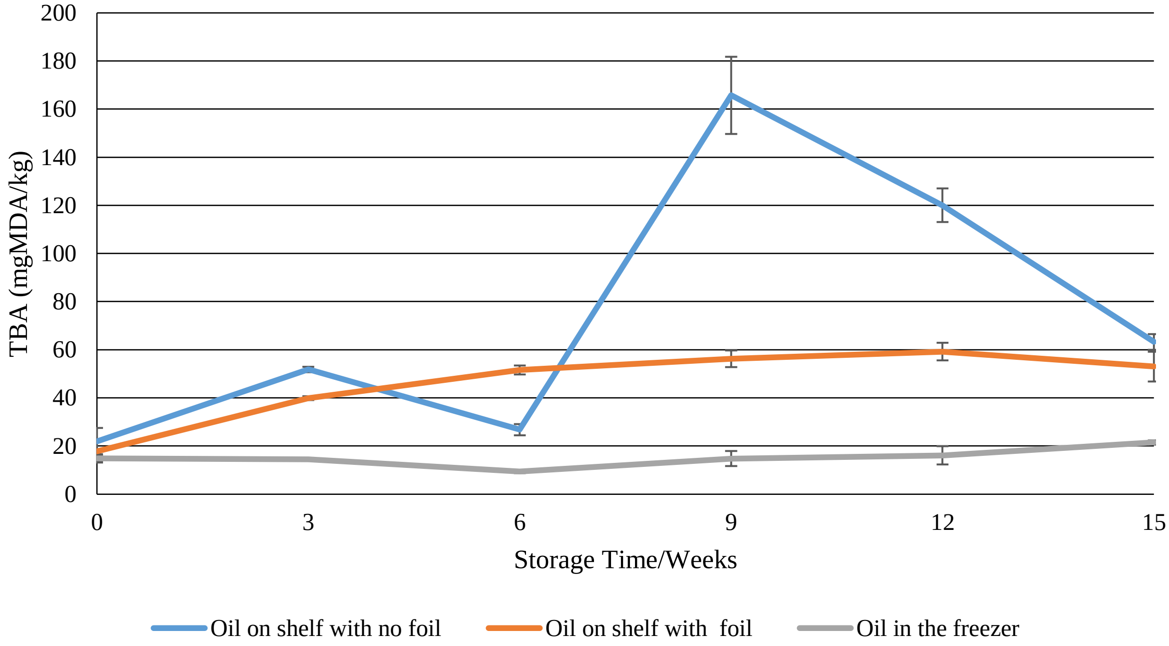


Figure 4.13: Changes in TBA value of cricket oil with time

For oil stored in the freezer, MDA levels increased more modestly from 14 mg MDA/kg to 21 mg MDA/kg**.** The modest increase in MDA for the oil stored in the freezer suggests that low temperatures are effectively slowing down the oxidation process. There is a gradual rise in the

TBA value of the cricket oil stored on shelf with foil. This gradual rise suggests that lipid oxidation is ongoing but at a controlled rate when using foil, which helps reduce exposure to light and air.

Cricket oil stored on the shelf without foil experienced an initial increase in MDA levels from 21 mg malondialdehyde per kilogram on week 0 to 51 mg MDA/kg by week 3. This early rise indicates the progression of oxidation as primary oxidation products, such as hydroperoxides, break down into MDA (Domínguez et al., 2019). The TBA value for oil stored on the shelf without foil decreased to 27 mg MDA/kg by week 6, likely due to the breakdown of some MDA or the formation of other oxidation products. By week 9, there was a sharp increase to 165 mg MDA/kg**,** indicating a period of intense secondary oxidation, where additional MDA was produced as primary oxidation products further decomposed (Aguilar Diaz De Leon & Borges, 2020). This level then decreased sharply to 63 mg MDA/kg by week 15, suggesting a stabilization phase where the formation of new MDA slowed, or some MDA reacted with other compounds.

4.7.2.2 Sensory shelf life of the cricket oil

4.7.2.2.1 Changes in colour

Figure 4.14 illustrates a gradual increase in the color intensity of both types of cricket oil, rising from 2.4 at week 0 to 4.8 at week 12. This increase suggests that as the oil ages, it undergoes chemical transformations that lead to a darker or more intense coloration, likely due to oxidation thus forming of degradation products (Tanga et al., 2021).

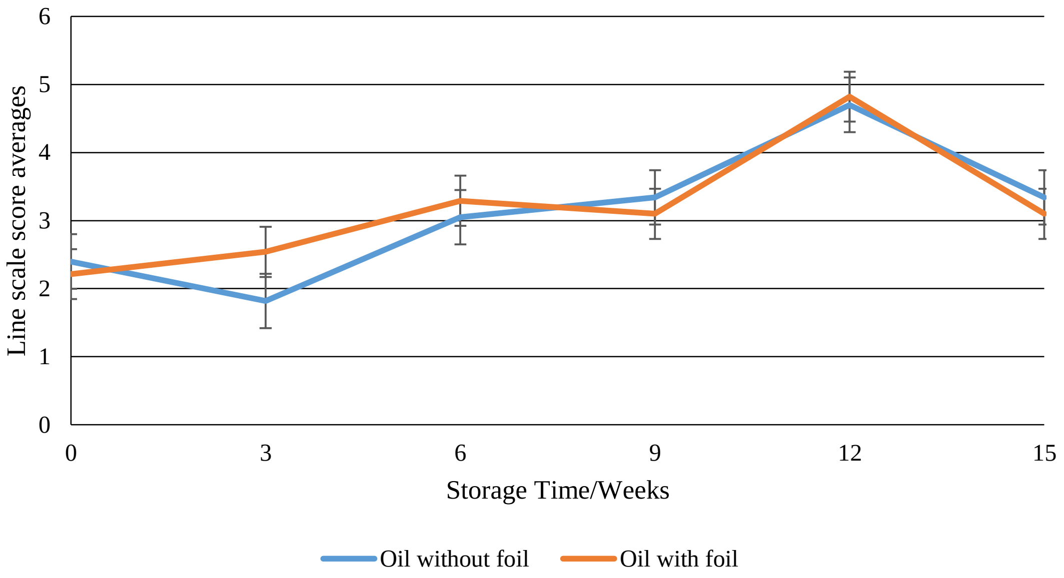


Figure 4.14: Changes in colour of cricket oil with time

Cricket oil stored without foil initially experienced a decline in color from 2.4 to 1.8 between week 0 and week 3. Between weeks 12 and 15, both types of cricket oil showed a sharp decrease in color intensity to 3. This sharp decline followed by stabilization may reflect changes in rate of oxidation or the development of different compounds that alter the color in varying ways over time (Gorman et al., 2024).

4.7.2.2.2 Changes in aroma

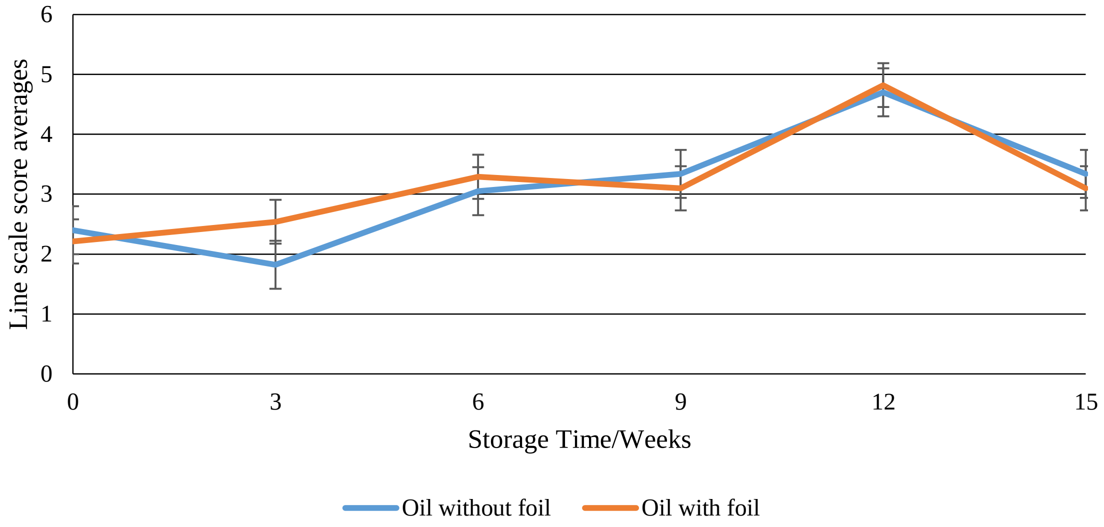


Figure 4.15: Changes in aroma of cricket oil with time

Figure 4.15 shows a gradual rise in the aroma intensity of both types of cricket oil, increasing from 2.4 at week 0 to 4.8 at week 12. This increase might be due to the buildup of oxidation products and other compounds that enhance the oil's aroma profile. It could also be a result of the loss or modification of volatile aromatic compounds over the storage period, potentially influenced by light and air exposure (Díaz-Montaña et al., 2023).

Cricket oil stored without foil initially showed a decrease in aroma intensity from 2.4 to 1.8 between week 0 and week 3. From weeks 12 to 15, both types of cricket oil experienced a sharp drop in aroma intensity to 3. This trend suggests that the aroma intensity was reducing due to degradation of aromatic compounds (Chen et al., 2022).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Most acceptable house cricket powder refining protocol employed defatting with petroleum ether and bleaching with sodium hypochlorite and hydrogen peroxide.

Refining house cricket powder improved the sensory acceptability and increased protein content by 8.4%.

Refining did not affect safety and stability of the house cricket powders (met Uganda's edible insect safety standard US 2146:2020). Therefore, refined house cricket powder can be stored in airtight containers at room temperature (24-28℃).

Saponification value, Iodine value, peroxide value of cricket oil were within the recommended standard limits however moisture, paranisidine and acid value were above the acceptable limits.

5.2 Recommendations

House cricket powder should be refined by defatting with petroleum ether and bleaching with a mixture of sodium hypochlorite and hydrogen peroxide.

Refined cricket powders should be incorporated in different staple flour to improve their protein content.

Studies may be done to determine the actual shelf life period for the house cricket powder.

Further studies may be done on refining cricket oil to improve its chemical and sensory stabilit**y.**

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APPENDICES

Appendix 1: Cricket powder refining methods generated by design expert software.

Number	**Defatting**	**Bleaching**	**Processing**	**Fat content**	**Aroma**	**Color brightness**	**Appearance**	**Overall - acceptability**	**Desirability**	
1	**PE soaking**	***CL/ROOH***	***Roasted & Dried***	**6.025**	**6.191**	**6.245**	**5.702**	**5.953**	**0.568**	**Selected**
2	PE soaking	*Chloroform*	*Dried*	6.025	6.191	6.245	5.702	5.953	0.568	
3	PE soaking	*None*	*Roasted & Dried*	6.025	6.191	6.245	5.702	5.953	0.568	
4	PE soaking	*Chloroform*	*Roasted & Dried*	6.025	6.191	6.245	5.702	5.953	0.568	
5	PE soaking	*CL/ROOH*	*Dried*	6.025	6.191	6.245	5.702	5.953	0.568	
6	PE soaking	*None*	*Dried*	6.025	6.191	6.245	5.702	5.953	0.568	
7	Folch	*Chloroform*	*Dried*	6.380	6.191	6.245	5.702	5.953	0.567	
8	Folch	*CL/ROOH*	*Dried*	6.380	6.191	6.245	5.702	5.953	0.567	
9	Folch	*Chloroform*	*Roasted & Dried*	6.380	6.191	6.245	5.702	5.953	0.567	
10	Folch	*None*	*Dried*	6.380	6.191	6.245	5.702	5.953	0.567	

11	Folch	*None*	*Roasted & Dried*	6.380	6.191	6.245	5.702	5.953	0.567
12	Folch	*CL/ROOH*	*Roasted & Dried*	6.380	6.191	6.245	5.702	5.953	0.567
13	New Method	*Chloroform*	*Dried*	7.267	6.191	6.245	5.702	5.953	0.564
14	New Method	*Chloroform*	*Roasted & Dried*	7.267	6.191	6.245	5.702	5.953	0.564
15	New Method	*CL/ROOH*	*Dried*	7.267	6.191	6.245	5.702	5.953	0.564
16	New Method	*CL/ROOH*	*Roasted & Dried*	7.267	6.191	6.245	5.702	5.953	0.564
17	New Method	*None*	*Roasted & Dried*	7.267	6.191	6.245	5.702	5.953	0.564
18	New Method	*None*	*Dried*	7.267	6.191	6.245	5.702	5.953	0.564
19	None	*CL/ROOH*	*Dried*	50.000	6.191	6.245	5.702	5.953	0.000
20	None	*CL/ROOH*	*Roasted & Dried*	50.000	6.191	6.245	5.702	5.953	0.000
21	None	*Chloroform*	*Roasted & Dried*	50.000	6.191	6.245	5.702	5.953	0.000
22	None	*None*	*Dried*	50.000	6.191	6.245	5.702	5.953	0.000

23	None	*None*	*Roasted & Dried*	50.000	6.191	6.245	5.702	5.953	0.000
24	None	*Chloroform*	*Dried*	50.000	6.191	6.245	5.702	5.953	0.000

Appendix 2: Panelists screening

All information submitted will be handled with utmost confidentiality.

Please mark or tick your answers accordingly.

Personal Details

Name ……………………………………………………………………………………………….

Age ……………………………………………………………………..

Telephone number ……………………………………………………………………………….

E.mail address ………………………………………………………….................................

Occupation ………………………………………………………………………………………

Are you interested in sensory evaluation of refined house cricket powder?	Yes	No
Are you available on Thursdays and Fridays between 10:00 and 12:00?	Yes	No
Are you allergic to any foods?	Yes	No
If you are, which foods?		
Do you have Diabetes?	Yes	No
Do you have dentures?	Yes	No
Do you have hypertension?	Yes	No
Do you have any health/personal condition that might impair your sensory acuity?	Yes	No

We thank you for your input and hope to invite you very soon to constitute our panel for Descriptive Sensory Evaluation

Triangulation test ballot

Name ……………………………………………

Date ……………………………………………

Instructions: Check samples from left to right. Two are identical; determine which is the odd sample. Describe the odd property. If no difference is apparent, you must guess.

Sample sets	Which is the odd sample	Describe the odd property
P		
Z		
B		
D		

Abstract thinking will be assessed by asking candidates to provide verbal descriptions of unidentified but commonly encountered aroma compounds. These odorous substances will be presented in liquid extract form within vials, at concentrations exceeding the recommended threshold levels. Candidates who correctly identify less than 65% of the odours will be deemed unsuitable to serve as panelists for this type of evaluation .Le nez du café (36 vials odors kit will be used)

Seven odours have been selected to be presented for identification coded P, D, Z, B, H,T, Y

Odour identification ballot

Name ……………………………………………

Date ……………………………………………

Please smell each of the samples presented and identify or describe the odour present. For example, tea-rose-like, caramel-like, potato-like, toast-like, earthylike, clove-like, apple-like and so on. Do not put the vial in direct contact with your nose and do not sniff.

P……………………………………..

D……………………………………..

Z……………………………………..

B……………………………………..

H……………………………………..

T……………………………………..

Y……………………………………..

Comment

……………………………………………………………………………………………

……………………………………………………………………………………………

Appendix 3: Sensory tool for colour ranking

RANKING BALLOT OF CRICKET POWDER

Name______________________________________ **Phone #**________________________

Instructions: Please observe house cricket powder samples provided and assign the sample with the brightest intensity a score 1 and increase numbering in order of decreasing colour intensity (1, 2, 3,4) with 4 being the least bright. Kindly assess the products in the sequence they are presented.

Set A		**Set B**	
Sample	**Score**	**Sample**	**Score**
541		800	
831		722	
769			
370			

Comment

………………………………………………………………………………………………………
……………………………………………………………………………………

Thank you for participating in this exercise

Appendix 4: Image of roasted house crickets