EFFECT OF CASSAVA (*Manihot esculenta* Crantz) FERMENTATION AND CRICKET (*Acheta domesticus*) ENRICHMENT ON PROTEIN QUALITY, SENSORY PROPERTIES, AND SHELF LIFE OF CASSAVA-CRICKET BISCUITS

KEVINE ODHIAMBO OTIENO

A Thesis Submitted to Graduate School in Partial Fulfilment of the Requirements for Master of Science Degree in Food Science of Egerton University

EGERTON UNIVERSITY

AUGUST, 2025

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work. It has not been presented in any other university either wholly or in part for award of an academic degree.

Signature: _  _____ Date: __12/08/2025_____

Kevine Odhiambo Otieno

KM16/12182/17

Recommendation

This thesis has been prepared with our guidance and has been submitted with our approval as the official university supervisors.

Signature: _  _____ Date: 17/09/2025_____

Prof. Patrick Muliro, PhD

Department of Dairy and Food Science and Technology,

Egerton University.

Signature: __  ___ Date: _22nd September 2025_____

Prof. Mary Omwamba, PhD

Department of Dairy and Food Science and Technology,

Egerton University.

COPYRIGHT

DEDICATION

This thesis is dedicated to every member of my family who is looking up to me for inspiration. I am trying my best under the current circumstances not to fail you.

ACKNOWLEDGEMENTS

I acknowledge the financial support accorded to me by the Kenya Climate Smart Agriculture Program (KCSAP). Special thanks to Egerton University for allowing me to undertake this course. I also acknowledge my supervisors; Prof. Patrick Muliro and Prof. Mary Omwamba for their guidance and support during my studies. I cannot forget the moral support offered by the fellow students at the department of Dairy and Food Science and Technology (DAFTECH), Egerton University.

ABSTRACT

Climate change and population growth, which have led to the subdivision of agricultural land, are threats to food security. Cassava (*Manihot esculenta Crantz*) and cricket (*Acheta domesticus*) are potential solutions to this threat. This study aimed to develop biscuits from fermented cassava enriched with cricket flour that meet the protein requirements of 5-13-year-olds. Cassava flour was fermented spontaneously and with *Aspergillus oryzae*, and mixed with cricket flour at three levels: 12%, 24%, and 36%. The blended flours were kneaded and baked at 180°C/7 minutes into biscuits then analysed for nutritional, microbial, shelf life, and sensory properties. Cricket flour was also analysed for amino acids and fatty acids. The results showed that cricket protein had significantly higher levels of all amino acids except threonine than the reference protein. It was richest in tyrosine, glutamic acid and histidine, with concentrations of 5.01, 4.76, and 4.01 g/100g, respectively. Cricket flour is a good source of retinol, with a concentration of 1.46 mg/100 g. The fat in cricket flour is richest in elaidic, palmitic, and stearic fatty acids, with concentrations of 43.13%, 21.83%, and 11.02%, respectively. Both method of fermentation and substitution level of cricket flour significantly improved the protein quality, sensory, microbial and shelf-life properties of the resultant biscuits. Biscuits made from spontaneous and *Aspergillus oryzae* fermented cassava flour had higher protein and ash content, and lower moisture and carbohydrate content than unfermented biscuits ($p<0.05$). Consumers rated the sensory attributes of biscuits lower as the cricket substitution level increased, regardless of the fermentation method used. Increasing the amount of cricket flour in biscuits significantly increased the intensity of colour, crumbliness, and bitterness, while significantly decreasing the intensity of consistency, density, baked aroma, sugary taste, and buttery flavour, irrespective of the fermentation method. Initial peroxide values of cassava-cricket biscuits were 2.9- 3.7 mEq of O_2/kg. During storage the activation energy for oxidative rancidity in cassava-cricket biscuits was from -8.84 to 10.07 1.07 J/K/Mol, enthalpy change was from -2462.71 to -2443.80 kJ/Mol, entropy change from -49.51 to -46.37 J/K/Mol and Gibbs free energy was from 11.242.18 to 12,160.12 kJ/Mol. The thermodynamic properties of the biscuits showed that the environment was anaerobic and had low water activity hindering oxidative rancidity during storage, so peroxide value could not be solely relied on to predict shelf life. These findings show that cassava and cricket utilization can open up a new frontier in the incorporation of insects in baked foods as protein sources, and address food security challenges in Sub-Saharan Africa.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistical Databases
MoALF	Ministry of Agriculture, Livestock and Fisheries

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Accessibility to adequate food of acceptable quality has continued to be an elusive pursuit for most of Sub-Sahara Africa where 40% of the population are food insecure (FAO, 2018). According to the Ministry of Agriculture, Livestock and Fisheries (MoALF) (2017), Kenya imports up to 40% of its food to bridge the national deficit. Extreme weather patterns have had negative impact on agricultural productivity, especially for cereal crops such as wheat whose yields have dropped by as much as 10% (Lesk et al., 2016). More than 370,000 children in Kenya are at risk of protein-energy malnutrition (MoALF, 2017). School going children aged between 5 and 13 years require between 19.7 – 35 mg of protein per day for growth and maintenance (National Academy of Sciences, 2010).

Globally, wheat is the second most popular cereal after rice. It is consumed in a variety of products such as pasta, noodles, biscuits, cakes, bread, *chapati* and buns. It is the most preferred flour by the baking industry given its rheological properties (Karaduman et al., 2019). Wheat would be a suitable food for addressing protein-energy malnutrition due to its popularity but for its gluten, which is unsuitable for consumers with gluten intolerance (Rostami et al., 2015). In addition, researchers have underscored the high expenses associated with commercial wheat production necessitating the need for an alternative source of baking flour (Fahad et al., 2018).

For centuries, the peoples of Africa, Asia and South American continents have considered cassava (*Manihot esculenta* Crantz) a staple food crop. It is a highly adaptable and resilient tropical tuber crop of the *Euphorbiaceae* family (Adebayo, 2008). Different African regions utilize cassava for consumption through different methods. In Nigeria, for instance, *gari* is the most common method of cassava consumption (Amarachi et al., 2015; Kolawole & Akingbala, 2011). In Kenya, cassava is utilized by roasting, milling into flour for use in *ugali* and biscuits, boiling of the fresh tubers and most recently, slicing into thin chips and crisps (Abong et al., 2016). The sweet cassava varieties are the most popular due to their low hydrogen cyanide content (<50 mg HCN/Kg) and one of the four important sources of starch besides maize, wheat and rice (Isendahl, 2011; Tagro & Kambire, 2010). In 2016, Sub-Saharan Africa produced 157,271,697 metric tonnes of cassava, of which 571,848 metric tonnes was from Kenya (FAOSTAT, 2016). Compared to wheat, cassava is relatively cheaper to produce but the value chain losses have been quantified to a tune of 23%. Cassava tubers can retain

starch quality for up to nine months underground, ideal for phased harvesting unlike wheat (Abong et al., 2016).

Cassava has relatively low protein content of about 2% compared to wheat with 12% protein content, necessitating compositing to boost the protein content of the final product (Abass et al., 2018; Jisha et al., 2010). Cassava flour is particularly deficient in methionine and cysteine (sulphur-containing amino acids), which has seen several researchers composite the flour with other cereals and legumes to improve its overall protein content (Jisha et al., 2010; Omwamba & Mahungu, 2014).

Research indicates that cassava flour can be substituted for wheat on a 1:1 ratio in the formulation of baked products (Falade & Akingbala, 2008). Cassava has no gluten hence suitable for substitution into baked food products for consumers with gluten intolerance. It also has minerals and vitamins that make it a suitable food source. According to USDA (2018), cassava has 1.36% protein, 0.28% fat, 38.1% carbohydrates, 1.8% dietary fibre and total energy is 160 kcal/100g. Cassava also has 16 mg calcium, 21 mg magnesium, 271 mg potassium, 20.6 mg vitamin C and 27 mg phosphorus, among other micronutrients. Its high fibre content facilitates slow release of sugar, which makes consumers feel fuller for longer (Oladiran et al., 2018). Cassava flour has been used successfully to bake gluten-free breads and crackers (Abass et al., 2018). Using cassava to produce baked products with longer shelf life can strategically help Kenya to address the challenges of hunger, malnutrition and food insecurity (FAO, 2018). However, the anti-nutrients and low protein content of cassava hampers its application in addressing protein-energy malnutrition despite its excellent starch.

Insects, especially house crickets (*Acheta domesticus*), are rich sources of protein with some studies indicating they contain as much as 60% protein content (Roos, 2018). They are easy to produce under very minimal costs and least carbon footprint (Halloran, 2017). Despite having been used to enrich wheat flour to make baked products (Homann et al., 2017; Roos, 2018), little has been done to produce baked products of cassava flour enriched with cricket protein. The current study delved into compositing of cassava flour with cricket flour to improve the protein quality of cassava biscuits.

1.2 Statement of the Problem

More than 370, 000 children face the risk of protein-energy malnutrition in Kenya (MoALF, 2017). Cassava, a hardy and resilient tropical tuber crop, is an excellent source of starch. It has been used in cassava-wheat and cassava-soy composite flours to produce baked products to enhance its utilization in the food value chain. However, unlike wheat, cassava lacks gluten, which is responsible for the functional properties of wheat flour. In addition to its

low protein content, cassava also contains cyanogenic glucosides that limit its consumption. House crickets are a viable protein source with up to 60% protein content. The insects are cheap and easy to produce in large quantities since they multiply rapidly. In addition, they have a small carbon footprint, which makes them an excellent alternative source of protein given the effects of climate change. Cricket flour protein has functional properties that can be harnessed to improve the quality of baked products. Through processing methods like fermentation and baking, the toxicity of cassava flour can be reduced to make it suitable for use as human food. Cricket flour can then be used to enrich cassava flour to bake cassava biscuits with acceptable protein content. In the end, this product can be used to address the problem of protein-energy malnutrition in children, contributing to the Sustainable Development Goals (SDGS) (e.g. zero hunger (SDG 2) and good health and wellbeing (SDG 3)). Therefore, this study aims to ferment cassava, enrich the flour with cricket and develop biscuits.

1.3 Objectives

1.3.1 General Objective

To contribute to nutrition and food security through the utilization of cassava flour enriched with cricket flour in biscuits.

1.3.2 Specific Objectives

i. To determine the effect of fermentation of cassava and enrichment with cricket on the chemical composition and protein quality of cassava-cricket biscuits.
ii. To determine the effect of fermentation of cassava and enrichment with cricket on the microbial and sensory properties of cassava-cricket biscuits.
iii. To determine the effect of fermentation of cassava and enrichment with cricket on the shelf life of cassava-cricket biscuits.

1.4 Hypotheses

i. Fermentation of cassava and enrichment with cricket has no significant effect on the chemical composition and protein quality of cassava-cricket biscuits.
ii. Fermentation of cassava and enrichment with cricket has no significant effect on the microbial and sensory properties of cassava-cricket biscuits.
iii. Fermentation of cassava and enrichment with cricket has no significant effect on the shelf life of cassava-cricket biscuits.

1.5 Justification

Production of cassava biscuits enriched with cricket flour will enhance utilization of cassava by making a convenient product that can be easily distributed. Cassava-cricket biscuits will provide an alternative snack for consumers with gluten intolerance who are unable to digest wheat-based baked products. With a new product that can be produced commercially, the industrial production of cassava biscuits will create demand for cassava flour, which was an incentive for farmers to invest in cassava farming (Onyediako & Adiele, 2022). This will not only improve the livelihoods of the farmers, but the government will also get additional revenues from the jobs created along the value chain and the ensuing economic activities. Cassava production will also reduce the pressure on cereals and by extension reduce greenhouse gas emissions since cassava has a smaller carbon footprint than cereals (Casolani et al., 2016). Enrichment of cassava biscuits with cricket flour will also provide an incentive for many farmers to adopt insect farming as a viable source of sustainable income. The protein enriched cassava biscuits will provide a cheaper alternative protein-energy rich food source to address the challenge of protein-energy malnutrition and contribute to the following Sustainable Development Goals (SDGs); 1 – no poverty, 2 – zero hunger, 9 – industry, innovation and infrastructure, and 12 – responsible consumption and production.

CHAPTER TWO
LITERATURE REVIEW

2.1 Global Demand for Food

According to estimates by the UN, 7.3 billion people constitute the current global population, which is projected to grow to 9.7 billion people by 2050. With this growth, the global food demand is expected to grow by between 59% and 98% by 2050 (Elferink & Schierhorn, 2016). With the pressure of climate change, the global food production is expected to reduce. Already, agricultural regions of Brazil and Australia are experiencing up to 23% drop in corn and cereal production.

To curb these challenges, there is need for research and adoption of newer crop varieties that can withstand these changes and increase yield to meet the demand. Highly resilient crops that can cope with the extreme weather conditions and resistant to a variety of diseases need to be adopted by the farmers. Root crops tend to withstand extreme climates much better than cereals. Root crops such as cassava are highly drought tolerant and resistant to many diseases and pests unlike cereals like wheat (Abong et al., 2016). Cassava production also has a lesser carbon footprint than wheat, which makes it a suitable candidate for climate smart agriculture. In many parts of Africa, insects have been used as sources of proteins over many centuries (Halloran, 2017). They are excellent sources of protein and have minimal impact to the environment, which makes them an excellent alternative protein source other than the usual intensively produce food animals like cattle, poultry, or swine.

2.2 Cassava as Food

Cassava is a perennial starchy root crop that can grow under conditions considered not ideal for cereal crops. It yields large quantities of high-quality starch and fibre, which makes it an ideal candidate crop with multiple applications. Given its resilience to extreme climatic conditions, cassava stands out as an ideal candidate crop to mitigate the negative effects of climate change to the food value chain. In Kenya, cassava is mostly grown in Western, Coastal and Eastern regions. These represent regions of high and low rainfall zones in the country, which demonstrates the resilience and adaptability of the crop to broad climatic zones. Total production of cassava in Kenya is shown in Table 2.1.

Cassava can be classified as being either sweet or bitter. Sweet varieties contain up to 50 mg HCN/Kg while bitter varieties contain more than 50 mg HCN/Kg cassava flour by weight (Ojiambo et al., 2017). Some of the sweet varieties grown in Western Kenya include *adhiambolera*, *palisa*, and *selele*. Drying and milling of the cassava flour, and consequent baking of the dough into biscuits sufficiently reduces the content of cyanogenic glucosides to

safe levels for consumption (Ojiambo et al., 2017). This makes fermentation and baking suitable processing methods for producing a safe ready to eat cassava-based product (proposed to be used in the current study).

Table 2.1: Cassava production data in Kenya from 2020-2023

Year	Area harvested (ha)	Yield (kg/ha)	Production (MT)
2023	76012	15626.5	1187700
2022	72247	15049.8	1087300
2021	61201	11632	711890
2020	61754	14358.9	886722

Data adapted from FAOSTAT (2024).

2.2.1 Nutritional Composition of Cassava

The nutritional profile of cassava is influenced by variety, time of harvesting, part of the plant harvested, and where the crop was grown. Generally, cassava is very highly starchy with up to 80% starch content of which 17% is amylose and 83% is amylopectin. Notably, cassava is also very low in protein with between 1-3% protein content, which necessitates its fortification with a protein source to make it a complete food (Kim & Iida, 2023). It is therefore necessary to find a suitable intervention for the low protein content of the cassava to remedy the deficient macronutrient in the final product. Previous studies (Aiking & Boer, 2018; Biró et al., 2020) have found insects to be suitable alternative protein sources for baked products. The nutritional profile of cassava is given in Table 2.2.

Table 2.2: Nutrient composition of cassava

Nutrient	Unit	Parts in 100g
Water	g	59.68
Energy	kcal	160
Protein	g	1.36
Total lipids	g	2.79
Carbohydrates	g	38.06
Total dietary fibre	g	1.8
Iron	mg	0.20
Magnesium	mg	16.19
Phosphorus	mg	27.33
Potassium	mg	265.07
Sodium	mg	10.02
Zinc	mg	1.74
Calcium	mg	32.51
Thiamin	mg	0.087
Riboflavin	mg	0.048
Niacin	mg	0.854
Vitamin B-6	mg	0.088
Folate	µg	27
Vitamin A	IU	13
Vitamin E (alpha-tocopherol)	mg	0.19
Vitamin K (phylloquinone)	µg	1.9

Source: (Kim & Iida, 2023; USDA 2018).

2.2.2 Utilization of Cassava

Cassava is a versatile crop with diverse applications globally. In Brazil, it is used for ethanol production, as food, and as filler material in the pharmaceutical industry; in Nigeria, it is consumed as gari, a popular traditional food; in Indonesia, it serves as a source of industrial-grade starch for various food industry applications; and in Ghana, it is processed into “starch, biomass, industrial flour, and feed formulations” (Poku et al., 2018). In Kenya and other Sub-Saharan African countries, cassava is predominantly cultivated on a small scale for subsistence. Despite being a staple since its introduction to the region in the 16th century, it is often perceived as a “poor people’s food” (Abong et al., 2016). Traditionally, cassava is consumed

boiled or roasted, milled into flour for *ugali*, or composited with cereals such as maize, sorghum, and finger millet for flour-based products like biscuits (Githunguri, 1995), while cassava crisps are a relatively recent innovation.

The presence of hydrogen cyanide, low protein content, and the tubers' susceptibility to rapid spoilage are major constraints to its wider utilization. Cassava naturally produces cyanogenic glucosides as a defence against pests; upon hydrolysis, these release hydrogen cyanide, which is toxic to humans. Chronic exposure can cause neurological disorders such as *konzo*, while acute poisoning can be fatal (Forkum et al., 2025; Nzwalo & Cliff, 2011). Bitter cassava varieties contain over 50 mg HCN/kg fresh weight, whereas ingestion of just over 10 mg HCN/kg body weight can cause poisoning (Ojiambo et al., 2017). A lack of consumer knowledge on identifying toxic varieties and applying detoxification methods discourages consumption.

Post-harvest handling further compounds these challenges. In Sub-Saharan Africa, particularly Kenya, rapid post-harvest physiological deterioration (PPD) limits the fresh root's shelf life to only a few days (Abong et al., 2016). Manual, labor-intensive harvesting often causes bruising, accelerating spoilage. Staggered harvesting, where roots are left in the ground until needed, is widely practiced to mitigate losses (Masamba et al., 2022). Transport over poor roads, often using basic vehicles or manual means, exacerbates damage and delays. Consequently, processing into shelf-stable products is prioritized. Drying roots into chips through traditional sun-drying or improved solar dryers extends storage to several months, with further milling into flour enabling preservation for up to a year under dry conditions (Njue & Wawire, 2021). Emerging solutions, such as varieties with delayed PPD, low-cost storage technologies (Tomlins et al., 2021), and small-scale mechanized chipping and grating equipment, hold potential to reduce losses. However, adoption remains limited by inadequate capital, technical knowledge, and infrastructure, perpetuating significant post-harvest losses.

2.2.3 Effect of Processing on Anti-nutrients in Cassava

The potent cyanogenic glucosides in cassava are its major anti-nutrients. Traditional methods of treating the cassava to reduce toxin content exist, the most widely used of which are fermentation and drying (Panghal et al., 2019). The properties of cassava flour makes it ideal for application in baking and production of extruded products. Given that baking is a high temperature processing method, it would significantly reduce the toxin content of the products made from cassava flour. In an experiment conducted by Cooke and Maduagwu (2007), dehydration process removed 10% of the bound cyanide content and 96% of the free cyanide

content, while soaking removed up to 55% of the bound cyanogens. Cooking removed 82% of the cyanogens in cassava (Ezeigbo et al., 2015).

2.3 Cassava Flour in Baking

There have been successful trials of cassava-wheat blend flours for baking of breads, cakes, and cookies. The rising demand for gluten-free baked products and the feasibility of fortification has fast-tracked the utilization of cassava flour in baking. Utilization of cassava flour in baking has been found to be a prime alternative for wheat to promote adoption of local produce and reduce wheat imports (Okoko et al., 2018). In bread baking, substituting wheat flour with cassava at 20% was found to produce bread of acceptable quality according to the Kenyan standards (Wambua et al., 2016). A blend of cassava-passion fruit flours have been used to produce cookies of acceptable consumer quality and commercialization potential (dos-Santos et al., 2017). Wahyuni et al. (2018) prepared brownies cakes of acceptable consumer quality using cassava flour even though they had a significantly lower shelf life as compared to brownies with wheat flour substitution.

2.4 Insects as Food

Insects serve as an important protein source for Asian, African, and Latin American populations. Even though social, economic and nutritional value of insects are marginalized, this seasonal resource has been exploited in more than 113 countries where over 300 ethnic groups utilize more than 1900 species of edible insects as a nutritive food source (Lacey, 2016; Mlcek et al., 2014). Despite the negative attitude towards insect as food propagated by the effect of colonization, the resource is currently gaining worldwide recognition as a cheap alternative protein source with little carbon foot print compared to the conventional protein sources (Mlcek et al., 2014).

2.4.1 The Rise of Entomophagy

Entomophagy entails the practice of consuming arthropods, especially insects, at their different growth stages by humans for food (van Huis, 2018). The definition of entomophagy surpasses the mere scope of insect consumption, but also embodies the consumption of non-insect arthropods such as spiders and scorpions, centipedes, shrimps, and lobsters (van Huis, 2018). The edible insect production, although still limited in scale, has continued to supplement livestock production as a source of food. In the accounts of Ayieko et al. (2016b), more than a thousand species of insects serve as a traditional part of the diet in diverse societies, both in Kenya and beyond. Examples of some of the edible insects include grasshoppers, caterpillars, beetles, grubs, crickets, and ants (Tao & Li, 2018). Africa and Asia exhibit the highest trends

of entomophagy. In Southeast Asia, for instance, various communities in China and Taiwan consume more than 178 species of insects for food. Still in Southeast Asia, FAO (2018) documented that entomophagy is part of traditional among Laos, with more than 95% of the community eating grasshoppers, crickets, and cricket eggs (Tang et al., 2019).

In Africa alone, various communities have been documented to feed on more than 250 species. In the Democratic Republic of Congo, Amadi and Kiin-Kabari, (2016) noted that communities draw more than 60% of their animal protein from insects. A similar case was observed in Zambia, where winged termites are the most preferred source of animal protein. Entomophagy is a significant part of the East African culture with longhorn grasshopper and winged termites established as a precious food among specific Ugandan communities and Western Kenya (Ayieko et al., 2016b). Although most edible insects are harvested from their habitats in the wild, the commercial rearing and production of such insects has recently begun and is currently emerging, supported by its nutritional value and ecological friendliness (Durst & Hanboonsong, 2015). If fully explored, entomophagy promises a sustainable food security system premised on its sound nutritional value and offers a hand in the ongoing global environmental preservation efforts.

2.4.2 Insect Production for Human Consumption

The growing demands for edible insects have created a market for exploration by commercially producing and selling such insects. Global developments in procuring edible insects in countries such as Thailand, Mexico, Peru, and Sub-Saharan Africa have opened up the space for more organized rearing and processing of edible insects for human consumption (Ayieko et al., 2016a). Peruvians, for example, preserve wildly growing prickly pear plants from which winged termites are harvested. Mexicans, on the other hand, the harvesting of aquatic hemipterans eggs, is favored by the existence of specific oviposition sites in large water bodies (van Huis, 2018). The Sub-Sahara African approach to weevil harvesting is facilitated by deliberate cutting of tropical palm trees upon which weevils lay eggs as they undertake metamorphosis. While these efforts aim at enhancing the production and multiplication of edible insects by manipulating their environments, they are merely considered as semi-cultivation efforts.

The extensive rearing of Crickets *(Acheta domesticus)*, palm weevils (*Rhynchophorus ferrugineus*), and water bugs (*Lethocerus americanus)* have been reported in Asian countries. Thailand has developed the production of edible cricket species for a period exceeding 15 years, with more than 20,000 sustaining its production both for domestic and international supply (Tao & Li, 2018). China has ventured into the rearing of water beetles. In Lao People's

Democratic Republic and Vietnam, cricket rearing takes place in small farms where farmers collect them from their natural habitats and keep them in confined farms by providing them with favorable conditions for growth and reproduction (Tao & Li, 2018). Success in Thailand's commercial cricket farming is primarily attributed to stable environmental conditions for rearing and timely, yet evidence-based dietary formulation and provision (van Huis, 2018). Tang et al. (2019) recommend sound feeding trends and favorable environmental rearing conditions for the successful growth, fecundity, survival, metamorphosis, and mating potentials of farmed edible insects. In Kenya, the Luo, Kisii, and Luhya communities living around Lake Victoria, for instance, regard black ants *(Carebara vidua)* as a suitable protein source for children and expectant women (Ayieko et al., 2016a). Besides, these communities have a particular preference for the lake fly. Despite this rich culture of entomophagy, Anese et al. (2016) report zero incidents of toxicity catastrophe traceable to insect-eating.

Harvesting of edible insects is achieved through extraction from nature with the help of hand gathering and other equipment such as hand-woven hoops and traps. This kind of harvesting, however, has been subject to extensive scholarly critique mainly regarding its sustainability (Ayieko et al., 2016b). However, tracing to the diverse nature of edible insects, together with the complexity of their habitat environments, there needs to exist opportunities that would guide improvements in harvesting criteria in pursuit of a broader sustainability framework backed with both biological and environmental knowledge (van Huis, 2018). In light of these recommendations, the need to preserve the genetic diversity of each insect colony would offer an additional advantage in the sustainability of future edible insect commercial production efforts (Ayieko et al., 2016b). There is a need for extensive investment in research towards establishing knowledge on the commercial domestication of grasshoppers, crickets, and winged termites as target insects for the Kenyan edible insect market.

2.4.3 Current Research on the Use of Insects as Human Food

Anthropo-entomophagy has received considerable scholarly attention, both in the past and currently. Food and Agriculture Organization (FAO, 2018) preempts that projected growth in the global population will double the demand for food, which will potentially exceed amounts sustainable by agricultural production. FAO (2018) also projects a double bulge in demand for animal protein, potentially unsustainable by conventional livestock production methodologies. Recent reports have also established that greenhouse emissions, as well as land and water, pollute resulting from extensive livestock production. Precisely, greenhouse gas emission has increased gradually by more than 51 percent in the fifty years ranging from 1961 to 2010, a factor that might further ramify livestock production (Jansson et al., 2019). The

aforementioned situation demands creative, yet affordable and sustainable interventional approaches that would sustain the high approaching demands of animal proteins, and food security in general.

It is in light of the denoted scenario that entomophagy has grown as a study discipline in the global efforts to develop new and more sustainable sources of food, precisely animal proteins. Jansson et al.(2019) outline that edible insects as a category of food, despite their potential contribution towards global food security issues, have remained underexplored. Both recent and current studies have proved that edible insects could contribute significantly towards global efforts to achieve food security and sustainability (Ayieko et al., 2016b). A different study by van Huis (2018) linked aspects such as the ease of accessibility of edible insects, high growth and feed conversion rates, coupled with their limited environmental implications to their sustainability as a source of food. Besides, studies have established high nutritive values from a wide variety of edible insects, mainly constituting proteins, fats, and essential minerals (Kim et al., 2019). However, even within similar groups of edible insects, van Huis et al. (2013) purport that their nutritive value would be diversified as the edible insects themselves are diversified in metamorphic stages, habitats, and diet. Insects are consumed in various forms, either whole, incorporated in other supplementary foods, or preserved and powdered.

The sustainability of edible insects as the human source has also received considerable scholarly attention. Van Huis et al. (2013) observed a significant shift in the conception of edible insects from being emergency food only consumable during famines, to stabilized staple food that is pursued for their tastes and nutritional relevance. Mopane caterpillar in the Southern regions of Africa and weaver ant eggs in Malaysia and China have been linked with high market values; hence they are considered as delicacies (Durst & Hanboonsong, 2015). The existing opportunity for large scale production is proven as both technically and financially feasible (van Huis et al., 2013). As noted by Melgar-Lalanne et al. (2019), established organizations and families are leading the way in the commercial farming, processing, and sales of edible insects, especially crickets in Thailand and other Southeast Asian countries. Apart from serving as human food, Imathiu (2020) recorded that the next couple of decades will witness a higher prevalence of insects as a feedstock for both aquaculture and poultry farming.

Kenya is not left behind in the research on entomophagy. Jaramogi Oginga Odinga University of Science and Technology (JOOUST) and Jomo Kenyatta University of Agriculture and Technology have led research on the sustainability of edible insects as human food (Ayieko et al., 2016b). A public exhibition of biscuits and crackers processed from

crickets was done by JOOUST to familiarize the Kenyan public with cricket as an edible insect (Ayieko et al., 2016a). In another study under the GREEiNSECT program, the willingness of Kenyans to pay for insect-based food was studied using bread baked from cricket powder for enhanced nutritional value. From this project, Ayieko et al. (2016a) noted a general positive preference by the Kenyan public to purchase and consume products made from edible insects. From these deductions, it is clear that a potential market for value-added cricket products could be present in Kenya.

2.4.4 Nutritive Value of Edible Insects

Table 2.3: Nutritional values for various edible insects

Insect species	*Parts/stage consumed*	*Crude Protein content (%)*	*Crude Fat content (%)*	*Energycontent(kcal/100 g fresh weight)*
Cockroach	Nymph	76.00	21.05	177
(*Blatta lateralis* and*Periplaneta Americana)*	Adult	66.00	19.85	
Honeybee	Adult	51.00	12.30	116
(Apis mellifera)	Pupa	49.00	20.10	
	Lava	42.00	18.91	
Termites	Without wings	33.00	36.80	535
(Macrotermesbellicosus)				
Silkworm	Lava	58.00	-	-
(Bombyx mori)	Pupa	-	35.00	94
Mopane Caterpillar	Lava	62.00	23.38	206
(Imbrasia truncate)				
House Cricket *(Acheta confirmata)*	Adult	62.00	21.14	122
Grasshopper	Adult	62.00	18.70	159
(Sphenariumhistrio)				

Source: Tang et al. (2019)

2.4.5 Edible Insects as a Rich Source of Proteins and Other Nutrients

The number of amino acids along with their distribution determines the value of protein in food as they also determine their molecular weights and size of charge. Most edible insects have protein contents ranging between 9-25% of their fresh weight (Amadi & Kiin-Kabari, 2016). The house cricket (*Acheta domesticus*) has a protein content ranging between 19-22% which is also incorporated with chitin. Mealworms (*Tenebrio molitor*) and beetles, (*Zophobas morio, Rhynchophorus palmarum, R. phoenicis, Callipogon barbatus*) have shown higher protein content when compared with house cricket (van Huis et al., 2013). Other studies have established that specific species of edible insects collected in forests have a crude protein content exceeding 70%.

Nonetheless, the quality of each protein source is assessed through reviewing its essential amino acid. The human body does not have the capacity to make its own essential amino acids, hence relies on dietary supply (van Huis, 2018). Examples of essential amino acids include phenylalanine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, valine, and histidine. The larva of an African palm weevil (*Rhynchophorus phoenicis*), the superworm (*Zophobas morio*), and locusts and grasshoppers: (*Locusta migratoria, Acridium melanorhodon*) have been recorded to be sufficient for the normal dietary human protein requirements (Ayieko et al., 2016b). Also, the rate at which these essentials amino acids are supplied should be sustainable and fall within the recommended range. The insects mentioned above have the capacity to supply dietary essential amino acids at rates between 10-30% which are the rates recommended by the World Health Organization.

Compared to conventional animal protein sources, edible insects have the potential to fill in nutritional deficits in dietary energy, fatty acids, and an assortment of various mineral salts. More than 50% of edible insects contain higher caloric value than most crop grains, such as soybean and maize (Aiking & Boer, 2018). The same study also established that grasshopper has 64% more caloric value compared to beef, and 70% more compared to fish. Edible insects also contain sufficient amounts of dietary proteins. Studies have linked edible insects with high protein value compared to other accessible sources of animal protein such as beef and fish. Adepoju and Omotayo (2014) also document that the protein value released by edible insects exceeds that from maize, soybean, and bean crops. Both house cricket and field cricket, for instance, have been found to supply higher dietary proteins than soybeans and beans (Jansson et al., 2019).

Entomophagy also provides other basic nutrients required by humans such as lipids and carbohydrates. Chitin, a fibrous compound which constitutes the exoskeletal structure of all arthropods is the main form of carbohydrate found in insects. Different insects have varied chitin composition with silkworm (*Bombyx mori*) having approximately 5.5% chitin while Yunae Palm Caterpillar (*Dendroli mushoui*) is recorded to have a chitin content exceeding 17.80% (Tang et al., 2019). Besides being a source of carbohydrates, chitin is also rich in dietary fiber, which constitutes more between 10-14% of dry insect. The value of these nutrients, however, varies from one insect species to another (Amadi & Kiin-Kabari, 2016). In termites (*Macrotermes sp*.), chitin is estimated to have dietary energy supply of 761kcal/ 100g, while caterpillars supply up to 457kcal/100g of energy on dry weight basis.

Edible insects are also considerable sources of dietary lipids in form of fats. Lipids are either naturally classified as polar or non-polar. Non polar lipids do not form aqueous phases in their natural forms while polar lipids form aqueous phases (Tao & Li, 2018). Cholesterol is the only lipid known not to form aqueous phases and have polar head groups. All other membrane-forming lipids such as triglyceride oils form aqueous phases even in their natural forms. Most edible insects have lipid contents ranging between 7% and 30%, mostly with highly concentrated unsaturated fats (Ayieko et al., 2016b). The entire Lepidoptera order, especially the moths and butterflies have an estimated fat content of 8.6-15.2g/100g while mealworms (*Tenebrio molitor*) record the highest fat content estimated at 6.0-12.7%.

2.4.6 Acceptability of Entomophagy

Consumer acceptance has posed a challenge to pro-entomophagy despite the high nutritive quality, non-toxicity, ease of access, and the sustainability with which it is associated. Ghosh et al. (2018) blames traditions, superstitions, and taboos, as well as personal perceptions as the primary functions influencing its acceptance at the community level. The study also registers that climatic, ecological factors and economic considerations also influence individual choices of insects as food. Long durations and financial costs that are required to harvest and process, or purchase edible insects before their consumption. Also, Rumphold and Langen (2019) in a study conducted at Wageningen academy to establish knowledge of how German consumers perceive entomophagy, it was determined that the willingness to taste was high and that consumers would be persuaded to purchase edible insect products by a piece of information. A study conducted by Alemu et al. (2015) to find out Kenyans willingness to pay for insect-based food established that Kenyans have a preference to pay for and consume products made from edible insects. It can be deduced that a potential market for value-added

cricket products could be present in Kenya. However, they observed that many Kenyans are still skeptical of consuming whole insects.

Table 2.4: Nutrient composition for various edible insects (% DM)

Insect species	Green long horned grasshopper (*Ruspoliad ifferens*)	Brown long horned grasshopper (*Ruspoliad ifferens*)	Termite/white ant (*Microtermes turbylanus*) (dewinged)	House cricket (*Acheta domesticus*)	Black ant (*Calebara vidua*)
Macronutrients					
Protein (g)	43	44	39	59	40
Fat (g)	48	46	44	25	47
*SFA (%)	38.3	39.1	35.1	52.6	
*MUFA (%)	26.6	26.3	52.8	40.5	
*PUFA (%)	34.4	33.8	12.2	6.8	
Crude fibre (g)	3.9	4.9		8.0	6.9
Ash (g)	2.8	2.6	7.6	4.0	1.6
Micronutrients					
Calcium (mg)	27.4	24.5	58.7	5.0	22.2
Magnesium (mg)	33.9	33.1		10.0	10.4
Iron (mg)	16.6	13.0	53.3	6.3	10.6
Zinc(mg)	17.5	12.4	8.1	15.0	5.6

Source: Münke-Svendsen et al. (2017).

Key: SFA= Saturated fatty acids; MUFA= Mono-unsaturated fatty acids; PUFA=Poly-unsaturated fatty acids

2.4.7 Role of Crickets in Human Nutrition

After successful trials of incorporating cricket flour in biscuits in a project at JOOUST, many farmers in Kenya have shown interest in commercial production of insects for human consumption (Münke-Svendsen et al., 2017). Insects have shown superior nutritional properties that have drawn the attention of scientists to their utilization in human food. The nutritional information of some of the most popular insects currently utilized for food in Kenya are profiled in Table 2.3.

Consumption of insects as an alternative source of proteins has been gaining traction globally. Several start-ups specializing in protein supplements made with cricket flour have

gained rapid popularity in the recent past. Exo^{TM}, Crowbar Protein, Entomo Farms, Protix B.V., Don Bugito, Bitty Foods, and Cricket Flours are some of these start-ups. Restaurant chains are now selling specialty cricket protein enriched cookies and delicacies (Lacey, 2016).

In Kenya, studies by Ayieko et al. (2010) document the various ways in which insects are prepared and consumed. The most common preparation methods include roasting, drying in the sun, and grinding into flour and mixing into other foods. The researchers also document products including sausages, loaves and even muffins that have been made with cricket flours. The increasing interest in insects as a source of protein is partly attributed to the declining consumption of meat due to health reasons and also due to the preference for a cheaper protein source of comparable quality. Trials are also being done to produce edible insect protein concentrates at JKUAT (Ndiritu, 2017).

Of the many species of crickets that exist worldwide, several of them have been subjected to nutritional analysis to ascertain their suitability for use in human nutrition. Some of the species that have been studied include the field cricket (*Gryllus*genus) and the house cricket (*Acheta* genus). Thailand's field cricket *Gryllusbimaculatus* (raw) was found to contain120 kcal/100g protein (in comparison, skinless chicken breast contains 150 kcal/100g protein). According to Lacey (2016), nutrient profile varies by type of cricket, with field cricket (58% protein and 10% fat) and house cricket (65% protein and 20% fat). Earlier studies had shown that the quality of house cricket protein is superior to soy protein (Mlcek et al., 2014). House crickets have sufficient amounts of vitamin B_{12} at 5.4 µg per 100 g in adults and 8.7 µg 100 g^{-1} in nymphs. This quantity exceeds the recommended daily intake at 2.4 µg (Lacey, 2016). This vitamin plays a key role in energy metabolism and helps prevent osteoporosis. Cricket flour also contains as high as 40% unsaturated fatty acids and non-trans fatty acids.

***Figure 2.1**: House cricket intensive production system (Photo by author)*

2.5 Carbon Footprint of Selected Foods

The carbon footprint (CF) of a product is defined as "the total greenhouse gas (GHG) emissions associated with the entire life cycle of that particular product and calculated from cradle to grave" (Zubelzu et al., 2015). Virtually every agricultural activity and product has an associated carbon footprint, with some outstripping others many times over. Reports indicate that producing one tonne of wheat emits over 200 kilograms of carbon (IV) oxide. This translates into over 2462 kg CO_2 Ha^{-1}, with fertilizers being the biggest contributors (Casolani et al., 2016).

Cassava is currently utilized on industrial scale to produce starch, which is a more refined product than whole wheat flour under a rigorous process. Despite the intensity of processing and multiple processes involved, processing one tonne of cassava into the final product only produces between 90 and 150 kilograms of carbon (IV) oxide. The biggest contributor being the effluent treatment process (Tran et al., 2015).

Production of animals as a source of protein in the human diet currently utilizes 70% of the world's agricultural land. Animals are responsible for 12% of the total greenhouse gas emissions (Sachs et al., 2010). Intensive livestock production calls for heavy use of antibiotics, fertilizers and other agro-chemicals which end up in waterways leading to increased pollution of the water sources. In addition, beef production puts more stress on the existing water resources since about 45,000 litres of water is required to produce one kilogram of beef (Lacey,

2016). Despite the limited data available on water use in cricket production, the figure is presumably lower than that of livestock production due to crickets' high feed conversion ratio tolerance to drought and short life cycle. Producing 1 kg of cricket protein requires 1.7 kg of feed as compared to 2.5, 5, and 10 kilograms required for chicken, pork and beef respectively (van Huis, 2013). In addition to this, up to 80% of the cricket body mass is edible compared to 55% for poultry and 40% for beef cattle (van Huis, 2013). Crickets can feed on organic waste and they do not produce methane hence are less detrimental to the environment as compared to livestock production. They require lesser space and can be produced indoors contrary to livestock production that requires clearance of large tracts of land for pastures.

2.6 Safety and Legal Issues on the Use of Crickets as Food

Insects have not been universally accepted into the food chain. This means that there exists little to no proper legislative framework to control its use as food. With limiting legislations, promoting the consumption of crickets as an alternative source of protein is an uphill task, especially in closely regulated markets as noted by Jansson, Hunter and Berggren (2019). Preparing crickets for human consumption through heating, boiling and frying has been found to reduce the risk of bacterial infection by either *Lysteria monocytogenes* or *Salmonella spp* (van Huis et al., 2013). Production of crickets as a monoculture, however, predisposes the insects to viral infections, for example, in Netherlands, a cricket farming company lost 50% of its crickets to a virus (Lacey, 2016). Integrating crickets with other insects has been suggested as a remedy to shield crickets from such vulnerabilities.

2.7 Review of Methods

Protein analysis often employs the Kjeldahl method, which determines total nitrogen content and converts it to protein using a factor (typically 6.25). This old method remains an AOAC/ISO reference due to its reliability across diverse matrices (Langyan et al., 2022). Recent innovations, such as micro-Kjeldahl protocols and automated digestion systems, have improved throughput and reduced sample preparation time (Langyan et al., 2022). However, Kjeldahl quantifies total nitrogen rather than true protein, leading to overestimation in the presence of non-protein nitrogen compounds. Alternatives like the Dumas combustion method offer faster analysis without hazardous chemicals, though they share the same limitation (Diego et al., 2022).

Protein quality evaluation combines digestibility data with amino acid composition to compute indices such as the Digestible Indispensable Amino Acid Score (DIAAS), which is preferred over the older PDCAAS for its amino acid–specific digestibility estimates (Guillermo

et al., 2024). In vivo assays remain the benchmark but are costly and time-intensive; thus, standardized in vitro protocols such as INFOGEST have been developed to simulate gastric and intestinal digestion phases. Post-digestion, protein hydrolysis and amino acid profiling (typically by acid hydrolysis followed by HPLC or GC) quantify individual amino acids, though certain amino acids are partially destroyed or modified during processing (Diego et al., 2022; Guillermo et al., 2024).

Fat analysis frequently involves determining fatty acid methyl esters (FAMEs) by gas chromatography (GC). Lipids are first extracted (traditionally by Folch or Bligh-Dyer methods) and then transesterified to FAMEs for GC–FID or GC–MS analysis, enabling precise quantification of saturated, unsaturated, and trans fatty acids (Hewavitharana et al., 2020). Recent advances include one-pot extraction–derivatization protocols and micro-scale methods that reduce solvent use.

Retinol (vitamin A) and tocopherols (vitamin E) are typically quantified via reversed-phase HPLC after saponification and extraction of the unsaponifiable fraction. Detection employs UV absorbance for retinol and fluorescence or UV for tocopherols (Słowik-Borowiec et al., 2023). Modern enhancements include simultaneous detection of multiple vitamins in a single HPLC run and advanced techniques such as LC–MS/MS or supercritical fluid chromatography for improved sensitivity (Liu et al., 2025). Despite these developments, HPLC remains the gold standard for fat-soluble vitamin analysis due to its specificity and reproducibility.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Research Sites

The product development and analytical was done at the Dairy and Food Science and Technology department at Egerton University. Amino acid and fatty acid profiling were done at BeCA-ILRI, Nairobi. Kisumu county was the site of interest for the Kenya Climate Smart Agriculture Project (KCSAP).

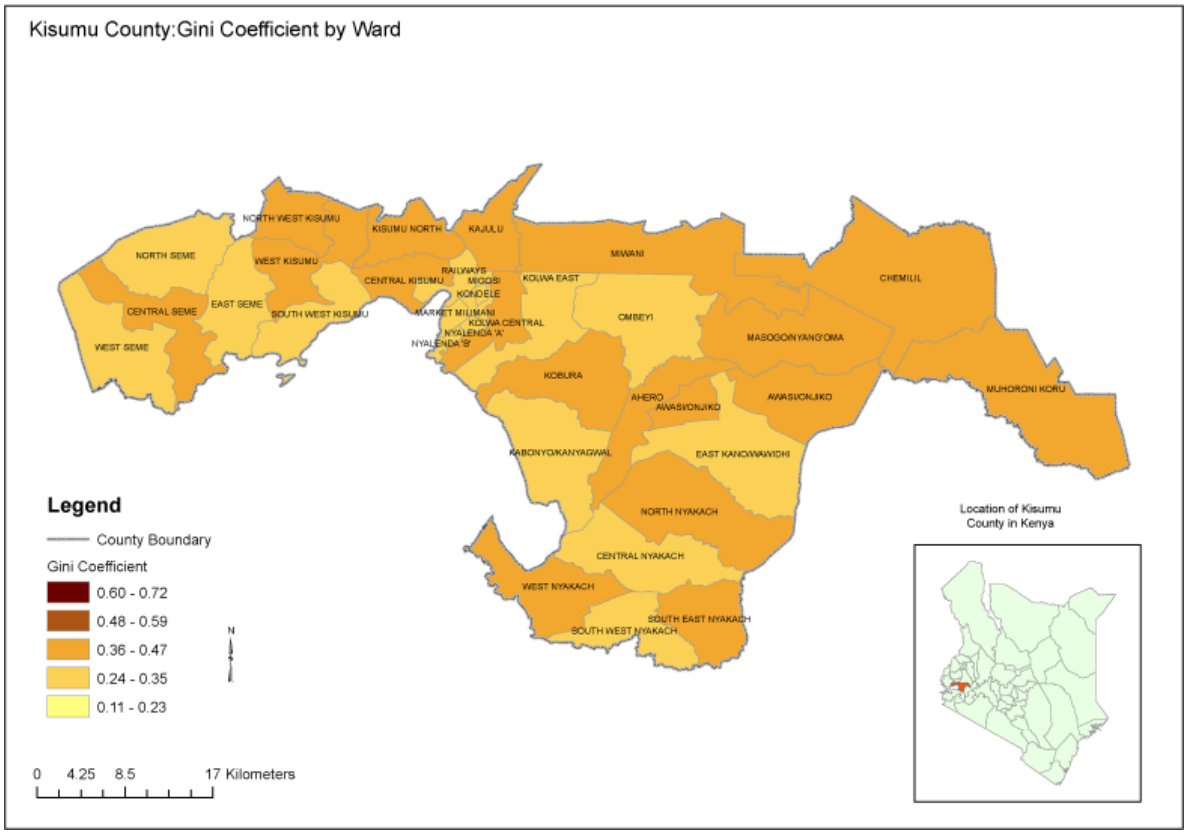


***Figure 3.1**: Map of Kisumu County*

Source: Independent Electoral and Boundaries Commission (IEBC), Kenya (as cited in (County Government of Kisumu, 2023)*)*

3.2 Materials

Sweet cassava tubers (*Selele* variety) were obtained from a local farmer's farm in Kolwa central, Kisumu, Kenya while the cricket flour was obtained from Mixa Farm in Kisumu, Kenya.

3.3 Experimental Design

This experiment was conducted using a 3 × 4 completely randomized design (CRD) in a factorial arrangement as shown in layout in Appendix I. The cassava flour was the main factor, divided into spontaneously fermented, controlled fermentation and unfermented

portions. These three portions of cassava flour were then mixed with cricket flour at four substitution levels (100:0, 88:12, 76:24, 6436) to find the best composite that will provide at least 60 percent of the daily protein requirements for children aged between 5 and 13 years to comply with the WHO recommendations (Table 3.1). Baking was done at 180°C for seven minutes. The control was 100% cassava flour (unfermented flour) without any cricket flour added.

Table 3.1: Recommended dietary allowances for carbohydrates and proteins

Age of Children (years)	Carbohydrate (grams/day)	Protein (grams/day)
4 – 8	130	19
9 - 13	130	34

Source: Institute of Medicine (2011)

The statistical model for the experimental design was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijkl} \quad \text{.....equation 3.1}$$

Where;

Y_{ijkl} = the observation on the response variable;

μ = the overall mean of the respective response variable (the measured chemical indicators of food quality);

α_i= the effect due to the i^{th} cassava fermentation;

β_j = the effect due to the j^{th} cricket flour substitution level;

$\alpha\beta_{ij}$ = the interaction effect between the i^{th} cassava fermentation and j^{th} cricket substitution level;

ε_{ijkl} = the random error associated with Y_{ijkl}.

3.4 Preparation of Cassava Flour

Processing of cassava flour was done by following the procedure by Iwe and associates with a little modification on the baking temperature (Iwe et al., 2017). The fresh tubers were weighed, peeled and washed with portable water. They were then chopped into chunks and solar dried until they attained 11% constant moisture content. The dried chunks were then milled in a hammer mill and sieved through 0.5 mm pore sized screen to produce the desired quality of cassava flour for baking.

The fermented flours were processed by first peeling the tubers, chopping them into small pieces and then putting the chopped pieces into a fermentation chamber where solid state fermentation proceeded for 72 hours. One batch underwent controlled fermentation where a pure *Aspergillus oryzae* culture was introduced. The other batch underwent spontaneous

fermentation where a mother culture had been prepared earlier by putting one kilogram of freshly peeled and chopped cassava into an airtight container and incubating it at 37°C for 48 hours. The mother culture was then introduced into the batch of freshly peeled and chopped cassava, mixed and incubated in an air tight container and 37°C for 72 hours. After fermentation, the fermented cassava pieces were washed in salt water (5%) to eliminate slime (Wahyuni et al., 2018). The cleaned cassava pieces were then sun dried to a constant moisture content of about 13%, then hammer milled and sieved through a 0.5 mm pore sized screen to produce the cassava flour for baking. The resultant flour was stored in airtight containers for analysis and further processing.

3.5 Preparation of Cricket Flour

Cricket flour was obtained from Mixa Farm in Kisumu. Mature crickets (3 months old) were harvested after being starved for 24 hours, being provided with water only to clean their guts. They were parboiled at 80°C for 40 minutes after which they were solar dried to a constant dry weight with moisture content of about 4%. The dried crickets were then milled into flour using a coffee grinder (Redberry RB134) at the farm. The cricket flour was kept in an air tight container for further processing.

3.6 Preparation of Biscuits

The three cassava flour batches flour were each blended with milled cricket flour for protein content enrichment at four substitution levels (100:0, 88:12, 76:24, 64:36). Pre-trials were done to fix the ratios. The cassava-cricket blended baking flour was used to prepare the biscuit dough according the process used by Olatunde and associates with modification on the baking temperature (Olatunde et al., 2016). Other biscuit making ingredients was added as described by Ade-Omowaye et al. (2008). The dough and the ingredients was mixed in a bowl with a hand mixer for 5-10 minutes until a uniform consistency is obtained. The mixed dough was then proofed for 30 minutes before baking. It was then spread on a flat surface at an average thickness of 0.5 centimetres. A biscuit cutter of 2-inch radius was used to cut out the pieces, which were transferred to the ungreased baking sheets and then baked in the pre-heated oven at 180°C for seven minutes. After baking, the biscuits were cooled and then weighed before storing in airtight containers for further analysis. The process is illustrated in the flow chart shown in Appendix IX.

3.7 Effect of Cassava Fermentation and Cricket Enrichment on the Proximate Composition and Protein Quality of Cassava-cricket Biscuits

3.7.1 Determination of Proximate Composition

i. Moisture content

Moisture content was determined according to the forced draft oven procedure (AOAC 2005, 9540:606). Two grams (2g) of the biscuit sample was weighed using analytical balance (Shimadzu AUW 220.max 220g), transferred to in aluminium dishes and dried at 105°C in the forced draft oven (Memmert GmbH Co KG, type UM400) for 3h, then cooled in a desiccator and reweighed after cooling down. Moisture content was determined according to equation 3.2;

$$\%\ \text{Moisture}\ \left(\frac{\text{wt}}{\text{wt}}.\right) = \frac{\text{Wt.of } H_2O \text{ in Sample}}{\text{Wt of wet sample}} \text{x}100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{equation 3.2}$$

ii. Ash

Ash content of the cassava-cricket biscuit samples was determined as per the protocol described in (AACC 2010, 08-01). In the method, 5g of the dry cassava-cricket biscuit sample was weighed in clean dry crucibles on an analytical balance (Shimadzu AUW 220 max 220g). The weighed sample was then charred in a fume hood using a bunsen burner before further incineration in a muffle furnace (Bamford, Sheffield England S30 2AU) for 5 hours at 550°C. The muffle furnace was turned off and allowed to cool overnight and opened the following morning when the temperature had dropped below 250°C. Crucibles were quickly transferred to a desiccator, and allowed to cool further to a constant dry weight before weighing. Proportion of the ash was calculated according to equation 3.3;

$$\%\ \text{Ash} = \frac{\text{Wt.after ashing} - \text{tare Wt.of crucible}}{\text{Wt.of dry sample} - \text{tare crucible wt}}\ \text{x}100 \ldots\ldots\ldots\ldots\ldots\ldots\text{equation 3.3}$$

iii. Crude protein

The crude protein content of the cassava-cricket biscuit samples was determined according to the improved Kjedahl protocol (AACC 2010, 46– 11), where about 0.2g of the sample was transferred into a digestion tube followed by 8g catalyst (96%$NaSO_4$ + 3.5% $CuSO_4$) and 10 ml concentrated H_2SO_4. Contents of the tube were digested at 350°C for 3h.45min until all fumes cleared and a clear light blue solution formed. The digest was allowed to cool for 1hr thereafter. A blank with all reagents except the sample was also prepared in a similar manner. 50ml of 2% boric acid was added into a conical flask followed by 1ml of methyl red-bromocresol green indicator. The digest was distilled with 50% NaOH solution (75ml), with the below boric acid capturing the liberated fumes. The distillate was titrated against 0.1N H_2SO_4.

Calculation of % crude protein in the sample.

0.1N H_2SO_4. = 0.0014gm Nitrogen

Moles of HCI = moles NH_3= moles N in the sample

$$\%N = NH_4Cl \times \text{Corrected acid} \frac{\text{volume}}{\text{gm}} \text{of sample} \times \frac{14gN}{\text{mole}} \times 100 \text{……....equation 3.4}$$

N = normality of HCI, in moles/l000 ml, corrected acid vol. = (ml std. acid for sample) - (ml std. acid for blank), 14 = atomic weight of nitrogen. Crude protein content was determined according to equation 3.5;

% Protein = % N × 6.25 (conversion factor) …….equation 3.5

iv. Crude fat

The protocol described in (AACC 2010, 30 – 10) was used to determine the crude fat content of the cassava-cricket biscuit samples. About 2g of the sample was added to thimbles in triplicates and placed in clean dry siphon tubes fitted to clean dried pre-weighed round bottom flask containing 60ml petroleum spirit (B.P 40-60°C) and 60ml diethyl. The setup was then fitted to a condenser and heated in a mantle and refluxed for 5h. The mantle was then switched off and the round bottom flasks transferred to a water bath to evaporate the solvent. The flasks were then dried in the oven for 1hr, followed by dessicator cooling and weighing. Spent thimbles were oven-dried at 100°C for 30min. Proportion for the fat content was determined according to equation 3.6;

$$\% fat = \frac{Wt.of\ extracted\ fat}{Wt.of\ dried\ sample} \times 100 \text{ ……equation 3.6}$$

v. Carbohydrate content determination

Carbohydrate content was determined according to equation 3.7;

$$100 - (\% moisture + \% protein + \% fat + \% ash) \text{…….equation 3.7}$$

3.7.2 Protein Quality Analysis

The protein quality of a food product defines its suitability to be used as an adequate source of dietary protein. The biscuits were subjected to protein quality analysis to assess the amino acid profile and to determine the *in-vitro* protein digestibility as outlined below.

i. Determination of in-vitro protein digestibility (IVPD)

To determine the digestibility of the proteins, the samples were subjected to pepsin digestion then followed by trypsin digestion in a procedure described by Hamaker et al. (1987). There was a modification on the method of determination for the residual protein content; Kjeldahl method was used instead of Dumas combustion method. A sample of 200 mg was digested with an enzyme of P7000-25G pepsin; activity ≥250 units/mg solid for 2 hours at 37°C. The procedure was repeated with trypsin enzyme (Pankreasprotease; activity min 200

FIPU/g, Merck Darmstadt, Germany) before being subjected to Kjeldahl digestion process. The digested products were pipetted off and the undigested part washed with distilled water. The remaining residue was dried for 6h in an oven at 100°C. After drying, the protein content of the residue was determined using Kjeldahl procedure (AOAC 2005, 955.04). The difference between the initial protein content and the protein content was expressed as a percentage of the initial protein content to give the protein digestibility of the samples.

ii. Amino acids profiling

Amino acids were profiled according to the protocol described in (AOAC, 2005: 994.12). Cricket sample was accurately weighed at about 0.2g put into test tubes containing 12 mL of 6M HCl, and then tubes flushed with nitrogen and capped immediately. These tubes were transferred into an oven to hydrolyse for about 24 hours. After 24 hours, samples retrieved and cooled to room temperature, followed by gentle drying to remove HCl. Samples were then re-reconstituted in 3mL of 0.02M HCl and respectively filtered carefully through 0.22μm filter paper to remove any contaminant before derivatization. A two-stage RP-HPLC-FLD procedure involving derivatization and identification and then quantification of the eluting fluorescent amino acid derivatives was used. This was followed by performic acid oxidation of cystine and methionine to cysteic acid and methionine sulfone. The performic acid was then decomposed by adding sodium metabisulfite. Hydrolysis was conducted with 6M HCl to liberate amino acids from the protein. Sodium citrate buffer was then added to dilute the hydrolysates and the pH adjusted to 2.2 before separating the individual amino acids through ion-exchange chromatography. Since hydrolysis destroys tryptophan and oxidation destroys tyrosine, it was not possible to determine these two. Agilent1100 series (USA) gradient HPLC system with autoinject or, column compartment, fluorescent detector (G1315B), vacuum degasser, and quaternary pump was used. Separation was performed on Eclipse XDBC18 Column (ID2.1 ×150mm, 5μ particle size) at 40°C. Fluorescent detector with excitation wave was used for peak monitoring. Amino acid solutions at five concentration levels (1.25 to 4.75 μM/mL) was used to determine the calibration curve. The amino acids were quantified by comparing their peak areas to the peak areas of standard amino acid solutions.

3.7.3 Determination of Fatty Acids Methyl Esters Composition

To determine the fatty acids, the protocol described by Szpyrka et al. (2022) was followed. Biscuit samples were prepared crude fat extracted using Soxhlet Method as described in Section 3.7.1. After extraction, the fat was saponified whereby 1 ml of test solutions was pipetted into a 15 ml screw cap centrifuge tube, followed by adding 3 ml of 0.5 N NaOH in

methanol, recapped the tube and shaken. The tube was place in a water bath at 85-100°C for about 8 minutes and then cooled. Cooled tubes were taken to the fume hood, 3ml of boron trifluoride (BF3) was then added, shaken and heated at 85-100°C for 10-15 minutes and later allowed to cool. Then 3 mL of hexane and 3 mL saturated NaCl solution were added, shaken vigorously then centrifuged at 559 G-force for 5 minutes. The tubes were left to stand until the hexane (upper) layer separates from the aqueous (lower) phase. Pasteur pipette was used to skim the upper layer and transfer it into a test tube through a cotton wool filter liner containing small amount of anhydrous Na_2SO_4. The aqueous phase was re-extracted twice more with 2 mL hexane passing it through the anhydrous layer as in the previous step. A small amount of anhydrous Na_2SO4 was then added. The solvent was evaporated off in by rotary evaporator under nitrogen stream. The FAMEs were then dissolved with hexane and diluted to an appropriate volume in a volumetric flask. 1ml of the solution was sampled and transferred into a GC sample vial with a screw cap. The GC used was Agilent GC with VF5-MS (5% Phenyl methylpolysiloxane), 30 m x 0.25 mm, 0.25 µm film, Manual- Syringe (10 µl), Injection port temperature of 280°C, carrier gas flow rate of 1 ml/min and sample injection volume 1 µl. Calculation of the individual fatty acid methyl ester in Kovats Linear retention Indices was done as per equation 3.8;

$$I = 100\ X\ (n + \frac{(t_{r(unknown)} - t_{r(n)}}{t_{r(N)} - t_{r(n)}})$$ ……..……...equation 3.8

Where;

I= Kovats retention index

n= the number of carbon atoms in smaller n-alkane.

N= the number of carbon atoms in the larger n alkane.

t_r= Retention time

The fatty acid methyl esters (FAMES) in the sample were then identified by comparing each of the obtained MS readings with the NIST Library. Also, the calculated Kovats linear retention Indices were compared against one obtained in a data base for the same capillary column stationary phase (http://www.pherobase.com/) using the formulae:

$$\%\ \text{Area of each fatty acids} = \frac{A_x\ \text{X}\ 100}{A_T}$$ ……..……..equation 3.9

Where;

Ax = area of each fatty acid methyl ester

AT = total area of all fatty acid methyl ester

100 = Conversion factor to report results in percentage

3.7.4 Determination of Retinol and Tocopherols

Retinols and tocopherols were determined according to Bhatnagar-Panwar et al. (2015) protocol. Where 0.5 g of the sample was weighed in triplicate and then put in a 25mL tube where 6 ml ethanol with 0.1% (BHT) was introduced and the mixture homogenized for 1 minute. 120 µL of potassium hydroxide 80% (w/v) was added in the homogenized solution and vortexed to mix. This was followed by incubate for 5 minutes at 85°C in a water bath. The tubes were retrieved from the water bath and immediately placed cooled in ice, after which 4 mL of deionized water was added to each tube and mixed in vortex. This was the followed with addition of 5 mL hexane with mixing in vortex. The samples were then centrifuged at 805 G-force for 5 minutes. The upper phase (hexane) was transferred to centrifuge tube by the use of Pasteur pipette. Extraction was also done 3 more times with 4×3×3 ml hexane and pooling the extract in to the 25 ml tube. 5ml deionized water was then added into the extract, vortexed for 1 minute in a centrifuge for 5 minutes at 805 G-force. Hexane was then recovered into a clean test-tube and then evaporated to complete dryness under nitrogen in the N-Evap. Reconstitution into 1 ml of methanol: tetrahydrofuran (85:15 v/v) vortexed and sonicated for 30 seconds, and then transferred 0.8 ml to HPLC vials. Reverse phase gradient HPLC system (Shimadzu Nexera UPLC system linked to SPD -M2A detector) w, Oven temperature-OFF, YMC C30, carotenoid column (3µm, 150X3.0 mm, YMC Wilmington, NC) and 10 µl Injection volume was used. Methanol/tert-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water) (mobile phase A) with methanol/tert-butyl methyl ether/water (8:90:2, v/v/v, with 1 % ammonium acetate in the water) (mobile phase B) with a total flow-rate 0.4 ml/min.

3.8 Effect of Cassava Fermentation and Cricket Enrichment on the Sensory Properties of Cassava-cricket Biscuits

3.8.1 Microbial Quality of Cassava-cricket Biscuits

Same day microbiological assessment of all the samples was done. The essence of the assay was to ensure product safety during handling and consumption. Total Viable Count (TVC), yeasts and moulds, and coliforms of aqueous sample extracts were assayed using the Food and Agriculture Organization (Andrews, 1992) protocols. Ten grams of each biscuit sample was homogenized in 90 mL of buffered peptone water and serially diluted. Total viable counts (TVC) were obtained by inoculating the dilutions of each sample onto plate count agar (PCA) then incubated at 37°C for 48 hours. Total coliform counts (TCC) were obtained by inoculating the dilutions onto violet red bile (VRB) agar and incubated at 37°C for 24 hours.

Yeasts and moulds were counted by inoculating the dilutions onto potato dextrose agar (PDA) and incubating at 25°C for 5 days. Microbial colonies were counted and expressed as colony-forming units per gram of biscuit (cfu/g).

3.8.2 Sensory Analysis

i. Descriptive sensory analysis

Descriptive sensory analysis was carried out by panellists who were trained for calibration purposes. Twelve panellists recruited from Egerton University DAFTEC Postgraduate club were screened through three training sessions of one hour each to be able to identify fundamental tastes. Descriptive attributes were parametrically evaluated on a 7-point scale for appearance, flavour and texture. Parameters such as glossiness, stickiness, springiness, colour, mouth feel, consistency of the cassava-cricket biscuits were evaluated by use of sensory descriptors and evaluation guidelines in the lexicon developed by the sensory panel according to the guidelines proposed by (Biró et al., 2020). The sensory analysis questionnaire is attached on Appendix III.

ii. Consumer acceptability test

Overall acceptability was carried out according to Muoki et al., (2015) using seven-point hedonic scale by engaging 64 panellists on acceptability test of the biscuits. Overall acceptability was scored by panellists; which were rated from 1 (dislike extremely) to 5 (like extremely) using a line scale and the biscuit samples were coded.

3.9 Effect of Cassava Fermentation and Cricket Enrichment on the Shelf-life of Cassava-cricket Biscuits

3.9.1 Determination of Peroxide Value (PV)

Acetic Acid-Chloroform method (AACC 2010, 58-16.01) protocol was used to determine the PV of the samples. About 5g of the cassava-cricket biscuit sample was weighed and added into 250-ml Erlenmeyer flask with a glass stopper, followed by 30ml acetic acid-chloroform solution. The Erlenmeyer flask was swirled to dissolve the sample, after which 0.5ml saturated KI was added. The solution was left standing with occasional 1-minute shaking intervals. 30ml distilled water added to the mix and the content was then titrated with 0.1N $Na_2S_2O_3$, with constant and vigorous shaking until yellow colour faded faintly. At this point, 0.5ml starch indicator solution was added and the titration continued under vigorous flask shaking towards endpoint. Total liberation of iodine from the chloroform layer was confirmed by the disappearance of the blue colour. Every day, a blank was prepared and titrated with 0.1ml 0.1N $Na_2S_2O_3$ solution. The PV was then calculated using equation 3.10;

$PV = \frac{Titre \times N \times 100}{W}$……..equation 3.10

Where PV= Peroxide Value meq O_2 /kg; Titre = (titration of sample - titration of blank), N = normality of $Na_2S_2O_3$, solution, and W= Weight of the sample

3.9.2 Accelerated Shelf-life Testing

Low-density polyethylene (LDP) bags were used to store the cassava-cricket biscuit samples at three different temperatures of 20°C, 30°C and 40°C. For each of these of these temperatures, biscuit samples were analyzed at 1, 3, 7, 14, 21 to 28 days for peroxide values. The first step was to plot concentration of PV against storage time for each of the three storage temperature levels to determine the kinetics order of reaction. Regression analysis was then used to interpret the results. The second step was to establish a regression plot of semi-logarithmic scale for the rate constants for each storage temperature (Ln *k*) against the inverse of absolute temperature ($\frac{1}{T}$) to get an Arrhenius equation based on equation 3.11;

$Ln\ k = Lnk_o - \frac{E_a}{R}\left(\frac{1}{T}\right)$……..equation 3.11

Where *k* = reaction rate constant; *R* = molar gas constant (8.314 J/K/mol), *T* = absolute temperature (K); E_a = apparent activation energy (J/mol), and k_o = pre-exponential factor.

Last step was to use the Arrhenius parameters from the plots to estimate the shelf life based on lipid oxidation according to equation 3.12 as described by Anese et al. (2012):

$Shelf\ life = \frac{[A_{lim}]-[A_0]}{K_T}$……equation 3.12

Where $[A_0]$ is the initial concentration of PV or aldehydes at time zero; K_T is zero-order rate constant at the selected storage temperature *T* in this case it was 20°C, 30°C and 40°C; $[A_{lim}]$ is the standard acceptable limit PV of 10 (meq O_2/kg) (Manzocco et al., 2020) was used to estimate shelf life of each storage temperature.

3.9.3 Estimation of Thermodynamic Properties

The food matrix under study necessitated the determination of thermodynamic properties, which were derived from absolute reaction rate theory using the mathematical expressions based on Erying's equation 3.13 (Taoukis et al., 1997):

$k = \frac{K_B T}{h} \times e^{\frac{\Delta S*}{R}} \times e^{\frac{-\Delta H*}{RT}}$……equation 3.13

$\Delta H^* = E_a - RT$

$\Delta G^* = \Delta H - T\Delta S$

Where K_B = Boltzmann's constant, 1.38×10^{-23} J/°K; h = Planck's constant, 6.63×10^{-34}J.S; ΔS^* = entropy of activation, J/mol °K; ΔH^* = enthalpy of activation, J/mol and ΔG^* = Gibbs free energy of activation, kJ/mol.

3.10 Data Analysis

Data from proximate, microbial, amino acids, sensory and microbial analyses are checked for normality before analysis using SAS® software version 9.4 at 95% confidence level. Analysis of variance (ANOVA) was done to test the study hypotheses and post-hoc analysis was by Tukey's HSD method. Correlation and Principal Component Analysis were also done on sensory data. The results have been presented as means± standard error in form of tables and graphs.

CHAPTER FOUR

RESULTS

4.1 Effect of Cassava Fermentation and Cricket Enrichment on Chemical Properties of Cassava-cricket Biscuits

4.1.1 Proximate Composition

As shown in Appendix IV, analysis of variance (ANOVA) determined that fermentation significantly only affected dry matter, protein and ash content while level of substitution with cricket flour had a significant effect on all proximate components in biscuits. Fermentation did not have a significant effect on the fat, fibre and carbohydrate content of the biscuits. The interaction between the fermentation process and the cricket substitution levels in biscuit showed a significant effect on all proximate components. As depicted in Table 4.1, biscuits made from *Aspergillus oryzae* fermented cassava flour had significantly higher protein and ash content, and comparatively lower moisture and carbohydrate content. Unfermented biscuits had the least protein content and highest fibre content in the array ($p<0.05$). Biscuits made from spontaneously fermented cassava flour had a significantly higher protein content when compared to the unfermented biscuits but lower than those made from *A. oryzae* fermented flour at $p<0.05$.

It was observed that as the cricket substitution level increased in the biscuit, there was a significant increase in dry matter, protein and ash as well as a significant decrease in fibre and carbohydrates. The biscuit sample with 36% cricket flour substitution had the highest moisture content while the sample with 24% cricket flour had the least moisture content of all the biscuit samples. The trend observed for the moisture content of the biscuits is an initial drop up with every additional substitute cricket flour added until 24% then it picks up an upward trend with more addition of the cricket flour into the mix.

A general trend was observed that irrespective of the fermentation method, biscuits began with a relatively higher moisture content at 0% cricket and dropped until 24% cricket, after which the moisture content began to rise. All the biscuits generally had moisture content of less than 5%, in all instances, biscuits with 24:76 cricket:cassava flour had the least moisture content for all the flour types while 36:64 cricket: cassava flour had the highest moisture content across the board. Irrespective of the fermentation method, protein and ash content in biscuits signficantly increased with substitution level but carbohydrates and fibre significantly decreased. Crude fat in biscuits showed a significant increase up to 24% subtitution level and decreased significantly at 36%.

Table 4.1: The interaction effect between the fermentation process and the substitution level of the cassava with crickets on proximate composition

Fermentation	% Cricket	Moisture	Protein	Ash	Fat	Fiber	CHO
Unfermented	0	$2.83^{bc}\pm0.21$	$3.97^{g}\pm0.13$	$3.16^{de}\pm0.15$	$37.76^{c}\pm0.60$	$23.70^{a}\pm0.83$	$28.58^{a}\pm0.50$
	12	$2.62^{c}\pm0.26$	$11.45^{f}\pm0.14$	$3.45^{d}\pm0.11$	$40.62^{b}\pm0.35$	$17.33^{c}\pm0.69$	$24.52^{b}\pm0.80$
	24	$0.92^{f}\pm0.05$	$24.47^{d}\pm0.51$	$3.89^{bc}\pm0.13$	$42.77^{c}\pm0.15$	$14.86^{d}\pm0.08$	$13.09^{d}\pm0.62$
	36	$3.57^{a}\pm0.22$	$34.53^{b}\pm0.60$	$4.53^{a}\pm0.17$	$39.66^{bc}\pm0.40$	$11.23^{e}\pm1.05$	$6.48^{e}\pm0.20$
Controlled	0	$2.38^{cd}\pm0.12$	$3.37^{g}\pm0.16$	$3.57^{cd}\pm0.12$	$40.60^{b}\pm0.43$	$22.63^{ab}\pm1.36$	$27.46^{a}\pm1.18$
	12	$2.09^{d}\pm0.08$	$14.83^{e}\pm0.21$	$4.14^{b}\pm0.10$	$40.06^{bc}\pm0.17$	$19.85^{b}\pm1.15$	$19.04^{c}\pm0.75$
	24	$1.14^{e}\pm0.07$	$25.13^{c}\pm0.58$	$4.11^{b}\pm0.10$	$39.08^{c}\pm1.02$	$16.77^{cd}\pm0.63$	$13.78^{d}\pm1.06$
	36	$2.96^{bc}\pm0.21$	$39.47^{a}\pm0.11$	$4.49^{ab}\pm0.11$	$37.69^{c}\pm0.32$	$12.68^{cd}\pm0.23$	$2.71^{f}\pm0.29$
Spontaneous	0	$2.68^{c}\pm0.13$	$3.59^{g}\pm0.30$	$2.95^{e}\pm0.14$	$40.24^{bc}\pm0.52$	$22.82^{a}\pm0.69$	$27.72^{a}\pm0.40$
	12	$3.12^{b}\pm0.16$	$15.73^{de}\pm0.29$	$2.74^{e}\pm0.09$	$39.97^{bc}\pm0.27$	$18.76^{bc}\pm0.87$	19.68 ± 1.39
	24	$1.62^{e}\pm0.13$	$26.20^{c}\pm1.34$	$3.42^{d}\pm0.23$	$39.65^{bc}\pm1.20$	$15.48^{d}\pm0.53$	$13.63^{d}\pm2.00$
	36	$2.95^{bc}\pm0.09$	$34.13^{b}\pm0.44$	$3.73^{c}\pm0.11$	$38.07^{c}\pm0.30$	$12.59^{e}\pm0.16$	$8.53^{e}\pm0.87$

Key: Means with the same alphabets down the column are not significantly different at $p<0.05$; CHO= Carbohydrates

Correlation analysis (Table 4.2) showed no significant correlation between moisture and protein, ash, fibre and carbohydrates. Also, there was no correlation between fat and ash content of the biscuits and no correlation between the fat and fibre, carbohydrate content of the biscuits. However, there was a significant correlation between fat and moisture, protein; protein and ash, fibre and carbohydrates; ash and fibre, carbohydrates; and fibre and carbohydrates.

Table 4.2: Correlation coefficients of proximate components of the prepared meal of fermented and non-fermented cricket substituted cassava flour product.

	Moisture	Protein	Ash	Fat	Fibre	CHO
Moisture	1.00					
Protein	0.18^{ns}	1.00				
Ash	-0.08^{ns}	0.61^{***}	1.00			
Fat	-0.35^{*}	0.34^{*}	-0.10^{ns}	1.00		
Fibre	-0.15^{ns}	-0.91^{***}	-0.62^{***}	0.16^{ns}	1.00	
CHO	-0.13^{ns}	-0.96^{***}	-0.67^{***}	0.17^{ns}	0.86^{***}	1.00

Key: Significance for ***= p<0.001; **= p<0.01 and * = p<0.05, and ns = not significant.

4.1.2 Protein Quality

i. Protein digestibility

Effect of substitution level under different fermentation methods on protein digestibility is shown in Figure 4.1. It is shown that biscuits from controlled fermentation had significantly highest protein digestibility while biscuits from the unfermented had the significantly least protein digestibility. It was also observed that the protein digestibility of biscuits from spontaneous and unfermented increased with the substitution level of crickets. On the contrary, biscuits from controlled fermentation showed an optimum protein digestibility of over 70% at 12% substitution levels.

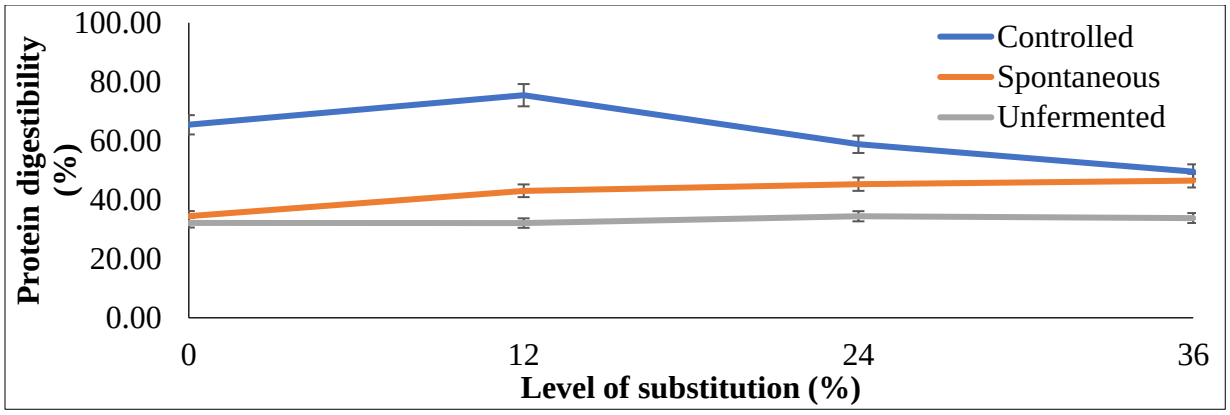


***Figure 4.1**: Effect of substitution level under different fermentation methods on protein digestibility*

ii. Amino acids profiling

The amino acid profile of protein from cricket is shown in Table 4.3. Protein from cricket is very rich in tyrosine at 5.01 g/100g, followed by glutamic acid at 4.76 g/100g and histidine at 4.01 g/100g but very poor in threonine at 0.37 g/100g.

Table 4. 3: Amino acid profile of cricket protein

Amino Acid	Cricket protein (g/100g)	Reference protein (g/100g)
Histidine	4.01^{a} ±0.42	0.38^{b} ±0.06
Isoleucine	1.00^{a} ±0.03	0.70^{b} ±0.02
Leucine	2.92^{a} ±0.23	1.09^{b} ±0.11
Lysine	1.92^{a} ±0.02	1.00^{b} ±0.07
Phenylalanine	1.64^{a} ±0.17	0.60^{b} ±0.05
Serine	1.58^{a} ±0.19	0.68^{b} ±0.08
Threonine	0.37^{b} ±0.02	0.63^{a} ±0.12
Tyrosine	5.01^{a} ±0.48	0.53^{b} ±0.03
Valine	0.90^{a} ±0.06	0.76^{b} ±0.02

Key: Means with the same letter across each column are not significantly different

4.1.3 Fatty Acids Methyl Esters in Cricket Flour

Fatty acid profile of cricket flour is given in Table 4.4. Cricket flour was found to be very rich in elaidic acid at 43.13%, followed by palmitic acid at 21.13%, stearic acid at 11.02% and linoleic acid at 9.28% while fatty acids were in trace amounts.

Table 4.4: Fatty acid profile of cricket flour

Fatty acid	Quantity (%)
Elaidic acid	43.13±5.15[a]
Palmitic acid	21.83±4.58[b]
Stearic acid	11.02±0.96[c]
Linoleic acid	9.28±1.33[c]
Azelaic acid	1.58±0.14[d]
Myristic acid	1.31±0.12[d]
Eicosanoic acid	1.22±0.36[de]
Hexadic-9-enoic acid	0.70±0.06[e]
Margaric acid	0.60±0.02[e]
Hexadecenoic acid	0.31±0.02[f]
Pentadecanoic acid	0.28±0.02[f]
Suberic acid	0.23±0.07[f]
Tetradecanoic acid	0.14±0.01[g]
Lauric acid	0.09±0.01[h]
Nonadecanoic acid	0.06±0.02[h]

4.1.4 Retinol and Tocopherols

The retinol and tocopherol content in biscuits and cricket flour are given in Table 4.5. It was found that cricket had the significantly highest levels of retinol and significantly lowest levels of tocopherol as compared to the biscuits. Also, it was observed that irrespective of the method, fermentation significantly influenced the levels of retinol and tocopherol in the biscuits. Biscuit from spontaneous fermentation with 12% cricket flour had the significantly highest gamma tocopherols while biscuits from unfermented with 0% cricket flour and biscuits from controlled fermented with 0% cricket flour had the significantly highest alpha tocopherols.

Table 4.5: Retinol and tocopherol content in biscuits and cricket flour

Biscuit	Retinol (mg/100g)	Gamma Tocopherol (mg/100g)	Alpha Tocopherol(mg/100g)
SP0	0.66 ± 0.01^{d}	2.91 ± 0.08^{c}	2.79 ± 0.18^{b}
SP1	0.76 ± 0.02^{cd}	3.46 ± 0.04^{a}	2.63 ± 0.02^{b}
CN0	1.14 ± 0.04^{b}	3.27 ± 0.06^{b}	3.43 ± 0.09^{a}
CN1	0.81 ± 0.08^{c}	2.77 ± 0.27^{cd}	2.70 ± 0.16^{b}
UN0	0.82 ± 0.05^{c}	2.39 ± 0.23^{d}	3.23 ± 0.18^{a}
UN1	0.88 ± 0.06^{c}	2.99 ± 0.14^{c}	2.38 ± 0.04^{c}
CRX	1.46 ± 0.14^{a}	0.85 ± 0.04^{e}	0.38 ± 0.02^{d}

Key: means with the same letter along the column are not significantly different; UN0= Unfermented with 0% cricket flour; UN1= Unfermented with 12% cricket; CN0= Controlled fermented with 0% cricket flour; CN1= Controlled fermented with 12% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP1= Spontaneously fermented with 12% cricket flour; SP2= Spontaneously fermented with 24% cricket flour; SP3= Spontaneously fermented with 36% cricket; CRX= Cricket flour.

4.2 Effect of Cassava Fermentation and Cricket Enrichment on the Microbial and Sensory Properties of Cassava-cricket Biscuits

4.2.1. Microbial Load in Cassava-cricket Biscuits

Effect on fermentation method and crickets' substitution level in biscuits on microbial load is shown in Figure 4.2. It was observed that biscuits from spontaneous and unfermented flours had the significantly highest TVC when compared biscuits from controlled fermentation as shown in Figure 4.2a. Also, it was observed that TCC and fungi (yeasts and moulds) did not grow in these cassava biscuits enriched with cricket flour.

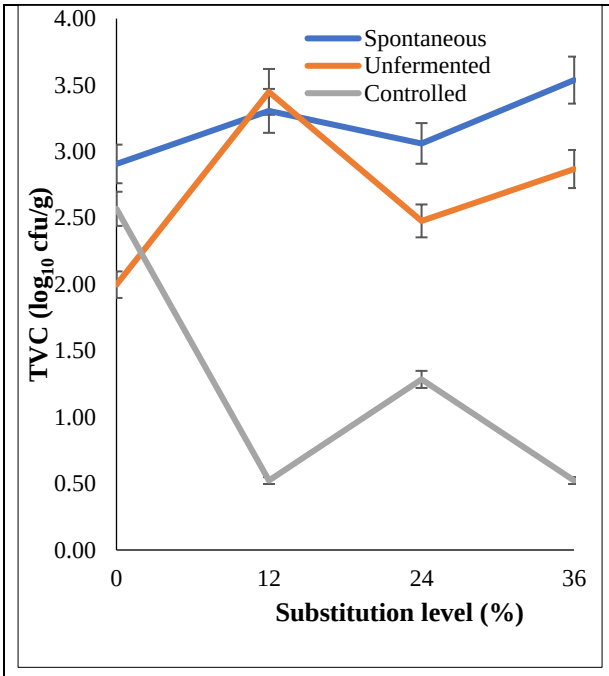


Figure 4.2a: Effect on substitution level and fermentation method on TVC in biscuits

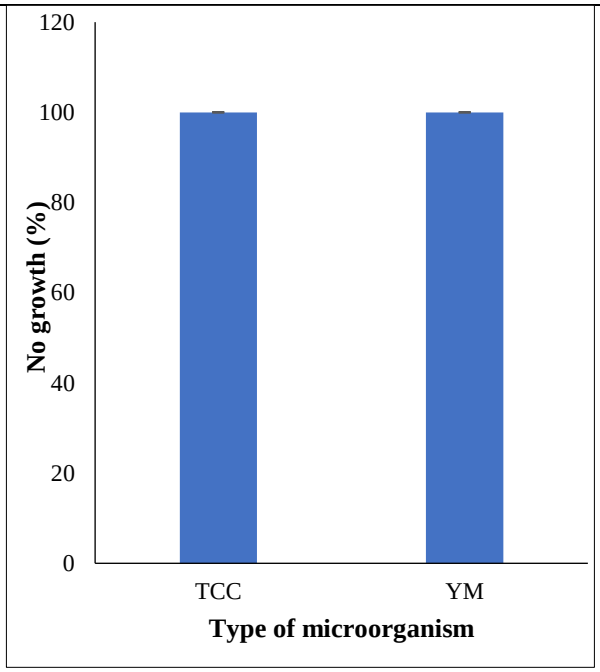


Figure 4.2b: Prevalence of TCC and yeasts and moulds in biscuits enriched with cricket flour

***Figure 4.2**: Microbial load in cassava-cricket biscuits*

4.2.2 Sensory Analysis

Mean scores for consumer rating of sensory attributes of cassava biscuits enriched with cricket flour are shown in Table 4.6. Biscuits with 0% cricket flour substitution and fermented spontaneously had the significantly highest mean scores for appearance at 6.40 and overall acceptability at 6.20 as compared to other biscuits. While biscuits with 24% cricket substitution level and fermented spontaneously scored significantly lowest on all attributes in relation to others. It was also observed that irrespective of the fermentation method used, increase in level of cricket substitution in the biscuits resulted in corresponding significant decrease in mean scores for all the attributes. Biscuits with 0% cricket substitution scored the highest irrespective of the fermentation method indicating that consumers liking of the product was negatively affected by cricket flour levels.

Table 4.6: Consumer acceptability mean scores for sensory attributes of cassava biscuits enriched with cricket flour

Biscuit	Appearance	Flavour	Texture	Overall
UN0	$5.40^{b}\pm0.49$	$5.53^{ab}\pm0.36$	$5.60^{ab}\pm0.38$	$5.67^{ab}\pm0.33$
UN1	$4.40^{c}\pm0.47$	$5.33^{abc}\pm0.35$	$5.00^{abc}\pm0.38$	$4.60^{abcd}\pm0.38$
UN2	$3.40^{d}\pm0.31$	$3.87^{bcd}\pm0.34$	$4.27^{abcd}\pm0.42$	$3.47^{cde}\pm0.26$
UN3	$3.07^{d}\pm0.36$	$3.33^{cd}\pm0.33$	$3.87^{bcd}\pm0.39$	$2.73^{ef}\pm0.30$
CN0	$5.93^{ab}\pm0.48$	$5.80^{a}\pm0.33$	$6.07^{a}\pm0.37$	$6.13^{a}\pm0.40$
CN1	$4.93^{bc}\pm0.40$	$4.93^{abcd}\pm0.40$	$5.07^{abc}\pm0.27$	$5.13^{abc}\pm0.31$
CN2	$4.93^{bc}\pm0.30$	$5.00^{abcd}\pm0.35$	$5.13^{abc}\pm0.35$	$4.80^{abcd}\pm0.38$
CN3	$4.07^{c}\pm0.40$	$4.40^{abcd}\pm0.46$	$4.27^{abcd}\pm0.45$	$4.33^{bcde}\pm0.45$
SP0	$6.40^{a}\pm0.34$	$5.87^{a}\pm0.34$	$5.67^{ab}\pm0.47$	$6.20^{a}\pm0.38$
SP1	$5.27^{b}\pm0.44$	$5.40^{ab}\pm0.46$	$5.13^{abc}\pm0.38$	$5.13^{abc}\pm0.39$
SP2	$1.20^{e}\pm0.11$	$2.00^{d}\pm0.39$	$2.53^{d}\pm0.47$	$1.53^{f}\pm0.29$
SP3	$3.20^{d}\pm0.33$	$3.60^{cd}\pm0.41$	$3.40^{cd}\pm0.43$	$3.13^{def}\pm0.42$

Key: Means with the same letter along the column are not significantly different; UN0= Unfermented with 0% cricket; UN1= Unfermented with 12% cricket; UN2= Unfermented with 24% cricket flour; UN3= Unfermented with 36% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN1= Controlled fermented with 12% cricket flour; CN2= Controlled fermented with 24% cricket flour; CN3= Controlled fermented with 36% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP1= Spontaneously fermented with 12% cricket flour; SP2= Spontaneously fermented with 24% cricket flour; SP3= Spontaneously fermented with 36% cricket flour.

Lexicon for sensory attributes of cassava biscuits enriched with crickets as generated by the panel is shown in Table 4.7. A total of 19 attributes were generated whereby flavour profile contributed the majority 9/19 (47%) while appearance properties contributed the least 3/19 (16%).

Table 4.7: Lexicon for sensory attributes of cassava biscuits enriched with cricket flour

Profile	Attribute	Description
Appearance	Colour	Intensity of brownness of the biscuits ranging from light to dark brown.
	Consistency	How smooth and cohesive the biscuits appear.
	Density	How compact the biscuits appear.
Flavour	Baked	Smell and taste of a regular baked product.
	Caramel	Flavour of burnt sugar in the biscuit.
	Salty	Perception of saltiness in the biscuit.
	Sugary	Perception of sweetness in the biscuit.
	Astringent	Leaves a puckering feeling on the tongue.
	Starchy	Tastes like regular flour.
	Buttery	Perception of butter taste in the biscuit.
	Bitter	Perception of bitterness/bad taste.
	Lingering	Leaves an after taste in the mouth.
Texture	Dryness	Absorbs saliva in the mouth and leaves the mouth dryer.
	Crumbliness	Falls into many pieces when external force is applied.
	Crunchiness	Easily breaks into definite part without crumbs.
	Grittiness	Leaves fine particles in the mouth when chewing.
	Particles	Particles are not cohesive.
	Stickiness	Sticks on teeth and roof of mouth during chewing.
	Oiliness	Leaves considerable amount of oil on fingers.

Attribute descriptors are adapted from (Morais et al., 2014).

Mean scores for intensities appearance and texture properties of biscuits enriched with cricket flour are shown in Table 4.8. and mean scores for intensities flavour properties are shown in Table 4.9. Irrespective of the fermentation used, increasing the substitution level of cricket flour in the biscuits did not significantly affect the intensities of 11 properties: oiliness, stickiness, particles, grittiness, dryness, baked aroma, caramel flavour, salty taste, astringency, starchy flavour and lingering taste. On the other hand, irrespective of the fermentation method, increasing the substitution level of cricket flour in the biscuits did significantly increase the intensities of colour,

crumbliness and bitterness. This is an indication that the cricket flour interfered with the appearance, dough properties hence crumbliness and introduced bitter taste. However, increasing the substitution level of cricket flour in the biscuits did significantly decrease the intensities of consistency, density, baked aroma, sugary taste and buttery.

Table 4.8: Mean scores for intensities appearance and texture properties of biscuits enriched with cricket (7-point hedonic scale (Muoki et al., 2015))

Biscuit	Colour	Consistency	Density	Dryness	Crumbliness	Crunchiness	Grittiness	Particles	Stickiness	Oiliness
UN0	2.67^{fg}±0.21	4.73^{bcd}±0.37	4.73^{abc}±0.44	3.73^{a}±0.43	4.27^{ab}±0.52	5.47^{ab}±0.43	4.33^{a}±0.41	2.93^{a}±0.46	3.27^{a}±0.42	4.13^{a}±0.49
UN1	4.07^{de}±0.25	4.20^{cde}±0.35	4.27^{c}±0.40	3.80^{a}±0.43	4.93^{ab}±0.36	4.73^{ab}±0.28	4.20^{a}±0.40	3.80^{a}±0.47	3.53^{a}±0.38	3.87^{a}±0.43
UN2	5.47^{bc}±0.26	3.07^{cde}±0.34	3.67^{c}±0.37	4.33^{a}±0.43	5.20^{ab}±0.28	4.93^{ab}±0.25	4.53^{a}±0.42	4.27^{a}±0.44	3.33^{a}±0.40	2.67^{a}±0.25
UN3	6.47^{ab}±0.19	2.67^{e}±0.49	3.67^{c}±0.44	4.40^{a}±0.50	5.40^{ab}±0.35	4.87^{ab}±0.45	4.40^{a}±0.55	3.80^{a}±0.43	3.13^{a}±0.38	2.67^{a}±0.30
CN0	1.20^{h}±0.11	6.20^{ab}±0.40	6.40^{a}±0.35	4.87^{a}±0.54	3.53^{b}±0.62	5.60^{ab}±0.36	3.53^{a}±0.47	3.27^{a}±0.45	4.00^{a}±0.54	3.93^{a}±0.56
CN1	3.33^{efg}±0.32	5.20^{abc}±0.37	5.07^{abc}±0.41	4.20^{a}±0.50	5.13^{ab}±0.46	3.93^{b}±0.47	3.47^{a}±0.32	3.73^{a}±0.43	4.27^{a}±0.49	3.87^{a}±0.45
CN2	4.67^{cd}±0.23	3.53^{cde}±0.40	4.33^{bc}±0.33	4.73^{a}±0.30	5.00^{ab}±0.28	5.07^{ab}±0.43	4.20^{a}±0.44	4.27^{a}±0.33	3.40^{a}±0.38	3.80^{a}±0.31
CN3	3.60^{def}±0.34	4.27^{cde}±0.45	4.40^{bc}±0.41	4.13^{a}±0.38	5.67^{a}±0.36	4.33^{ab}±0.46	3.53^{a}±0.49	3.93^{a}±0.51	3.47^{a}±0.50	4.27^{a}±0.47
SP0	1.53±0.19	6.60^{a}±0.21	6.13^{ab}±0.40	4.27^{a}±0.53	4.00^{ab}±0.56	5.87^{a}±0.45	3.53^{a}±0.49	2.87^{a}±0.34	2.93^{a}±0.44	3.33^{a}±0.41
SP1	2.47^{gh}±0.26	4.73^{bcd}±0.41	4.93^{abc}±0.41	3.67^{a}±0.37	5.07^{ab}±0.41	4.67^{ab}±0.40	3.80^{a}±0.35	2.93^{a}±0.28	3.00^{a}±0.46	3.87^{a}±0.42
SP2	6.80^{a}±0.11	3.93^{cde}±0.43	4.40^{bc}±0.32	5.53^{a}±0.46	5.40^{ab}±0.36	5.00^{ab}±0.44	4.67^{a}±0.42	4.13^{a}±0.46	3.40^{a}±0.52	2.87^{a}±0.40
SP3	5.67^{bc}±0.23	4.13^{cde}±0.34	4.00^{c}±0.37	4.73^{a}±0.33	5.40^{ab}±0.32	4.07^{ab}±0.33	4.20^{a}±0.44	4.27^{a}±0.46	3.80^{a}±0.48	3.80^{a}±0.39

Key: Means with the same along the column are not significantly different; UN0= Unfermented with 0% cricket flour; UN1= Unfermented with 12% cricket flour; UN2= Unfermented with 24% cricket flour; UN3= Unfermented with 36% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN1= Controlled fermented with 12% cricket flour; CN2= Controlled fermented with 24% cricket flour; CN3= Controlled fermented with 36% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP1= Spontaneously fermented with 12% cricket flour; SP2= Spontaneously fermented with 24% cricket flour; SP3= Spontaneously fermented with 36% cricket flour;

Table 4.9: Mean scores for intensities flavour properties of biscuits enriched with cricket (7-point hedonic scale (Muoki et al., 2015))

Biscuit	Baked	Caramel	Salty	Sugary	Astringent	Starchy	Buttery	Bitter	Lingering
UN0	$5.40^{a}\pm0.35$	$4.07^{a}\pm0.43$	$2.27^{a}\pm0.25$	$5.60^{a}\pm0.32$	$4.00^{a}\pm0.49$	$4.47^{a}\pm0.45$	$5.73^{ab}\pm0.37$	$2.00^{cd}\pm0.31$	$5.27^{a}\pm0.43$
UN1	$4.67^{a}\pm0.49$	$4.80^{a}\pm0.39$	$3.33^{a}\pm0.39$	$5.13^{ab}\pm0.38$	$4.27^{a}\pm0.46$	$4.60^{a}\pm0.43$	$5.13^{abc}\pm0.40$	$3.07^{abcd}\pm0.44$	$4.40^{a}\pm0.46$
UN2	$4.07^{a}\pm0.50$	$4.93^{a}\pm0.48$	$3.87^{a}\pm0.50$	$4.33^{abc}\pm0.25$	$4.40^{a}\pm0.45$	$4.60^{a}\pm0.46$	$4.20^{bc}\pm0.37$	$4.07^{abc}\pm0.46$	$4.80^{a}\pm0.35$
UN3	$3.87^{a}\pm0.49$	$5.47^{a}\pm0.47$	$3.80^{a}\pm0.55$	$4.27^{abc}\pm0.36$	$4.47^{a}\pm0.39$	$4.40^{a}\pm0.39$	$4.07^{bc}\pm0.43$	$4.67^{ab}\pm0.42$	$4.47^{a}\pm0.31$
CN0	$5.27^{a}\pm0.49$	$4.20^{a}\pm0.49$	$2.87^{a}\pm0.49$	$5.27^{ab}\pm0.43$	$3.93^{a}\pm0.43$	$4.73^{a}\pm0.52$	$4.93^{abc}\pm0.57$	$1.73^{d}\pm0.42$	$3.67^{a}\pm0.42$
CN1	$5.20^{a}\pm0.33$	$4.60^{a}\pm0.29$	$3.33^{a}\pm0.36$	$4.67^{ab}\pm0.33$	$4.07^{a}\pm0.47$	$5.13^{a}\pm0.35$	$5.00^{abc}\pm0.41$	$2.73^{bcd}\pm0.44$	$3.93^{a}\pm0.48$
CN2	$4.67^{a}\pm0.39$	$5.00^{a}\pm0.32$	$3.73^{a}\pm0.40$	$4.93^{ab}\pm0.42$	$4.27^{a}\pm0.43$	$4.67^{a}\pm0.43$	$4.73^{b}\pm0.34$	$2.73^{bcd}\pm0.45$	$4.07^{a}\pm0.42$
CN3	$4.87^{a}\pm0.42$	$4.20^{a}\pm0.48$	$4.13^{a}\pm0.42$	$4.40^{abc}\pm0.41$	$4.67^{a}\pm0.41$	$5.47^{a}\pm0.31$	$5.13^{abc}\pm0.34$	$3.33^{abcd}\pm0.54$	$3.67^{a}\pm0.37$
SP0	$5.67^{a}\pm0.35$	$5.27^{a}\pm0.36$	$2.40^{a}\pm0.21$	$5.47^{a}\pm0.41$	$4.13^{a}\pm0.54$	$5.20^{a}\pm0.35$	$5.67^{ab}\pm0.35$	$2.13^{cd}\pm0.40$	$4.00^{a}\pm0.56$
SP1	$5.40^{a}\pm0.36$	$5.07^{a}\pm0.25$	$2.87^{a}\pm0.24$	$5.27^{ab}\pm0.28$	$4.53^{a}\pm0.43$	$5.53^{a}\pm0.34$	$6.00^{a}\pm0.28$	$2.47^{cd}\pm0.41$	$4.07^{a}\pm0.40$
SP2	$4.07^{a}\pm0.55$	$4.60^{a}\pm0.58$	$2.60^{a}\pm0.46$	$2.93^{c}\pm0.38$	$4.73^{a}\pm0.43$	$3.60^{a}\pm0.52$	$3.00^{c}\pm0.39$	$5.13^{a}\pm0.58$	$5.27^{a}\pm0.50$
SP3	$3.80^{a}\pm0.38$	$4.33^{a}\pm0.42$	$3.93^{a}\pm0.37$	$3.60^{bc}\pm0.35$	$4.53^{a}\pm0.40$	$4.27^{a}\pm0.36$	$3.40^{c}\pm0.40$	$3.80^{abcd}\pm0.50$	$4.80^{a}\pm0.37$

Key: Means with the same along the column are not significantly different; UN0= Unfermented with 0% cricket flour; UN1= Unfermented with 12% cricket flour; UN2= Unfermented with 24% cricket flour; UN3= Unfermented with 36% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN1= Controlled fermented with 12% cricket flour; CN2= Controlled fermented with 24% cricket; CN3= Controlled fermented with 36% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP1= Spontaneously fermented with 12% cricket flour; SP2= Spontaneously fermented with 24% cricket flour; SP3= Spontaneously fermented with 36% cricket flour;

Loading matrix of sensory attributes on principal components is shown in Table 4.10 and Figure 4.3. As observed, principal component analysis reduced the sensory attributes to 10 from 19 as given in Table 4.7. It was found that the remaining 9 attributes could be grouped into 3 major principals. First major component shows that increasing the cricket substitution levels in the cassava (unfermented, spontaneous and *A. oryzae* fermented) biscuits caused increase in bitter taste by over 65%, particles by 64% and salty taste by 56% while reducing baked aroma by 56%, buttery taste by 59% and sugary taste by 70%. Therefore, this observation could be linked to the chitin increase as a result of substitution level increase. Second component shows that increasing the cricket substitution levels in the cassava biscuits caused increase in crunchiness by 54%, starchy flavour by 54% and stickiness by 48% while the third component had only one attribute, dryness, that increased by 61%.

Table 4.10: Loading matrix on principal component analysis

Sensory attribute	PC 1	PC 2	PC 3
Bitter taste	0.655	-	-
Particles	0.641	-	-
Salty taste	0.560	-	-
Crunchiness	-	0.544	-
Starchy flavour	-	0.539	-
Dryness	-	-	0.610
Baked aroma	-0.569	-	-
Stickiness	-	0.485	-
Buttery	-0.591	-	-
Sugary taste	-0.708	-	-

Key: PC= Principal Component

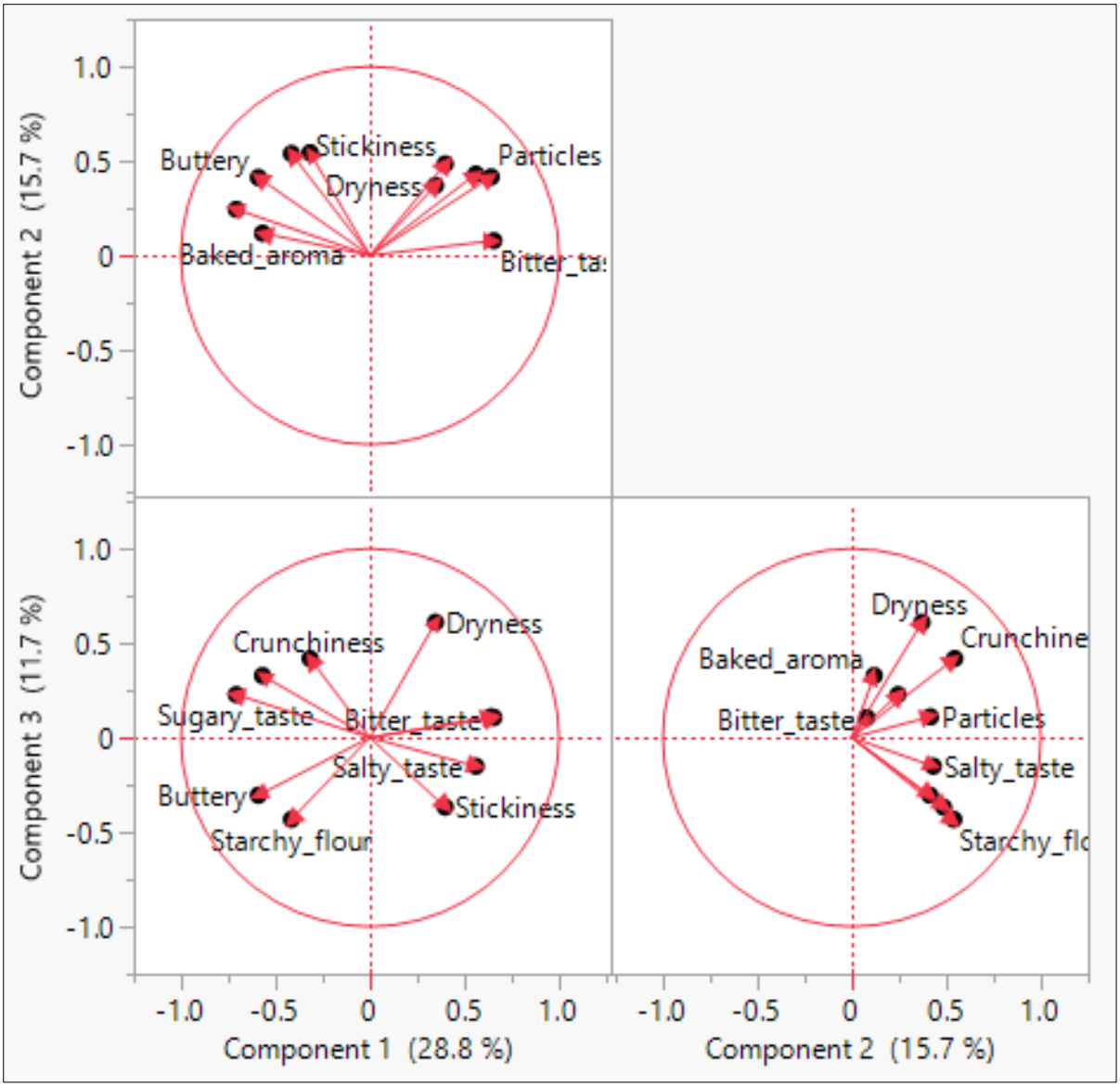


***Figure 4.3**: Loading of attributes on principal components*

4.3 Effect of Cassava Fermentation and Cricket Enrichment on Shelf-life of Cassava-cricket Biscuits

4.3.1 Peroxide Value

The initial peroxide values of fermented cassava biscuits enriched with cricket flour are shown in Figure 4.3. Mean peroxide values in the biscuits ranged between 2.9- 3.7 mEq of O_2/kg.

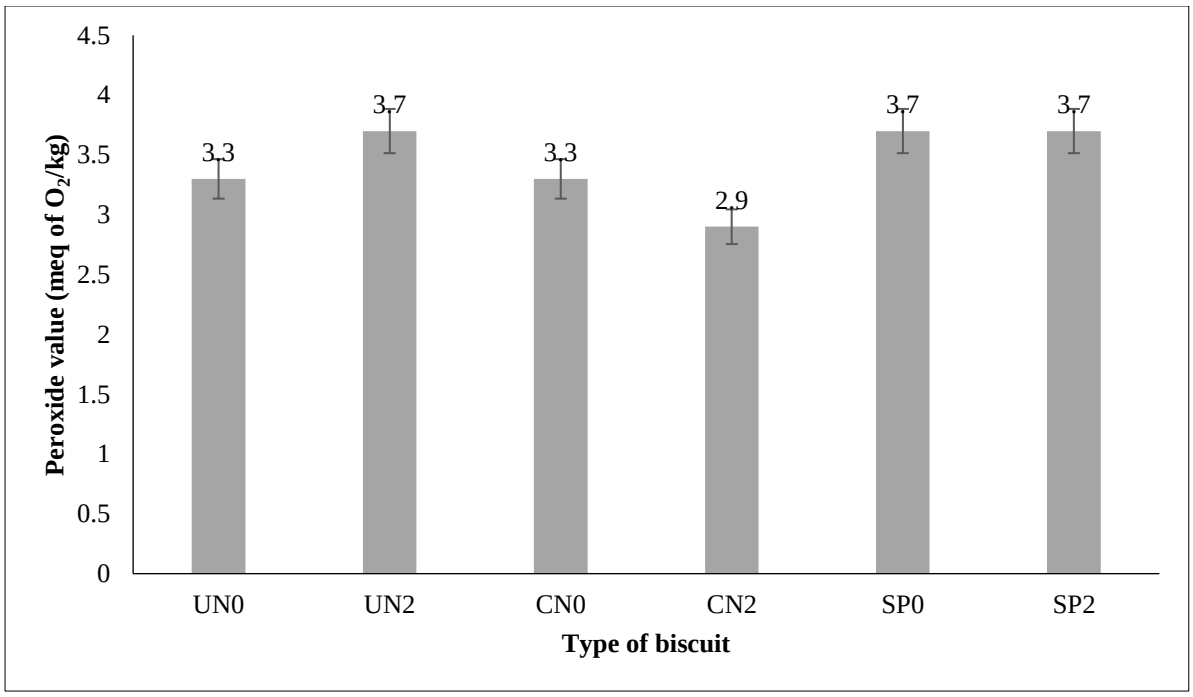


***Figure 4.4**: Initial peroxide values of fermented cassava biscuits enriched with cricket flour*

Key: UN0= Unfermented with 0% cricket flour; UN2= Unfermented with 24% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN2= Controlled fermented with 24% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP2= Spontaneously fermented with 24% cricket flour.

4.3.2 Accelerated Shelf-life Test (ASLT)

Oxidative rancidity reaction kinetics in fermented cassava biscuits enriched with cricket flour was measured by peroxide value during storage are shown in Figure 4.4. It was observed that during storage, oxidative rancidity reactions in the biscuits was a zero order.

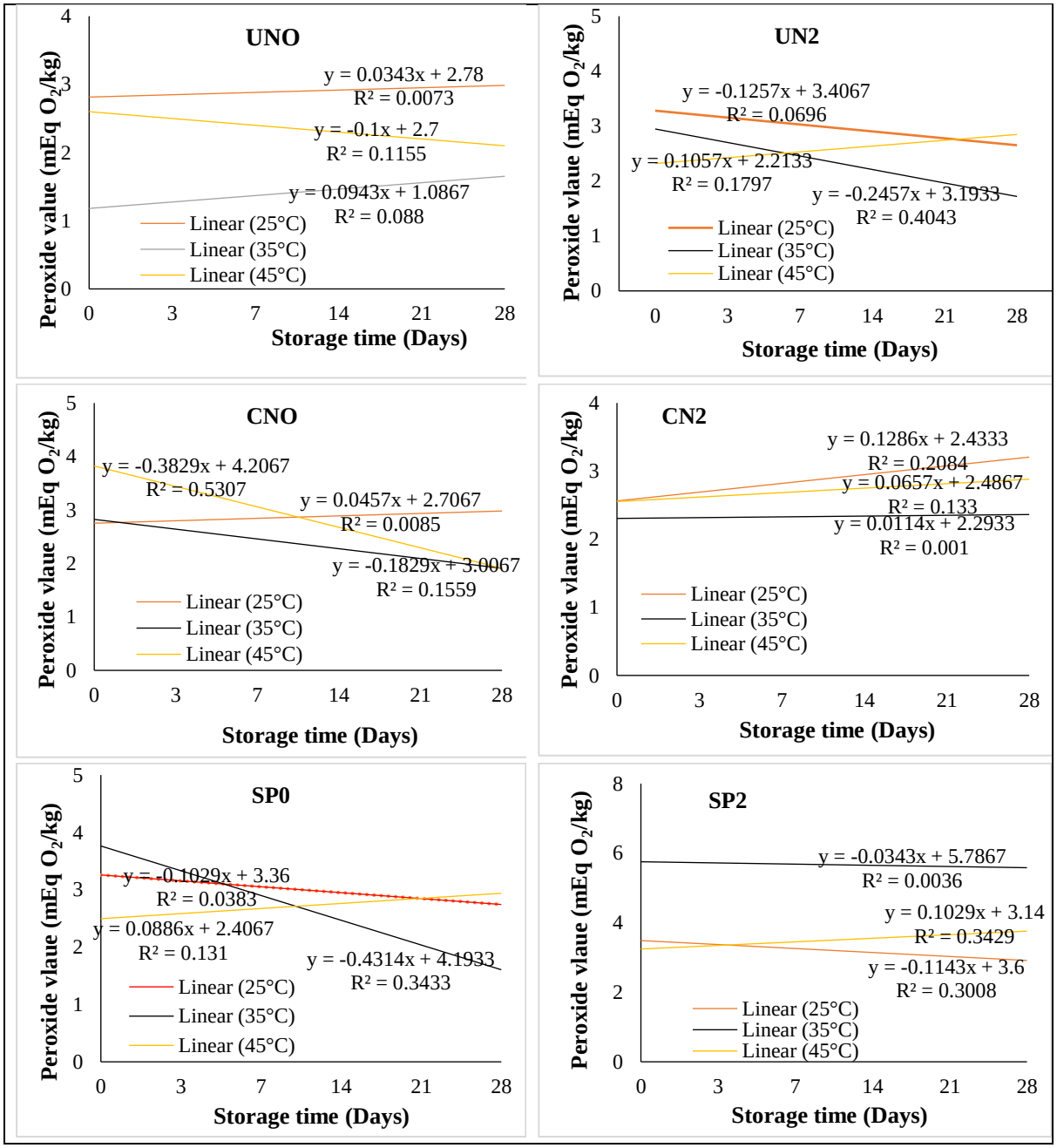


***Figure 4.5**: Oxidative rancidity reaction kinetics in fermented cassava biscuits enriched with cricket flour*

Key: UN0= Unfermented with 0% cricket flour; UN2= Unfermented with 24% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN2= Controlled fermented with 24% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP2= Spontaneously fermented with 24% cricket flour.

The Arrhenius equations for the reaction kinetics in fermented cassava biscuits enriched with cricket flour are shown in Figure 4.5. It was observed that the rate of oxidative rancidity reduced with increase in storage temperature for all the biscuits except two: the unfermented with 0% cricket and the controlled fermented with 0% cricket.

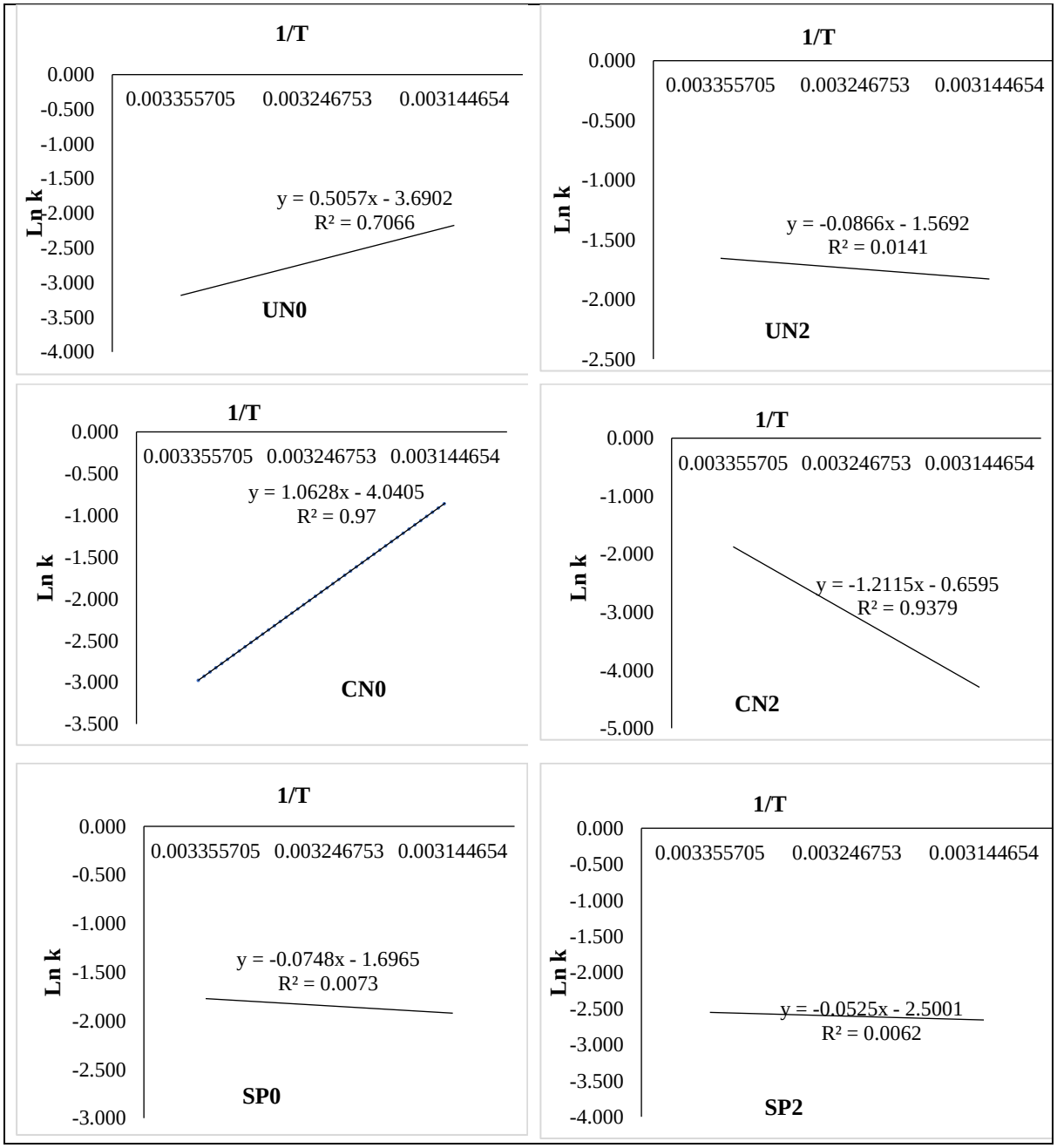


***Figure 4.6**: Arrhenius equations for the reaction kinetics in fermented cassava biscuits enriched with cricket flour*

Key: UN0= Unfermented with 0% cricket flour; UN2= Unfermented with 24% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN2= Controlled fermented with 24% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP2= Spontaneously fermented with 24% cricket flour.

Predicted shelf-life of cassava biscuit enriched with cricket are shown in Figure 4.6. It was observed that cassava biscuit with from spontaneously fermentation with 24% cricket flour had the longest predicted shelf life of 120 days. Biscuit from controlled fermentation with 24% cricket recorded the least predicted shelf life of about 6 days.

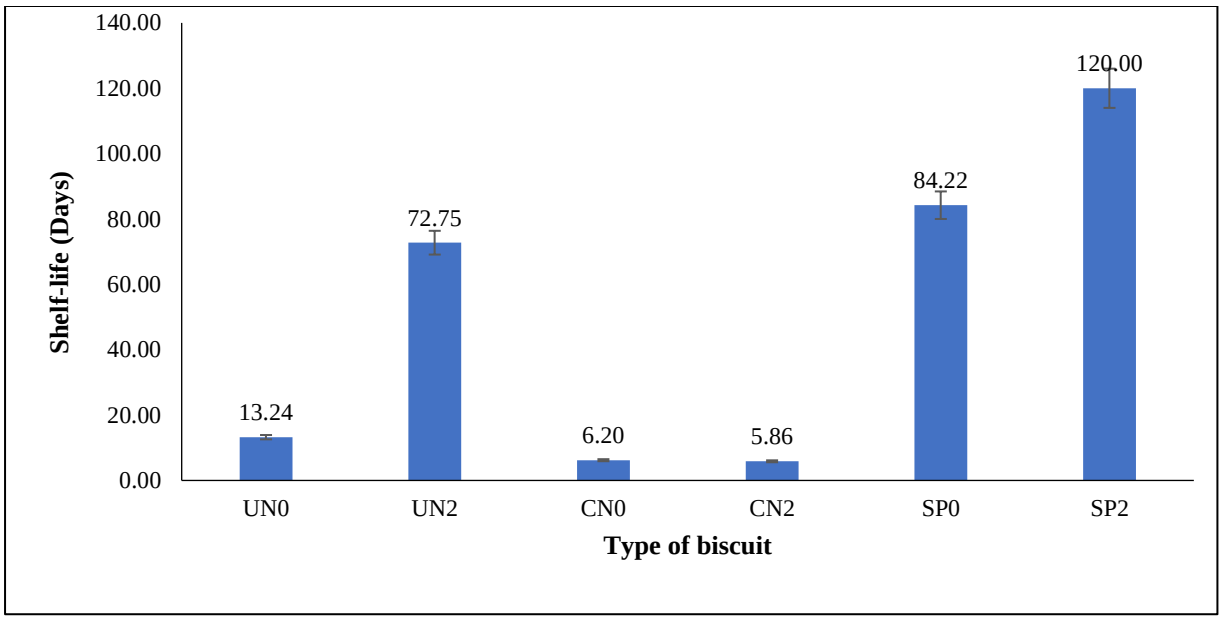


***Figure 4.7**: Predicted shelf-life of cassava biscuit enriched with cricket*

Key: UN0= Unfermented with 0% cricket; UN2= Unfermented with 24% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN2= Controlled fermented with 24% cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP2= Spontaneously fermented with 24% cricket flour.

4.3.3 Thermodynamic Properties

Thermodynamic properties of oxidative rancidity in cassava biscuits enriched with cricket flour are given in Table 4.11. The results showed that activation energy for oxidative rancidity in cassava-cricket biscuits was from -8.84 to 10.07 J/K/Mol, enthalpy change was from -2462.71 to -2443.80 kJ/Mol, entropy change from -49.51 to -46.37 J/K/Mol and Gibbs free energy was from 11,242.18 to 12,160.12 kJ/Mol.

Table 4.11: Thermodynamic properties of cassava-cricket biscuits during storage

Biscuit	Arrhenius equation	Ea (kJ/K/Mol)	ΔH (kJ/Mol)	ΔS (J/K/Mol)	ΔG (kJ/Mol)
UN0	Y= 0.506X - 3.690	-4.20	-2458.08	-47.25	11,487.49
UN2	Y= -0.087X - 1.569	0.72	-2453.16	-49.01	12,012.65
CN0	Y= 1.063X - 4.041	-8.84	-2462.71	-46.51	11,264.20
CN2	Y= -1.212X - 0.660	10.07	-2443.80	-46.37	11,242.18
SP0	Y= 0.075X – 1.697	-0.62	-2454.50	-49.16	12,054.71
SP2	Y= -0.053X – 2.500	0.44	-2453.44	-49.51	12,160.12

Key: UN0= Unfermented with 0% cricket flour; UN2= Unfermented with 24% cricket flour; CN0= Controlled fermented with 0% cricket flour; CN2= Controlled fermented with 24%

cricket flour; SP0= Spontaneously fermented with 0% cricket flour; SP2= Spontaneously fermented with 24% cricket flour.

CHAPTER FIVE
DISCUSSIONS

5.1 Nutritional Composition of Fermented Cassava-cricket Biscuits

The findings in this study showed that both the inclusion level of cricket flour in cassava and the fermentation of these rations significantly influenced the nutritional composition of the biscuits. In particular, as indicated in Table 4.1, the reduction in carbohydrate content observed in fermented biscuits can be attributed to utilization by the microorganisms during the fermentation process. The use of a pure strain culture of *A. oryzae* yielded the best results in terms of reducing the carbohydrate content of the biscuits, compared to the spontaneous fermentation process. As indicated in Table 4.1, inclusion level of cricket flour in cassava also had an effect nutritional composition of the biscuits. The trend observed on moisture content is due to the effect of the cricket flour on the biscuit matrix (Machado & Thys, 2019), which allowed more moisture to escape up to a point. However, adding more cricket flour did not further modify the nutritional characteristics of the biscuit matrix, leading to retention of more moisture in the biscuit. The fat content of the biscuits did not vary much except for the sample with 36% cricket flour added.

All the biscuits generally had moisture content of less than 5%, which is in line with the recommendations by Chavan et al. (2016) as indicated in Table 4.1. These findings are in tandem with a relatable study by Osimani et al. (2018). This is an indication that *Aspergillus oryzae* is utilizing the fibre and carbohydrates during fermentation to metabolites such as organic acids that contribute towards preservation of the product as well as sensory properties (Abrão et al., 2022). Also, fatty acids and amino acids are produced during the fermentation and this could explain the increase in protein and crude fat (Ichikawa et al., 2022) as shown in Table 4.2. Therefore, as seen from Tables 4.1, both fermentation and substitution level of cassava flour with cricket flour had a significant effect on the protein and other components of the biscuits. When comparing the biscuits made with fermented flour (both controlled and spontaneously fermented flours), there was a significant increase in the protein content of the fermented flour biscuits over the unfermented flour biscuits. Biscuits made from *Aspergillus oryzae* fermented cassava flour attained the highest level of protein content at the highest level of cassava flour substitution with cricket flour. The increase in protein content of the biscuits can be directly attributed to the addition of the protein-rich cricket flour, and its non protein nitrogen sources that are also included during conversion since Kjeldahl method does not discriminate nitrogen source (Kowalczewski et al., 2021). The findings are concurrent with

Bawa et al. (2020) who also found that cricket flour contributed to the increase in protein content of the final product in their respective studies.

While addition of cricket flour increased the protein content of the biscuits, an inverse trend was observed for both the fiber and carbohydrate content of the biscuits. Fermentation had a significant effect ($p<0.5$) on carbohydrate content of the biscuit, with *Aspergillus oryzae* fermented flour biscuits reporting the least carbohydrate content. This can be attributed to the utilization of the carbohydrate by the microorganisms during fermentation of the cassava, in line with similar attribution by Magdi (2011) in a different study.

The ash content of the biscuits were observed to increase significantly with the increased substitution of cassava flour with cricket flour. This can be attributed to the high chitin and chitosan content of the cricket flour (Ibitoye et al., 2018). The increased ash content of the biscuits is indicative of the increase in the mineral content of the biscuits, which shows that the use of cricket flour can be recommended to improve the nutrient content of cassava biscuits. Generally, the fat content of the biscuits ranged from 37.69±0.32% to a high of 42.77±0.15% and is mostly attributed to the margarine used in baking the biscuits.

As indicated in Figure 4.1, these findings concur with a previous study by Bas and El (2022) who showed that enriching biscuits with cricket doubles the protein digestibility as compared to standard ones. The increase in protein digestibility during fermentation is due *Aspergillus oryzae* producing protease enzymes. Protease breaks down proteins into smaller molecules, such as amino acids and peptides which makes the proteins easier to digest and absorb. Increasing protein digestibility during fermentation with *Aspergillus oryzae* also improves the nutritional value of food.

When compared to the reference protein, cricket protein had significantly higher quantities of all amino acids except threonine. Hence, threonine can be categorized as the first limiting amino acid in protein from cricket flour. From these findings, it can be deduced that enriching cassava flour with cricket flour, not only does it increase the quantity of protein but also improves on the quality due to high quantities of essential amino acids as shown in Table 4.5. It has shown that cassava is also a poor source of protein and other nutrients. Enriching cassava with cricket flour can help to improve its nutritional value and make it a more sustainable food source. But as seen in Table 4.1, controlled fermentation gave significantly higher protein and ash as compared to the other methods. Fermentation improves protein and ash but at the same time causing reduction in fibre and carbohydrates. Therefore, enriching cassava fermented with *Aspergillus oryzae* with cricket flour is a promising way to improve

the nutritional value and sustainability of this staple food. This can help to improve the health and well-being of people in developing countries.

In Table 4.4, the fatty acid profile of cricket flour is similar to that of other insects, with a high proportion of unsaturated fatty acids. Earlier similar study showed that the most abundant fatty acids in cricket flour are linoleic acid (omega-6), oleic acid (omega-9), and palmitic acid. Linoleic acid is an essential fatty acid, which means that it cannot be made by the body and must be obtained from the diet. Oleic acid is a monounsaturated fatty acid that has been shown to have beneficial effects on heart health. Palmitic acid is a saturated fatty acid that has been linked to an increased risk of heart disease (Ghosh et al., 2017). In this case, elaidic which is an oleic acid trans isomer (Plötz et al., 2017) was predominant.

On retinol and tocopherol as given in Table 4.5, it can be concluded that the possible source tocopherol in the biscuit was the margarine which was used as an ingredient while the main source of retinol could be fat from cricket flour. A different study found that the retinol content of house crickets (*Acheta domesticus*) was 0.35 μg/g (Ayieko et al., 2016b).

5.2 Microbial Load and Sensory Properties of Fermented Cassava-cricket Biscuits

Similar to nutritional composition, the findings of this study also show that that both the inclusion level of cricket flour in cassava and the fermentation of these rations significantly influenced the sensory and microbial load of the biscuits. As shown in Figure 4.2, it is required that in biscuits, which is a ready-to-eat product, TVC should be $< \log_{10} 5.0$ and coliforms should be nil (Nethathe et al., 2023). Coliform are indicators of contamination due to unhygienic handling of the product (Al-Nasiry, 2020; Soare et al., 2022). Yeasts and moulds are indicators of contamination from the environment (Hagler, 2006). Therefore, this is an indication that good hygienic practices were observed during production of cassava biscuits enriched with cricket flour. Also, chitin from the exoskeleton of crickets, has been shown to be effective against a wide range of microbes, including bacteria, fungi, and viruses (Nowakowski et al., 2022). It can inhibit the growth and proliferation of these microorganisms by disrupting their cell membranes, interfering with their enzyme systems, and causing damage to their DNA (Park & Kim, 2010). Acceptable microbial quality of the biscuits ascertained the safety of the product for consumer studies.

As shown in Table 4.6, consumer liking of the cassava-cricket biscuits varied significantly. According to Biró et al. (2020), the consumer acceptance of insects such as crickets as food depends on many influencing factors but negative attitude towards them remains to be the main one. Table 4.9, signifies that enriching cassava with cricket flour to make biscuits affects flavour and textural profiles more than appearance. As seen in Tables 4.8

and 4.9, this observation can be due to cricket flour containing high levels of chitin, a natural compound that has a bitter taste. Chitin which is a major component of insect exoskeletons could also be contributing towards making biscuits more crumbly. In addition, cricket flour can also absorb moisture, which can make biscuits dry and hard (Kewuyemi et al., 2020; Melgar-Lalanne et al., 2019). Colour and aroma of baked products is mainly due to caramelization and Maillard reactions (Lukinac et al., 2022) and therefore inclusion of cricket flour into biscuits could be interfering with these reactions as seen in changes in their intensities. In Figure 4.3, PCA is a powerful tool that can be used to reduce the dimensionality of descriptive sensory data. This can make it easier to visualize and interpret the data, and it can also be used to identify the most important sensory attributes. PCA helps to simplify and identify the most significant patterns or dimensions of variation in the data based on the covariance structure of sensory attributes and clusters them on the principle of linear combinations of the original variables (Næs et al., 2021) as shown in Table 4.12. This could explain the decreasing mean scores rating in consumer acceptability with increase in cricket flour substitution levels in cassava biscuits as shown in Table 4.8. Ayensu et al., (2019) observed high levels of acceptability of orange fleshed sweet potato biscuits fortified with palm weevil larvae by pregnant women in a different study conducted in Ghana. The difference in texture of fermented vs unfermented biscuits was not significant, which differs from the expected outcomes according to Wang and Wang (2024) who posit that fermentation results in softer fluffier texture. This observation is attributed to high fiber content of the biscuits (Caponio et al., 2022).

5.3 Shelf-life Properties of Fermented Cassava-cricket Biscuits

According Manzocco et al. (2020) peroxide value (PV) is an indicator of lipids oxidative rancidity or degree of oxidation in food products and the recommended maximum acceptability limit for sunflower oil (10 mEqO_2/kg oil). Therefore, the initial values were very high as compared to earlier findings which showed that PV in biscuits were < 1mEqO_2/kg (Nwosu & Akubor, 2018), < 2.25 mEq of O_2/kg (Haider et al., 2022) and 0.86 mEq of O_2/kg (Dahal et al., 2022). When the peroxide value is between 30 and 40 mEqO_2/kg, a rancid taste is noticeable. Using the unfermented biscuit with 0% cricket flour substitution which had PV of 3.3 mEq of O_2/kg as a reference point, these high values could be attributed to the margarine that was used in the preparation of the biscuits. Peroxides are formed when lipids in the food are exposed to oxygen. The higher the peroxide value, the more oxidized the product is (Zhang et al., 2022). In biscuits, peroxide values can be used to determine the shelf life of the product. Biscuits with high peroxide values will have a shorter shelf life and will taste rancid sooner

than biscuits with low peroxide values. There are a number of factors that can affect the peroxide value of biscuits, including the type of fat used, the storage conditions, and the presence of antioxidants. By controlling these factors, it is possible to extend the shelf life of biscuits and prevent them from becoming rancid.

In Figure 4.5, however, these reactions had very low coefficients of determination (R^2) of less than 0.4. Accelerated shelf-life testing (ASLT) is a method of predicting the shelf life of foods by exposing them to accelerated conditions, such as high temperature. ASLT can be used to determine the PV of foods under these conditions. This information can be used to predict the shelf life of foods under normal storage conditions. However, peroxide value is not always a reliable predictor of shelf life because it does not measure all of the oxidation products that can give food a rancid odour and taste. Therefore, PV should not be used as the sole criterion for determining the shelf life of a food. A study by Anese et al. (2012) corroborates findings in Figure 4.6, observed a nonlinearity in the Arrhenius plot behaviour of lipid oxidation. In Figure 4.7, however, this predicted shelf life based on oxidative rancidity alone can be said not to be reliable due to the thermodynamic properties involved during the reaction as explained in Section 4.3.3 and attributed to by Rahman et al. (2019) study findings.

As seen in Table 4.11, according to Damodaran (2017) “activation energy is energy change required to elevate a chemical entity from a ground state to a transition state”, whereupon reaction can occur. It was noted that irrespective of the fermentation method, biscuits with no cricket flour substitution had negative (lowest) activation energy as compared to their counterparts with cricket flour. The enthalpy energy values were all negative which indicates that of oxidative rancidity in cassava-cricket biscuits was not temperature dependent. This could explain the low values of coefficient of determination in Figure 4.5, which could be as a result of the biscuit food matrix, especially the low water activity and moisture content. Also, it was observed that oxidative rancidity in cassava-cricket biscuits exhibited negative change in activation entropies thereby hindering the reaction. This hindrance is from the formation of a highly structured shell around molecules which makes oxygen unavailable for the reaction making the environment anaerobic. This can be seen in high positive values for Gibbs free energy. According to Leng et al. (2018), anaerobic reactions occur when the net Gibbs free energy is negative.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following are the conclusions from this study:

i. This study showed that the quality of cassava biscuits can be improved by fermenting with *Aspergillus oryzae* and adding cricket flour to improve the dry matter content, protein and ash content. Even though the addition of cricket flour reduces the fibre content of the biscuits, it only does so to the extent comparable to the fibre content of whole wheat flour of about 15%. Adopting fermented cassava flour enriched with cricket flour for baking will improve utilization of the product to create highly nutritious biscuits that can be used to address hunger problems in Sub-Sahara Africa.

ii. This study showed that fermentation with *Aspergillus oryzae* and inclusion of cricket flour in cassava flour significantly affected the sensory properties of the resultant biscuits. Bitter taste, salty taste and particles increased significantly which resulted in decreasing degree of consumer acceptability.

iii. This study also showed that fermentation with *Aspergillus oryzae* and inclusion of cricket flour in cassava flour significantly increased the shelf-life of the resultant biscuits.

6.2 Recommendations

From this study, the following recommendations can be made:

i. Fermentation of cassava with *A. oryzae* and enrichment with cricket flour improves nutritional profile of cassava-cricket biscuits making them an ideal ready to eat food.

ii. Cassava-cricket biscuits with 12% cricket flour inclusion and controlled fermentation is the recommended formula for the best results, according to the findings.

iii. Cassava fermentation and cricket enrichment of cassava-cricket biscuits is a reliable method for diversifying cassava and improving its utility long after harvesting of the tubers.

6.3 Further Studies

Future studies should explore the use of sensory analysis in addition to peroxide value in shelf life determination.

REFERENCES

AACC International. (2010). *AACC International Approved Methods of Analysis* (11th ed.). St Paul, MN: AACC International PRESS.

Abass, A. B., Awoyale, W., Alenkhe, B., Malu, N., Asiru, B. W., Manyong, V., & Sanginga, N. (2018). Can food technology innovation change the status of a food security crop? A review of cassava transformation into "bread" in Africa. *Food Reviews International, 34*(1), 87-102. https://doi.org/10.1080/87559129.2016.1239207

Abong, G., Shibairo, S., Wanjekeche, E., Ogendo, J., Wambua, T., Lamuka, P., Arama, P., Okoth, M., Mulwa, R. M., Kamidi, M., Mcosore, Z., & Masha, C. (2016). Post-Harvest Practices, Constraints and Opportunities Along Cassava Value Chain in Kenya. *Current Research in Nutrition and Food Science, 4*(2), 114-126. https://doi.org/10.12944/CRNFSJ.4.2.05

Abrão, F. O., Duarte, E. R., Pessoa, M. S., Santos, V. L., Gonçalves, D. B., Lima, S. S., Saliba, E. d., & Rodriguez, N. M. (2022). Aspergillus spp. isolated from the bovine gastrointestinal tract improve organic acid profiles in Urochloa decumbens fermentation. *Biocatalysis and Agricultural Biotechnology, 42*, 102360. https://doi.org/10.1016/j.bcab.2022.102360

Adebayo, A. (2008). *Recent development in cassava processing, utilization and marketing in East and Southern Africa and lessons learned.* UK: International Institute of Tropical Agriculture.

Ade-Omowaye, B. I., Akinwande, B. A., Bolarinwa, I. F., & Adebiyi, A. (2008). Evaluation of Tigernut (Cyperus esculentus) -Wheat Composite Flour and Bread. *African Journal of Food Science, 2*, 87-91. https://doi.org/10.5897/AJFS.9000134

Adepoju, O., & Omotayo, O. (2014). Nutrient Composition and Potential Contribution of Winged Termites (Marcrotermes bellicosus Smeathman) to Micronutrient Intake of consumers in Nigeria. *British Journal of Applied Science and Technology, 4*(7), 1149-1158. https://doi.org/10.9734/BJAST/2014/4420

Aiking, H., & Boer, J. D. (2018). Protein and sustainability – the potential of insects. *Journal of Insects as Food and Feed, 5*(1), 3-7. https://doi.org/10.3920/JIFF2018.0011

Alemu, M., Vedel, S., Olsen, S., Pambo, K., & Owino, K. (2015). *Consumer acceptance and willingness to pay for edible insects as food in Kenya: The case of white-winged termites.* University of Copenhagen.

Al-Nasiry, B. S. (2020). Detection of bacterial contamination in filled and dried biscuit products of young children. *Annals of Tropical Medicine and Health, 23*(16), 231-602. https://doi.org/10.36295/ASRO.2020.231602

Amadi, E., & Kiin-Kabari, D. (2016). Nutritional Composition and Microbiology of Some Edible Insects Commonly Eaten in Africa, Hurdles, and Future Prospects: A Critical Review. *Journal of Food: Microbiology, Safety & Hygiene, 1*(1), 671. https://doi.org/10.4172/2476-2059.1000107

Amarachi, U.-A. D., Oluwafemi, C. J., & Umezuruike, L. O. (2015). Post harvest handling and storage of fresh cassava roots: A review. *Food Bioprocess Technology, 8*, 729–748. https://doi.org/10.1007/s11947-015-1478-z

Andrews, W. (1992). *Manuals of Food Quality Control: 4. Microbiological analysis.* Rome, Italy: FAO.

Anese, M., Lanciotti, R., Gardini, F., & Lagazio, C. (2012). Case Studies. In M. C. Nicoli (Ed.), *Shelf Life Assessment of Food* (pp. 280-310). Florida, USA: Taylor and Francis Group.

AOAC. (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg: AOAC International.

Ayensu, J., Lutterodt, H., Annan, R. A., Edusei, A., & Loh, S. P. (2019). Nutritional composition and acceptability of biscuits fortified with palm weevil larvae (Rhynchophorus phoenicis Fabricius) and orange-fleshed sweet potato among pregnant women. *Food Science and Nutrition, 7*, 1807–1815. https://doi.org/10.1002/fsn3.1024

Ayieko, M., Kinyuru, J., Ekesi, S., & Roos, N. (2016). *Insects as food and feed in Kenya – past, current, and future perspectives.* Bondo, Kenya: Jaramogi Oginga Odinga University of Science and Technology (JOOUST).

Ayieko, M., Ogola, H., & Ayieko, A. (2016). Introducing rearing crickets (gryllids) at household levels: adoption, processing and nutritional values. *Journal of Insects as Food and Feed, 2*(3), 203-211. https://doi.org/10.3920/JIFF2015.0080

Ayieko, M., Oriaro, V., & Nyambuga, I. A. (2010). Processed products of termites and lake flies: improving entomophagy for food security within the Lake Victoria region. *African Journal of Food, Agriculture, Nutrition and Development, 10*(2), 2085–2098. https://doi.org/10.4314/ajfand.v10i2.53352

Bas, A., & El, S. N. (2022). Nutritional evaluation of biscuits enriched with cricket flour (Acheta domesticus). *International Journal of Gastronomy and Food Science, 29*, 100583. https://doi.org/10.1016/j.ijgfs.2022.100583

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020). Effect of Diet on the Growth Performance, Feed Conversion, and Nutrient Content of the House Cricket. *Journal of Insect Science, 20*(2), 10. https://doi.org/10.1093/jisesa/ieaa014

Berggren, Å., Janson, A., & Low, M. (2019). Approaching Ecological Sustainability in the Emerging Insects-as-Food Industry. *Trends in Ecological Evolution, 34*(2), 132-138. https://doi.org/10.1016/j.tree.2018.11.005

Bhatnagar-Panwar, M., Bhatnagar-Mathur, P., Bhaaskarla, V., Reddy, S., & Sharma, K. (2015). Rapid, accurate and routine HPLC method for large-scale screening of pro-vitamin A carotenoids in oilseeds. *Journal of Plant Biochemistry and Biotechnology, 24*, 84-92. https://doi.org/10.1007/S13562-013-0239-1

Biró, B., Sipos, M. A., Kovács, A., Badak-Kerti, K., Pásztor-Huszár, K., & Gere, A. (2020). Cricket-enriched oat biscuit: Technological analysis and sensory evaluation. *Foods, 9*(11), 1561. https://doi.org/10.3390/foods9111561

Caponio, G. R., Difonzo, G., Gennaro, G. d., Calasso, M., Angelis, M. D., & Pasqualone, A. (2022). Nutritional Improvement of Gluten-Free Breadsticks by Olive Cake Addition and Sourdough Fermentation: How Texture, Sensory, and Aromatic Profile Were Affected? *Frontiers in Nutrition, 9*(830932). https://doi.org/10.3389/fnut.2022.830932

Casolani, I., Pattara, C., & Liberatore, L. (2016). Water and Carbon Footprint Perspective in Italian Durum Wheat Production. *Land Use Policy, 58*, 394-402. https://doi.org/10.1016/j.landusepol.2016.07.014

Cooke, R. D., & Maduagwu, E. (2007). The effect of simple processing on cyanide content of cassava chips. *International Journal of Food Science & Technology, 13*(4), 299-306. https://doi.org/10.1111/j.1365-2621.1978.tb00807.x

County Government of Kisumu. (2023). *County Integrated Development Plan (2023-2027).* Kisumu, Kenya: County Government of Kisumu.

Dahal, S., Dangal, A., Pradhananga, M., Timsina, D., & Timsina, P. (2022). The preparation and quality evaluation of biscuit using composite flour by mixing wheat flour, chickpea flour, and Peanut flour. *International Journal on Food, Agriculture and Natural Resources, 3*(1), 14-19. https://doi.org/10.46676/ij-fanres.v3i1.58

Damodaran, S. (2017). Water and ice relations in foods. In S. Damodaran, & K. L. Parkin (Eds.), *Fennema's food chemistry* (pp. 19-90). CRC Press.

Diego, H., Javier, F., & Pilar, G.-C. (2022). Comparative study of the most commonly used methods for total protein determination in milk of different species and their

ultrafiltration products. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.925565

dos-Santos, S. S., Melo, D. M., Silva, L. A., Zago, T. A., Pontes, A. R., & Pinedo, R. A. (2017). Technological Use of Cassava and Passion Fruit Flours in Preparing Cookies. *Journal of Culinary Science and Technology, 15*(1), 54–63. https://doi.org/10.1080/15428052.2016.1204971

Durst, P., & Hanboonsong, Y. (2015). Small-scale production of edible insects for enhanced food security and rural livelihoods: Experience from Thailand and Lao People's Democratic Republic. *Journal of Insects as Food and Feed, 1*(1), 25-31. https://doi.org/10.3920/JIFF2014.0019

Elferink, M., & Schierhorn, F. (2016, April 07). Global Food Demand is Rising. Can We Meet It? *Harvard Business Review*. https://hbr.org/2016/04/global-demand-for-food-is-rising-can-we-meet-it

Ezeigbo, O. R., Ekaiko, M. U., & Ibegbulem, Z. O. (2015). Effect of Cooking Time on Starch and Cyanide Contents of Freshly Harvested Cassava Tubers Used for Tapioca Production. *British Biotechnology Journal, 8*(4), 1-6. https://doi.org/10.9734/BBJ/2015/16944

Fahad, S., Adnan, M., & Basir, A. (2018). *Global Wheat Production.* London, UK: Intech Open.

Falade, K. O., & Akingbala, J. O. (2008). Improved Nutrition and National Development Through the Utilization of Cassava in Baked Foods. In IUFoST, G. Robertson, & J. Lupien (Eds.), *Using Food Science and Technology to Improve Nutrition and Promote National Development.* Academic Press.

FAO. (2018). *The State of Food Security and Nutrition in the World; Building Climate Resilience for Food Security and Nutrition.* Rome, Italy: Food and Agriculture Organization of the United Nations.

FAOSTAT. (2016). *Production Crops Africa. Food and Agriculture Organization of the United Nations Statistics Division*. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#search/cassava%20production%20kenya

FAOSTAT. (2024, October 18). *FAOSTAT: Crops*. Retrieved November 23, 2019, from FAOSTAT. http://www.fao.org/faostat/en/#data/QC

Forkum, A. T., Wung, A. E., Kelese, M. T., Ndum, C. M., Lontum, A., Kamga, E. B., Nsaikila, M. N., & Okwen, P. M. (2025). Safety of cassava and cassava-based products: a

systematic review. *Frontiers in Sustainable Food Systems, 9*(1497609.). https://doi.org/10.3389/fsufs.2025.1497609

Ghosh, S., Ghosh, S., & Saha, S. (2017). Nutritional Values and Functional Properties of House Cricket (Acheta domesticus) and Field Cricket (Gryllus bimaculatus). *Food Science & Nutrition, 5*(1), 226-235. https://doi.org/10.3136/fstr.25.597

Ghosh, S., Jung, C., & Meyer-Rochow, V. B. (2018). What Governs Selection and Acceptance of Edible Insect Species? In S. Ghosh, C. Jung, & V. B. Meyer-Rochow, *Edible Insects in Sustainable Food Systems* (pp. 331-351). New York City: Springer Publishing Company.

Githunguri, C. M. (1995). Cassava food processing and Utilization in Kenya. In T. A. Egbe, A. D. Brauman, & S. T. Griffon, *Transformation Alimentaire du Manioc.* (pp. 120-132). Ostrom.

Guillermo, S.-S., Beatriz, M., André, B., Didier, D., Lotti, E., & Isidra, R. (2024). Current advances for in vitro protein digestibility. *Frontiers in Nutrition, 11*. https://doi.org/10.3389/fnut.2024.1404538

Hagler, A. N. (2006). Yeasts as Indicators of Environmental Quality. In G. R. Péter, *Biodiversity and Ecophysiology of Yeasts. The Yeast Handbook* (pp. 515-532). Springer, Berlin, Heidelberg.

Haider, N. N., Altemimi, A. B., George, S. S., Baioumy, A. A., El-Maksoud, A. A., Pasqualone, A., & Abedelmaksoud, T. G. (2022). Nutritional Quality and Safety Characteristics of Imported Biscuits Marketed in Basrah, Iraq. *Applied Sciences, 12*(18), 9065. https://doi.org/10.3390/app12189065

Halloran, A. M. (2017). *The impact of cricket farming on rural livelihoods, nutrition and the environment in Thailand and Kenya.* Copenhagen, Denmark: University of Copenhagen.

Hamaker, B., Kirleis, A., Butler, L., Axtell, J., & Mertz, E. (1987). Improving the in vitro protein digestibility of sorghum with reducing agents. *Applied Biology, 84*(3), 626–628. https://doi.org/10.1073/pnas.84.3.626

Hewavitharana, G. G., Perera, D. N., Navaratne, S., & Wickramasinghe, I. (2020). Extraction methods of fat from food samples and preparation of fatty acid methyl esters for gas chromatography: A review. *Arabian Journal of Chemistry, 13*(8), 6865-6875. https://doi.org/10.1016/j.arabjc.2020.06.039

Homann, A., Ayieko, M., Konyole, S., & Roos, N. (2017). Acceptability of biscuits containing 10% cricket (Acheta domesticus) compared to milk biscuits among 5-10-year-old

Kenyan schoolchildren. *Journal of Insects as Food and Feed, 3*(2), 95 - 104. https://doi.org/10.3920/JIFF2016.0054

Ibitoye, E. B., Lokman, I. H., Hezmee, M. N., Goh, Y. M., Zuki, A. B., & Jimoh, A. A. (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials, 13*(2), 025009. https://doi.org/10.1088/1748-605X/aa9dde

Ichikawa, N., Ng, L. S., Makino, S., Goh, L. L., Lim, Y. J., Ferdinandus, Sasaki, H., Shibata, S., & Lee, C.-L. K. (2022). Solid-state fermented okara with Aspergillus spp. improves lipid metabolism and high-fat diet induced obesity. *Metabolites, 12*(3), 198. https://doi.org/10.3390/metabo12030198

Imathiu, S. (2020). Benefits and food safety concerns associated with the consumption of edible insects. *Journal of the National Society of Nutrition and Food Science, 18*(1), 1-11. https://doi.org/10.1016/j.nfs.2019.11.002

Institute of Medicine. (2011). *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: The National Academies Press.

Iwe, M. O., Michael, N., Madu, N. E., Obasi, N. E., Onwuka, G. I., Nwabueze, T. U., Onuh, J. O., & Asumugha, V. U. (2017). Production and Evaluation of Bread Made from High Quality Cassava Flour (HQCF) and Wheat Flour Blends. *Agrotechnology, 6*(3), 1-8. https://doi.org/10.4172/2168-9881.1000166

Jansson, A., Hunter, D., & Berggren, Å. (2019). *Insects as food – an option for sustainable food production?* Uppsala, Sweden: Swedish University of Agricultural Sciences, the research platform Future Food.

Jisha, S., Sheriff, J., & Padmaja, G. (2010). Nutritional, Functional and Physical Properties of Extrudates from Blends of Cassava Flour with Cereal and Legume Flours. *International Journal of Food Properties, 13*(5), 1002-1011. https://doi.org/10.1080/10942910902934090

Karaduman, Y., Önder, O., Sayaslan, A., & Aydin, N. (2019). Utilisation of GlutoPeak tester on whole-wheat flour for gluten quality assessment. *Quality Assurance and Safety of Crops & Foods, 11*(3), 295-304. https://doi.org/10.3920/QAS2018.1319

Kewuyemi, Y. O., Kesa, H., Chinma, C. E., & Adebo, O. A. (2020). Fermented edible insects for promoting food security in Africa. *Insects, 11*(5), 283. https://doi.org/10.3390/insects11050283

Kim, D.-S., & Iida, F. (2023). Nutritional composition of Cassava (Manihot esculenta) and its application to elder-friendly food based on enzyme treatment. *International Journal of Food Properties*, *26*(1), 1311–1323. https://doi.org/10.1080/10942912.2023.2213410

Kim, T.-K., Yong, H. I., Kim, Y.-B., Kim, H.-W., & Choi, Y.-S. (2019). Edible Insects as a Protein Source: A Review of Public Perception, Processing Technology, and Research Trends. *Food Science of Animal Resouces*, *39*(4), 521-539. https://doi.org/10.5851/kosfa.2019.e53

Kolawole, O. F., & Akingbala, J. O. (2011). Utilization of Cassava for Food. *Food Reviews International*, *27*(1), 51-83. https://doi.org/10.1080/87559129.2010.518296

Kowalczewski, P. Ł., Gumienna, M., Rybicka, I., Górna, B., Sarbak, P., Dziedzic, K., & Kmiecik, D. (2021). Nutritional Value and Biological Activity of Gluten-Free Bread Enriched with Cricket Powder. *Molecules*, *26*(4), 1184. https://doi.org/10.3390/molecules26041184

Lacey, R. (2016). *Crickets as Food: The Perceptions of and Barriers to Entomophagy and the Potential for Widespread Incorporation of Cricket Flour in American Diets.* Ann Arbor, Michigan.: University of Michigan.

Langyan, S., Bhardwaj, R., Radhamani, J., Yadav, R., Gautam, R. K., Kalia, S., & Kumar, A. (2022). A Quick Analysis Method for Protein Quantification in Oilseed Crops: A Comparison With Standard Protocol. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.892695

Latimer, W. (Ed.). (2012). *Official Method of Analysis of AOAC International* (19th ed., Vol. Method 991.39). Maryland, USA: AOAC International.

Leng, L., Yang, P., Singh, S., Zhuang, H., Xu, L., Chen, W.-H., Dolfing, J., Li, D., Zhang, Y., Zeng, H., Chu, W., & Lee, P.-H. (2018). A review on the bioenergetics of anaerobic microbial metabolism close to the thermodynamic limits and its implications for digestion applications. *Bioresource Technology*, *247*, 1095-1106. https://doi.org/10.1016/j.biortech.2017.09.103

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, *529*, 84–87. https://doi.org/10.1038/nature16467

Liu, Y., Lv, M., Wang, Y., Wei, J., & Chen, D. (2025). Analytical Strategies for Tocopherols in Vegetable Oils: Advances in Extraction and Detection. *Pharmaceuticals*, *18*(8), 1137. https://doi.org/10.3390/ph18081137

Lukinac, J., Komlenić, D. K., Čolić, M. L., Nakov, G., & Jukić, M. (2022). Modelling the browning of bakery products during baking: a review. *Ukrainian Food Journal, 11*(2), 217-234. https://doi.org/10.24263/2304-974X-2022-11-2-3

Machado, C. d., & Thys, R. C. (2019). Cricket powder (Gryllus assimilis) as a new alternative protein source for gluten-free breads. *Innovative Food Science & Emerging Technologies, 56*, 102180. https://doi.org/10.1016/j.ifset.2019.102180

Manzocco, L., Romano, G., Calligaris, S., & Nicoli, M. C. (2020). Modeling the effect of the oxidation status of the ingredient oil on stability and shelf life of low-moisture bakery products: The case study of crackers. *Foods, 9*(6), 749. https://doi.org/10.3390/foods9060749

Masamba, K., Changadeya, W., Ntawuruhunga, P., Pankomera, P., Mbewe, W., & Chipungu, F. (2022). Exploring Farmers' Knowledge and Approaches for Reducing Post-Harvest Physiological Deterioration of Cassava Roots in Malawi. *Sustainability, 14*(5), 2719. https://doi.org/10.3390/su14052719

Melgar-Lalanne, G., Hernández-Álvarez, A.-J., & Salinas-Castro, A. (2019). Edible Insects Processing: Traditional and Innovative Technologies. *Comprehensive Reviews in Food Science and Food Safety, 18*(1), 44-49. https://doi.org/10.1111/1541-4337.12463

Mlcek, J., Rop, O., Borkovcova, M., & Bednarova, M. (2014). A Comprehensive Look at the Possibilities of Edible Insects as Food in Europe – a Review. *Polish Journal of Food and Nutrition Sciences, 64*(3), 145-157. https://doi.org/10.2478/v10222-012-0099-8

MoALF. (2017). *National food and Nutrition security policy Implementation framework.* Nairobi: Ministry of Agriculture, Livestock and Fisheries.

Morais, E., Cruz, A., Faria, J., & Bolini, H. (2014). Prebiotic gluten-free bread: Sensory profiling and drivers of liking. *LWT -Food Science and Technology, 55*(1), 248-254. https://doi.org/10.1016/j.lwt.2013.07.014

Münke-Svendsen, C., Kipkoech, C., Kinyuru, J., Ayieko, M. A., Homan, A., & Roos, N. (2017). *Technical Brief #5: Nutritional Properties of Insects for Food in Kenya.* Copenhagen, Denmark: GREEiNSECT Project.

Muoki, P. N., Kinnear, M., Emmambux, M. N., & de Kock, H. L. (2015). Effect of the addition of soy flour on sensory quality of extrusion and conventionally cooked cassava complementary porridges. *Journal of the Science of Food and Agriculture, 95*(4), 730-738. https://doi.org/http://hdl.handle.net/2263/44137

Næs, T., Tomic, O., Endrizzi, I., & Varela, P. (2021). Principal components analysis of descriptive sensory data: Reflections, challenges, and suggestions. *Journal of Sensory Studies, 36*(5), e12692. https://doi.org/10.1111/joss.12692

National Academy of Sciences. (2010). *Dietary Reference Intakes for Energy, Carbohydrate. Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005).* Washington, DC: National Academy of Sciences.

Ndiritu, A. K. (2017). *Physical, chemical and functional characterization of edible cricket (Acheta domesticus) protein concentrate.* Nairobi: Jomo Kenyatta University of Agriculture and Technology.

Nethathe, B., Phato, A. A., Mpho, E. M., & Shonisani, E. R. (2023). Microbial safety of ready-to-eat food sold by retailers in Thohoyandou, Limpopo province, South Africa. *Cogent Food and Agriculture, 9*(1), 2185965. https://doi.org/10.1080/23311932.2023.2185965

Njue, R., & Wawire, N. (2021). Performance evaluation of a cassava solar dryer in Busia County. *African Journal of Agricultural Research, 17*(10), 1296-1301. https://doi.org/10.5897/AJAR2021.15719

Nowakowski, A. C., Miller, A. C., Miller, M. E., Xiao, H., & Wu, X. (2022). Potential health benefits of edible insects. *Critical Reviews in Food Science and Nutrition, 62*(13), 3499-3508. https://doi.org/10.1080/10408398.2020.1867053

Nwosu, A. N., & Akubor, P. I. (2018). Acceptability and storage stability of biscuits produced with orange peel and pulp flours. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 12*(12), 8-15. https://doi.org/10.9790/2402-1212010815

Nzwalo, H., & Cliff, J. (2011). Konzo: From Poverty, Cassava, and Cyanogen Intake to Toxico-Nutritional Neurological Disease. *PLOS Neglected Tropical Diseases, 5*(6), e1051. https://doi.org/10.1371/journal.pntd.0001051

Ojiambo, O. C., Nawiri, M. P., & Masika, E. (2017). Reduction of cyanide levels in sweet cassava leaves grown in Busia County, Kenya based on different processing methods. *Food Research, 1*(3), 97-102. https://doi.org/10.26656/fr.2017.3.024

Okoko, J. U., Alonge, A. F., & Ngoddy, P. O. (2018). A review of rheological properties of high quality cassava flour (HQCF) composites. *Arid Zone Journal of Engineering, Technology and Environment, 14*(4), 208-218. https://doi.org/https://azojete.com.ng/index.php/azojete/article/download/267/183

Oladiran, D. A., Emmambux, M. N., & de Kock, H. L. (2018). Extrusion cooking of cassava-soy flour with 200 g/kg wheat bran promotes slower oral processing during

consumption of the instant porridge and higher derived satiety. *LWT, 97*, 778-786. https://doi.org/10.1016/j.lwt.2018.07.068

Olatunde, S. J., Oyeyinka, S. A., Adetola, R. O., Oyeyinka, A. T., & Owolabi, T. O. (2016). Physico-Chemical Properties of Pro-Vitamin A Cassava-Wheat Composite Flour Biscuit. *Food in Health and Disease, 5*(1), 20-26. https://doi.org/https://hrcak.srce.hr/169139

Omwamba, M., & Mahungu, S. (2014). Development of a Protein-Rich Ready-to-Eat Extruded Snack from a Composite Blend of Rice, Sorghum and Soybean Flour. *Food and Nutrition Sciences, 5*(14), 1309-1317. https://doi.org/10.4236/fns.2014.514142

Onyediako, P. O., & Adiele, J. G. (2022). Enhanced Cassava Production for Food Security and Economic Development in Nigeria: A review. *Nigerian Agricultural Journal, 53*(3), 204-211. https://doi.org/https://www.ajol.info/index.php/naj/article/view/243491

Panghal, A., Munezero, C., Sharma, P., & Chhikara, N. (2019). Cassava Toxicity, Detoxification and its Food Application: A Review. *Toxin Reviews, 40*(1), 1-16. https://doi.org/10.1080/15569543.2018.1560334

Park, B. K., & Kim, M. M. (2010). Applications of chitin and its derivatives in biological medicine. *International Journal of Molecular Sciences, 11*(12), 5152-5164. https://doi.org/10.3390/ijms11125152

Plötz, T., Krümmel, B., Laporte, A., Pingitore, A., Persaud, S. J., Jörns, A., Elsner, M., Mehmeti, I., & Lenzen, S. (2017). The monounsaturated fatty acid oleate is the major physiological toxic free fatty acid for human beta cells. *Nutrition & Diabetes, 7*(12), 305. https://doi.org/10.1038/s41387-017-0005-x

Poku, A., Birner, R., & Gupta, S. (2018). Making Contract Farming Arrangements Work in Africa's Bioeconomy: Evidence from Cassava Outgrower Schemes in Ghana. *Sustainability, 10*(5), 1604.

Rahman, T., Sulaiman, N. F., Turmala, E., Andriansyah, R. C., Luthfiyanti, R., & Triyono, A. (2019). Shelflife prediction of biscuits prepared from modified suweg (Amorphophallus campanulatus B) flour using Arrhenius model. *IOP Conference Series: Earth and Environmental Science. 251*, pp. 1-9. Bristol, England: IOP Publishing Ltd. https://doi.org/10.1088/1755-1315/251/1/012035

Roos, N. (2018). Insects and Human Nutrition. In A. Halloran, R. Flore, P. Vantomme, & N. Roos, *Edible Insects in Sustainable Food Systems* (pp. 83-91). New York: Springer.

Rostami, K., Rostami-Nejad, M., & Al-Dulaimi, D. (2015). Post gastroenteritis gluten intolerance. *Gastroenterol Hepatol Bed Bench, 8*(1), 66-70.

Rumphold, B., & Langen, N. (2019). Potential of enhancing consumer acceptance of edible insects via information. *Journal of Insects as Food and Feed, 5*(1), 45-53. https://doi.org/10.3920/JIFF2018.0041

Sachs, J., Remans, R., Smukler, S., Winowiecki, L., Andelman, S. J., Cassman, K. G., Castle, D., DeFries, R., Denning, G., Fanzo, J., Jackson, L. E., Leemans, R., Leemans, J., Milder, J. C., Naeem, S., Nziguheba, G., Palm, C. A., Reganold, J. P., Richter, D. D., Scherr, S. J., . . . Sanchez, P. A. (2010). Monitoring of the World's Agriculture. *Nature, 466*(7306), 558−560. https://doi.org/10.1038/466558a

Słowik-Borowiec, M., Głąb, N., Stach, S., & Szpyrka, E. (2023). A Miniaturized Sample Preparation Method for the Determination of Vitamins A and E in Food Products. *Molecules*, *28*(8), 3449. https://doi.org/10.3390/molecules28083449

Soare, C., Mazeri, S., McAteer, S., McNeilly, T. N., Seguino, A., & Chase-Topping, M. (2022). The microbial condition of Scottish wild deer carcasses collected for human consumption and the hygiene risk factors associated with Escherichia coli and total coliforms contamination. *Food Microbiology, 108*, 104102. https://doi.org/10.1016/j.fm.2022.104102

Szpyrka, E., Podbielska, M., & Książek-Trela, P. (2022). Simple Method for Fatty Acids Determination in Food, Superfood and Spice Samples by GC-MS Technique. *Acta Universitatis Cibiniensis Series E Food Technology, 26*(2), 171-182. https://doi.org/10.2478/aucft-2022-0014

Tagro, G., & Kambire, O. (2010). Biochemical and microbial changes during traditional spontaneous lactic acid fermentation process using two varieties of cassava for production of a “Alladjan” starter. *International Food Research Journal, 17*(3), 563-573.

Tang, C., Yang, D., Liao, H., Sun, H., Liu, C., Wei, L., & Li, F. (2019). Edible insects as a food source: a review. *Food Production, Processing, and Nutrition, 8*(1), 859-861. https://doi.org/10.1186/s43014-019-0008-1

Tao, J., & Li, Y. O. (2018). Edible insects as a means to address global malnutrition and food insecurity issues. *Food Quality and Safety, 2*(1), 17-26. https://doi.org/10.1093/fqsafe/fyy001

Taoukis, P., Labuza, T., & Saguy, I. (1997). Kinetics of food deterioration and shelf-life prediction. In K. Valentas, J. Rotstein, & E. Singh (Eds.), *The Handbook of Food Engineering Practice* (pp. 363-405). New York: CRC Press.

Tomlins, K., Parmar, A., Omohimi, C. I., Sanni, L. O., Adegoke, A. F., Adebowale, A.-R. A., & Bennett, B. (2021). Enhancing the Shelf-Life of Fresh Cassava Roots: A Field Evaluation of Simple Storage Bags. *Processes, 9*(4), 577. https://doi.org/10.3390/pr9040577

Tran, T., Da, G., Moreno-Santander, M. A., Veles-Hernandez, G. A., Giraldo-Toro, A., Piyachomkwan, K., Sriroth, K., & Dufour, D. (2015). A Comparison of Energy Use, Water Use and Carbon Footprint of Cassava Starch Production in Thailand, Vietnam and Colombia. *Resources, Conservation and Recycling, 100*, 31-40. https://doi.org/10.1016/j.resconrec.2015.04.007

USDA. (2018, April 1). *National Nutrient Database for Standard Reference Legacy Release*. Retrieved June 27, 2019, from United States Department of Agriculture. https://ndb.nal.usda.gov/ndb/foods/show/301800?manu=&fgcd=&ds=&q=Cassava,%20raw

van Huis, A. (2013). Potential of Insects as Food and Feed in Assuring Food Security. *Annual Review of Entomology, 58*, 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

van Huis, A. (2018). Insects as Human Food. In A. van Huis, *Ethnozoology* (pp. 195-213). Wageningen, The Netherlands: Wageningen University & Research.

van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible Insects: Future Prospects for Food and Feed Security*. Rome, Italy.: Food and Agriculture Organization for the United Nations.

Wahyuni, S., Holilah, Asranudin, & Noviyanti. (2018). Estimation of Shelf Life of Wikau Maombo Brownies Cake Using Accelerated Shelf Life Testing (ASLT) Method with Arrhenius Model. *IOP Conference Series: Earth and Environmental Science* (p. 122). Bristol, England: IOP Publishing. https://doi.org/10.1088/1755-1315/122/1/012082

Wambua, M., Matofari, J. W., Faraj, A. K., & Lamuka, P. O. (2016). Effect of Different Cassava Varieties (Manihot esculenta) and Substitution Levels in Baking of Wheat-cassava Composite Bread on Physical Properties and Sensory Characteristics. *African Journal of Food Science and Technology, 7*(6), 131-139. https://doi.org/10.14303/ajfst.2016.071

Wang, Z., & Wang, L. (2024). Impact of sourdough fermentation on nutrient transformations in cereal-based foods: Mechanisms, practical applications, and health implications. *Grain & Oil Science and Technology, 7*(2), 124-132. https://doi.org/10.1016/j.gaost.2024.03.001

Zubelzu, S., Álvarez, R., & Hernández, A. (2015). Methodology to calculate the carbon footprint of household land use in the urban planning stage. *Land Use Policy, 48*, 223-235. https://doi.org/10.1016/j.landusepol.2015.06.005

APPENDICES

Appendix I: Setup of the Completely Randomised Design Used in the Experiment

		Cricket substitution (%)			
Flour type %w/w		0	12	24	36
	Unfermented	100	88	76	64
	Controlled	100	88	76	64
	Spontaneous	100	88	76	64

Appendix II: Sensory Panel Recruitment Form

Sensory Panel Recruitment Form

I…… have voluntarily agreed to take part in this study. I understand that I will not directly benefit from this study. I have had the study explained to me and I understand that it entails sensory evaluation of cassava-cricket biscuits. My participation in this study involves tasting of the cassava-cricket biscuits and profiling the predetermined sensory properties according to my perception against the standards. I confirm that I am not allergic to cassava-cricket biscuits and do not have any issue consuming a food product containing cassava-cricket biscuits. I understand that the results will be kept for [duration] after the date of examination and will be treated confidentially. My identity will remain anonymous after the study and this will be done by coding my details.

…… ……

Signature of participant Date

……

Signature of researcher

I believe that the participant is giving informed consent to participate in this study.

Appendix III: Score Sheet for Sensory Analysis

Score sheet for a 7-scale descriptive sensory evaluation

Panellist code……………………………… Name of Panellist………………………………

Date………………………

Instructions:

You are provided with coded samples. You are required to score and record each sample as per your judgement of the attributes listed on the left side of the table in the appropriate box. You will use a descriptor scale to score the sensory attributes accordingly. You can score 7 = most similar, 6 = more similar, 5 = similar, 4 = neither similar nor dissimilar, 3 = dissimilar, 2 = more dissimilar, 1= most dissimilar.

- Please rinse your mouth before starting.
- Evaluate the product in front of you by looking at it, feeling it and tasting it.
- Assign an appropriate score for each of the listed parameters/components.

Biscuits specific evaluation

APPEARANCE

Colour: Brown

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Less						**More**

Consistency: visual uniformity of the sample/level of smoothness

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**

	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						**More**

Density: compactness of the cross-section

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						**More**

AROMA

Baked: aroma associated with baked products.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						**More**

FLAVOUR

Caramel: flavour associated with caramelization.

Sample	1	2	3	4	5	6	7
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						More

Salty: associated with iodized salt.

Sample	1	2	3	4	5	6	7
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						More

Sugary: sweetness associated with baking sugar.

Sample	1	2	3	4	5	6	7
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						More

Astringency: causes puckering/shrinking of the tongue surface.

Sample	1	2	3	4	5	6	7

	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Less						**More**

Starchy: associated with high starch foods.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Less						**More**

Buttery: associated with baking butter/margarine.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Less						**More**

Bitterness: produces bitter taste in the mouth.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**

	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						**More**

Lingering: how long the after-taste lasts in the mouth.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Less						**More**

TEXTURE

Dryness: amount of saliva required when chewing

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Moist						**Dry**

Cohesiveness: how particles tend to agglomerate/stay together during chewing

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Particulate						**Cohesive**

Fracturability: the force with which the sample raptures

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Crumbly						**Brittle**

Grittiness: Amount of small, hard particles between teeth during chew

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	None						**High**

Particles: quantity of particles left in the mouth

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	None						**Many**

Stickiness: degree to which residues stick to the teeth.

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	None						**High**

Oiliness: amount of oil left in the mouth

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Less						**More**

BISCUIT LIKING QUESTIONS

You will now use a 5-point hedonic scale to score your overall liking of the biscuits considering all characteristics **(APPEARANCE, FLAVOUR and TEXTURE)** indicate your overall opinion by checking one box [√]. You can score 5 = like extremely, 4 = like, 3 = neither like nor dislike, 2 = dislike, 1= dislike extremely.

Sample	**1**	**2**	**3**	**4**	**5**

[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
Dislike extremely		**Neither like nor dislike**		**Like extremely**

Please re-taste the product as needed and indicate how much you LIKE or DISLIKE the following. *Check* the box that represents your response **[√].**

Overall appearance

Sample	**1**	**2**	**3**	**4**	**5**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Dislike extremely		**Neither like nor dislike**		**Like extremely**

Overall flavour

Sample	**1**	**2**	**3**	**4**	**5**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**

	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Dislike extremely		**Neither like nor dislike**		**Like extremely**

Overall texture

Sample	**1**	**2**	**3**	**4**	**5**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	[ ]	**[ ]**	**[ ]**	**[ ]**	**[ ]**
	Dislike extremely		**Neither like nor dislike**		**Like extremely**

Appendix IV: The ANOVA Table Showing Fermentation and Enrichment Effects on Proximate Composition

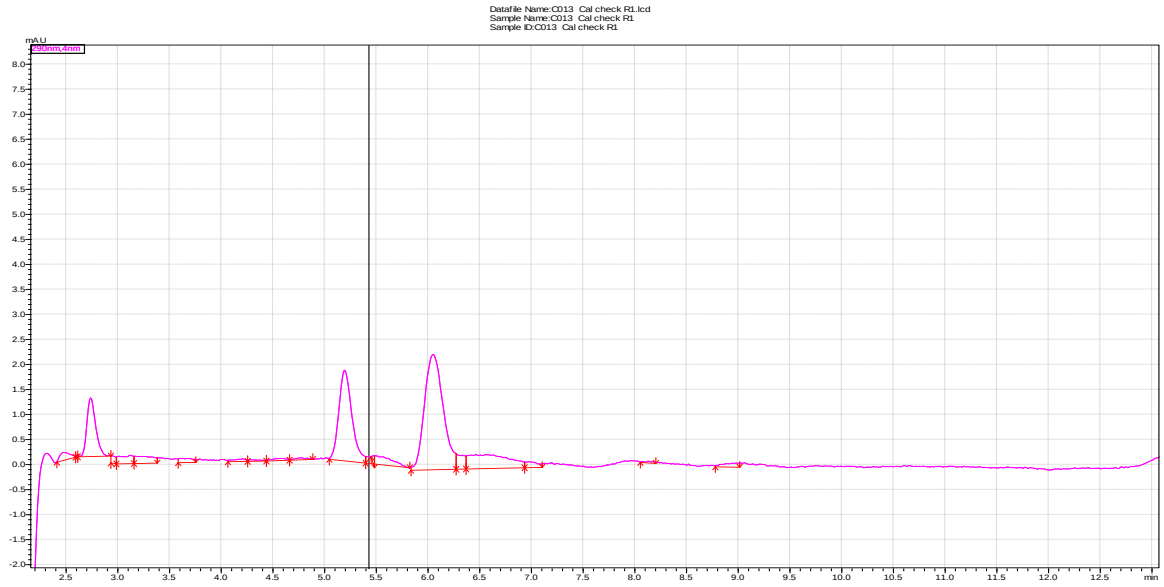
SOV	**D.o.F**	**Moisture**	**Protein**	**Ash**	**Fat**	**Fiber**	**CHO**
Fermentation	2	0.67^{***}	13.42^{***}	2.30^{***}	2.50^{ns}	4.35^{ns}	4.35^{ns}
Ratio	3	6.15^{***}	1765.13^{***}	1.80^{***}	7.38^{***}	191.21^{***}	101.92^{***}
Fermentation*ratio	6	0.35^{***}	10.32^{***}	0.15^{*}	6.72^{***}	2.08^{*}	1.11^{*}
Rep	2	0.04	1.01	0.09	0.65	1.42	1.42
Error	13	0.08	0.79	0.051	1.00	1.87	1.88
R^2	-	92.82	99.67	90.79	75.60	93.54	93.54
CV	-	11.56	4.50	6.14	2.53	7.88	7.88
MSD	-	0.36	1.16	0.30	1.31	1.79	2.18

Key: Source of Variation, D.O.F= Degree of Freedom, R^2 = Coefficient of determination, CV = Coefficient of Variation, and MSD = Minimum significant difference

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.735
Bartlett's Test of Sphericity	Approx. Chi-Square	315.077
	df	45
	Sig.	0.000

Appendix V: Tocopherol Elution Chart



Extracted Standard chromatogram (290nm)

RETENTION TIME	COMPOUND
2.742	Retinol
5.403	Gamma Tocopherol
6.288	Alpha Tocopherol

Appendix VI: NACOSTI Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **355238**

Date of Issue: **19/March/2020**

RESEARCH LICENSE

This is to Certify that Mr.. Kevine Otieno of Egerton University, has been licensed to conduct research in Kisumu on the topic: Assessment of Protein, Sensory Qualities and Shelf-Life of Fermented Cassava Biscuits Enriched with Cricket Flour. for the period ending : 19/March/2021.

License No: **NACOSTI/P/20/4029**

355238

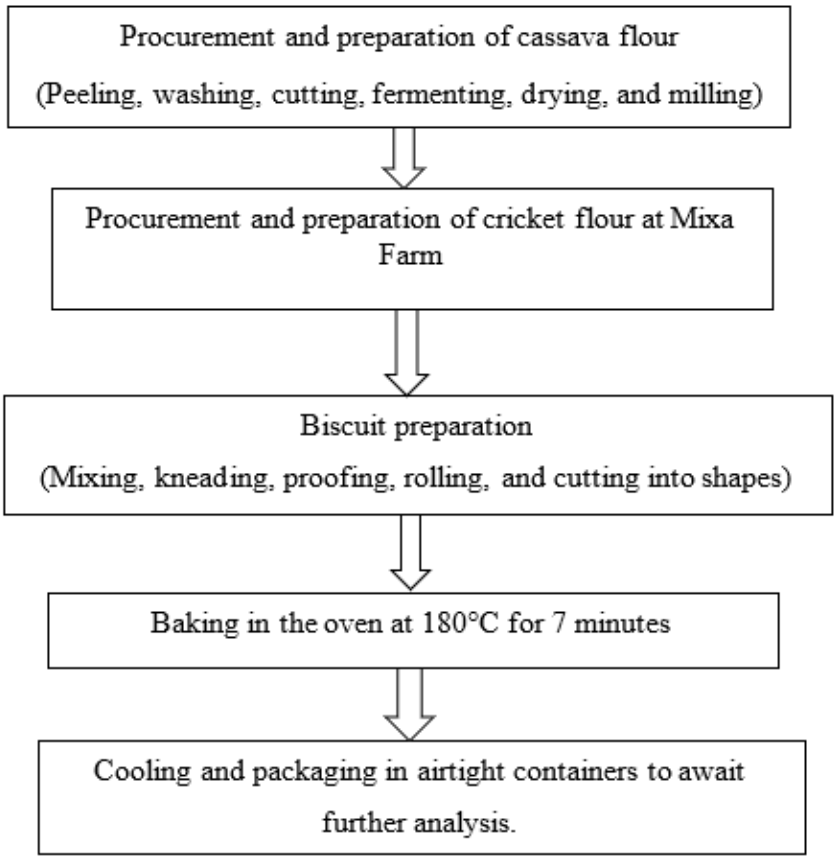
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix VI: Biscuit Processing Flow Chart



Appendix VIII: Cassava-cricket Biscuits (Image by Author)

Appendix IX: Journal Paper Abstract

E. Afri. Agri. For. J (2021,Volume 85, 1-4, Pg. 40-47)

NUTRIENT COMPOSITION OF BISCUITS CONTAINING FERMENTED CASSAVA AND CRICKET FLOUR

K. O. Otieno#, P. S. Muliro and M. N. Omwamba

Department of Dairy, Food Science and Technology, Egerton University P.O. Box 536-20115, Egerton.

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a highly adaptable root crop that can be utilized as an excellent source of carbohydrate. The objective of this study was to determine the effects of fermentation of cassava and compositing with cricket flour (*Acheta domesticus*) on the nutrient content of the biscuits. The treatments (fermentation and blending ratios) were arranged in a 3 × 4 factorial arrangement under completely randomized design (CRD). The biscuits were baked in a fan-assisted oven at 170 °C for 7 minutes. Biscuit samples were subjected to proximate analysis. All samples showed an increase in protein and ash content ($P<0.05$) on fermentation with increased level of cricket flour. At the same time, fibre and carbohydrate decreased ($P<0.05$) with an increase in cricket flour content in the mix. The drop in carbohydrate content is attributable to the utilization of carbohydrates by the microorganisms during fermentation. The fat content of the biscuits did not vary among the treatments ($P>0.05$) and is attributed to the use of margarine during preparation. There was a positive ($P<0.001$) correlation ($R^2=99.67$) between blending and protein quantities in the biscuits indicating that cricket flour can be a valuable source of protein for enriching cassava to make biscuits hence enhancing productivity and diversification of food sources to tame the protein-energy malnutrition in Kenya.

Key words: *cassava biscuits, cricket flour, insect protein*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a highly adaptable and resilient tropical root crop of the *Euphorbiaceae* family that is a staple food crop in Africa, Asia and South America (Adebayo, 2008). Cassava is prepared and consumed in different forms across the African continent. For instance in Nigeria, *gari* is the most common method of cassava preparation and consumption (Kolawole and Akingbala, 2011; Amarachi *et al*., 2015). *Gari* is a fine to coarse textured cassava tuber flour made by grating the cassava tubers, squeezing out the starch and leaving it to ferment for 3-7 days. It can then be fried with or without palm oil (Kolawole and Akingbala, 2011). It can also be used to make bread (Iwe *et al*., 2017). In Kenya, cassava is roasted, milled and blended with other flours for use in *ugali* and porridge, boiling of the fresh tubers and most recently, slicing into thin chips and frying in oil to make crisps (Abong *et al.,* 2016). The sweet cassava varieties are the most popular due to their low hydrogen cyanide content (<50 mg HCN/kg) and one of the four important sources of starch besides maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (Tagro and Kambire, 2010; Isendahl, 2011). In 2016, Sub-Saharan Africa produced 157, 271, 697 tonnes of cassava, of which 571, 848 tonnes were from Kenya (FAOSTAT, 2016). Compared to wheat, cassava is relatively cheaper to produce but the value chain losses have estimated at 23%. Cassava tubers can retain starch quality for up to nine months and can therefore be harvested piecemeal unlike wheat (Abong *et al*., 2016).

Cassava has relatively low protein content of about 2% compared to wheat (12%), necessitating compositing to increase the protein content of the final cassava-based product (Jisha *et al*., 2010; Abass *et al*., 2018). Cassava flour is particularly deficient in the sulphur-containing amino acids methionine and cysteine, which has seen several researchers mix the flour with other cereals and legume flours to improve its overall protein content (Jisha *et al.,* 2010; Omwamba and Mahungu, 2014).

Research indicates that cassava flour can be substituted for wheat on a 1:1 ratio in the formulation of baked products (Falade and Akingbala, 2008). Cassava has no gluten hence suitable for baking food products for consumers with gluten intolerance. It also has minerals

#Corresponding author:kevobiero@gmail.com