# QUALITY AND STABILITY OF CRICKET (*Acheta domesticus*) ENRICHED CASSAVA (*Manihot esculenta* Crantz) FLOUR

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**20/U/GMFT/13333/PD**

**A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF SCIENCE IN FOOD TECHNOLOGY OF KYAMBOGO UNIVERSITY**

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# DECLARATION

## Declaration by Candidate

I, Jjoloba Wilberforce, declare that this dissertation is my original piece of work and has never been submitted to any university or institution of higher learning for the award of a degree.

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## DEDICATION

To my Dad, Ssalongo Wasswa Kayiira and my wife, Jackieline Nakiberu Jjoloba for always believing in me and supporting my research ideas as well as striving to ensure I succeed in my education and life as a whole.

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## ABSTRACT

Cassava, being a starchy staple, contains minimal protein and other nutrients, which limits its utilisation as a complete food. Incorporating edible insects, such as house crickets, has shown promise in improving the nutritional profile of staple foods like maize and millet. However, the impact of house cricket powder incorporation on cassava flour properties remains relatively unexplored. This study aimed to enhance the nutritional composition of cassava flour using cricket powder without compromising its nutritional, functional, safety, shelf life and sensory properties. By so doing, the study contributes to the resilience and well-being of populations reliant on cassava as a staple food.

Four improved cassava varieties i.e. Mkumba, NAROCASS 1, NASSE 3 and NASSE 14 and one local variety i.e. Magana were selected based on their agronomical properties and processed into cassava flour. House crickets underwent two treatments prior to processing into cricket powder. Composites were formulated using Design expert software after which they were screened to obtain the sensorialy acceptable formulation for chemical, functional, microbial and shelf life studies. Standard methods were used for all analyses. Findings were analysed using ANOVA.

Sensory analysis revealed a significant negative impact of cricket powder on colour, aroma, aftertaste, and overall acceptability. Protein content significantly increased by 5.54% ($p < 0.05$) and fat content 2.21% ($p < 0.05$) in cassava-cricket composite flours. Solubility decreased while swelling capacity and oil absorption capacity increased with cricket powder incorporation. Chemical analysis showed absence of aflatoxin and pesticide residues, while microbiological assays showed absence of Salmonella sp. and *Staphylococcus aureus*. Over a 24-week storage period, cassava-cricket composite flours' moisture content, peroxide value, and acid value, remained with the range recommended by Codex Alimentarius and East African standards. Total plate count and total coliform counts declined consistently during the shelf life study period, indicating microbial suppression. Sensory attributes and levels of yeast and moulds remained consistent throughout storage, suggesting the stability of cassava-cricket composite flours over 24 weeks. Findings suggest that cricket powder enhances the nutritional value of cassava flour without compromising safety, functionality, shelf life or sensory acceptability. Further studies could explore the use of cricket powder in different food matrices.



Key words: Cassava-cricket composite flour, nutritional enrichment, acceptability, safety, cassava-cricket stiff porridge

# CHAPTER ONE: INTRODUCTION

## 1.1 Background

Approximately 600 million people in Asia, Africa, and Latin America rely on cassava as a means of livelihood with a significant portion of this population, exceeding 300 million people, situated in the sub-Saharan African region (FAO, 2018; Dada et al., 2018). Cassava production in East Africa was estimated at 35,754,620 metric tonnes (MT) with Uganda contributing approximately 2,660,000 MT in 2019 (FAOSTAT, 2024). Ninety-three percent of cassava produced in Uganda is reported as food supply quantity (FAOSTAT, 2024). In Uganda, approximately 200,000 MT of cassava is consumed as flour annually in various domestic and industrial applications (Silva et al 2017; Kleih et al. 2012). However, cassava is primarily composed of carbohydrates making up about 71 to 84 g/100 g, fat up about 1.08 g/100 g, and low levels of essential nutrients such as protein (1 to 3 g/ 100g), vitamins (0.08 to 29.1 mg / g) and minerals (27.2 to 451 mg/ g). This nutritional deficiency poses a challenge for populations heavily reliant on cassava as a dietary staple, as it contributes to malnutrition and associated health issues. The cassava flour protein content is reported to be lower than that of common staples consumed in Uganda such as maize (*Zea mays*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) (Liang et al., 2019). As a result, cassava has been characterized as lacking in nutritional richness (Ogunyemi et al., 2018; Abdulrahman and Omoniyi 2016; Emmanuel et al., 2012).

There has been a growing emphasis over the last several years on enhancing the nutritional quality of cassava flour by incorporating substitute sources of protein (Udomkun et al., 2019; Lima et al., 2013; Montagnac et al., 2009). One such potential protein source is powder derived from the house cricket *Acheta domesticus*. House crickets possesses substantial nutritional value having a high crude protein content of 64.38 to 70.75 g/100 g on dry weight basis. Based on their protein content, these edible insects show promise for utilization in both human and

animal nutrition (Bbosa et al., 2019; Klunder et al., 2012). House crickets contain crude fat (9.80 - 22.80 g/100 g), crude fibre (14.92 to 22.08 g/100 g), nitrogen-free extract (carbohydrate) (11.80-12.27 g/100 g), ash (3.57 - 9.10 g/100 g), omega-3 fatty acids (~0.25 g/100 g), omega-6 fatty acids (~1.55 g/100 g), and iron (~5.0 mg/100 g), and energy (414.14 - 455.19 Kcal/100 g) on a basis of dry weight (Bbosa et al., 2019; Finke, 2015; Rumpold and Oliver, 2013). Their potential lies in serving as dietary supplements fulfilling the recommended dietary intake (RDI) for protein (FAO, 2013). Cricket farming is considered sustainable and environmentally friendly due to its negligible greenhouse gas emission, low land and water demands, and efficient feed conversion (Murugu et al., 2021; Megido et al., 2017; Fuah et al., 2016). Incorporation of cricket powder into cassava could therefore enhance the nutritive value of the cassava flour, and therefore offer a promising solution to address both nutritional deficiencies and sustainability concerns.

## 1.2 Problem statement

In Uganda, cassava holds the second highest significance as a food crop, following cooking bananas, with an average consumption of 60.76 kg per capita/year (FAOSTAT, 2022). The low content of protein and other nutrients limits its utilization as a complete food and is thus regarded as a nutritionally poor crop. On the other hand, house crickets is high in protein (64.38 - 70.75 g/100 g) on a basis of dry weight, and iron (5.0 mg/100 g) ( Bbosa et al., 2019; Mariod et al., 2017). The Food and Agricultural Organisation (FAO) recommends use of insects in food production because of their high nutrient density (FAO, 2013). Simeon et al. (2022) have used cricket powder to enrich millet flour in a study. Studies by Olaniran et al. (2020), Dada et al. (2018), Lima et al. (2013), have been dedicated to enhancing the protein concentration of cassava derivatives using, cow pea-orange fleshed sweet potato blend, cow peas and cassava leaves, respectively. However, little is known regarding the effect of enrichment of cassava flour with house cricket powder.

## 1.3 Objectives

### 1.3.1 General objective

To enhance the nutritional and sensory properties of cassava flour through incorporation of cricket (*Acheta domesticus*) flour.

### 1.3.2 Specific objectives

1. To determine the sensorialy acceptable level of enrichment of cassava flour with cricket powder
2. To determine the nutritional (protein, fat, ash, fibre, carbohydrate, moisture) and functional (water holding capacity, oil absorption capacity, bulk density, swelling capacity, solubility, dispersibility) properties of the sensorialy acceptable cassava-cricket composite flour
3. To assess the effect of cricket powder incorporation on safety (*E.coli*, *Staphylococcus aureus*, Enterobacteriaceae, Yeasts and Moulds, *Salmonella typhimurium*, aflatoxins, pesticide residues) and shelf life (total plate count, Yeasts and Moulds, acid value, peroxide value, aroma, colour) of the sensorialy acceptable cassava-cricket composite flour at ambient temperature storage

## 1.4 Hypotheses

1. There is an acceptable level of cricket powder enrichment in cassava flour that maintains or enhances sensory acceptability.
2. The nutritional (protein, fat, ash, fibre, carbohydrate, moisture) and functional (water holding capacity, oil absorption capacity, bulk density, swelling capacity, solubility, dispersibility) properties of acceptable cassava-cricket composite flour are not significantly different from that of cassava flour
3. The safety (*E.coli*, *Staphylococcus aureus*, Enterobacteriaceae, Yeasts and Moulds, *Salmonella typhimurium*, aflatoxins, pesticide residues) and shelf life (total plate count,

Yeasts and Moulds, acid value, peroxide value, aroma, colour) of acceptable cassava-cricket composite flour are not significantly different from that of cassava flour at ambient temperature storage

## 1.5 Justification

Enriching cassava flour with cricket powder could significantly increase its protein content and improve its overall amino acid profile. The enrichment can elevate the nutritional significance of cassava-based products and contribute to combating protein-energy malnutrition, especially in communities where the main food is cassava. This could contribute to achieving of sustainable development goal 2 aimed to end hunger, achieve food security and promote sustainable agriculture.

Incorporating house crickets as a protein source aligns with the principles of sustainable food production, given their lower land, water and feed requirements in comparison to conventional livestock, along with reduced greenhouse gas (Murugu et al., 2021; Megido et al., 2017; Fuah et al., 2016)

The research addresses the issue of food security by exploring ways to enhance nutritional composition of a widely consumed staple food such as cassava. By enhancing the nutritional value and durability of cassava flour, this study seeks to increase the food systems' resilience and improve the well-being of populations reliant on cassava as a primary food source.

Understanding the acceptability and perception of cricket enriched cassava products among consumers is crucial for the successful adoption of the products. Investigating consumer attitudes, preferences and willingness to incorporate these products into their diets provide valuable insights for product development and market acceptance

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Composite flours and their role in addressing malnutrition

Composite flour is described as a blend of whole gluten-free flour derived from mixtures of flours from various sources such as grains, tubers, roots, legumes and pulses. It is intended for the creation of both conventional and innovative food products (Lakshmi et al., 2015). By blending flours from various sources, composite flour can offer a diverse array of vital nutrients, encompassing vitamins and minerals, proteins, and dietary fibre (Banua et al., 2023). This nutrient diversity can help combat multiple forms of malnutrition, such as in iron, zinc, calcium, and vitamins deficiencies. Incorporating protein-rich flours, such as legumes or insect flours, into composite blends can significantly boost the protein concentration in the end product (Udomkun et al., 2019; Dada et al., 2018).

Protein malnutrition can be addressed by providing a balanced source of amino acids contributing to overall better health and growth, especially in children. According to the Uganda Demographic Health Surveys (UDHS) of 2016, 39% of children under five years are seriously stunted (Tiara, Muhammad and Masruhim, 2016). Stunting is attributed to a protein intake below the recommended dietary requirements (Endrinikapoulos et al., 2023).

## 2.2 Cassava

### 2.2.1 Description

A vital staple food that is widely consumed around the world, particularly in tropical and subtropical areas, is cassava. (FAOSTAT, 2022). Cassava also known as *muwogo* in Luganda, *singkong* in Indonesia *nwugo* in Nigeria, is valued for its versatility, affordability, and ability to thrive in diverse agro-ecological conditions (Nanyonjo et al., 2021; Nakabonge et al., 2018).

### 2.2.2 Cassava production

World Production is estimated at 299,028,225 MT with Nigeria and Democratic Republic of Congo being the top two producers (Table 2.1). Uganda stands as the 18th cassava producer globally and ranks eleventh on the African continent after Benin, Cameroon, Malawi, Mozambique, Côte d'Ivoire, Tanzania, Angola, Ghana, Democratic republic of Congo and Nigeria. (Table 2.1, FAOSTAT, 2024). However, cassava has certain nutritional and functional limitations that require consideration.

Table 2.1: Top 20 nations that cultivate cassava on a significant scale globally.

| SN | Country | Quantity produced per year (MT ) | | | | |
|---|---|---|---|---|---|---|
| | | 2017 | 2018 | 2019 | 2020 | 2021 |
| 1. | Nigeria | 61,350,199 | 65,350,850 | 56,969,160 | 55,565,610 | 58,237,500 |
| 2. | Democratic Republic of Congo | 37,699,983 | 38,873,036 | 40,050,112 | 42,769,463 | 45,673,454 |
| 3. | Thailand | 30,495,190 | 29,368,185 | 31,079,966 | 28,999,122 | 35,094,485 |
| 4. | Ghana | 19,008,724 | 20,845,960 | 22,750,000 | 24,368,000 | 24,997,000 |
| 5. | Brazil | 18,501,645 | 17,877,163 | 17,593,186 | 18,197,572 | 18,220,656 |
| 6. | Indonesia | 19,053,748 | 16,119,020 | 16,350,000 | 16,271,000 | 15,731,000 |
| 7. | Cambodia | 13,817,300 | 13,750,100 | 13,512,800 | 13,757,218 | 17,048,500 |
| 8. | Vietnam | 10,267,568 | 9,847,074 | 10,174,858 | 10,504,203 | 10,565,589 |
| 9. | Angola | 8,326,745 | 8,730,517 | 9,000,432 | 9,592,870 | 9,866,553 |
| 10. | Tanzania | 4,025,265 | 8,372,217 | 8,184,093 | 7,088,393 | 6,150,411 |
| 11. | Côte d'Ivoire | 5,367,000 | 5,608,044 | 5,8772,30 | 6,443,565 | 6,302,334 |
| 12. | Mozambique | 3,867,000 | 6,346,023 | 6,019,201 | 6,026,000 | 6,218,141 |
| 13. | India | 4,171,000 | 4,950,000 | 4,976,000 | 6,060,000 | 6,941,000 |
| 14. | Malawi | 4,960,556 | 5,410,506 | 5,708,032 | 5,858,745 | 6,101,396 |
| 15. | Cameroon | 5,322,429 | 5,658,753 | 5,676,050 | 5,779,727 | 5,904,383 |
| 16. | China | 4,958,762 | 5,011,700 | 5,042,748 | 5,067,415 | 5,036,849 |
| 17. | Benin | 3,959,450 | 3,819,804 | 4,525,450 | 4,161,660 | 4,218,992 |
| 18. | Uganda | 2,729,260 | 4,390,231 | 2,660,000 | 2,084,000 | 2,257,790 |
| 19. | Burundi | 2,285,265 | 2,386,709 | 2,408,958 | 2,426,653 | 2,494,775 |
| 20. | Central African Republic | 1,461,120 | 1,519,565 | 1,580,347 | 1,031,200 | 1,062,100 |

Source: (FAOSTAT, 2024)

### 2.2.3 Cassava consumption

According to the Food and Agricultural organisation, Democratic Republic of Congo ranks as the largest cassava consumer in the world (Table 2.2, FAOSTAT, 2024). Uganda ranks 15th globally and 13th in Africa after Tanzania, Cameroon, Malawi, Benin, Mozambique, Nigeria, Côte d'Ivoire, Central African Republic, Burundi, Angola, Ghana and Democratic republic of Congo. (Table 2.2, FAOSTAT, 2024).

Table 2.2: Cassava consumption by the top 20 cassava-producing countries

| SN | Country | Cassava consumption (kg /capita/year ) | | | | |
|---|---|---|---|---|---|---|
| | | 2017 | 2018 | 2019 | 2020 | 2021 |
| 1. | Democratic Republic of Congo | 410.49 | 409.65 | 409.07 | 393.36 | 407.85 |
| 2. | Cambodia | 256.87 | 251.87 | 261.14 | 260.73 | 245.83 |
| 3. | Ghana | 229.02 | 246.34 | 224.36 | 226.32 | 230.78 |
| 4. | Angola | 204.15 | 216.36 | 222.41 | 237.58 | 242.25 |
| 5. | Burundi | 190.14 | 199.27 | 189.22 | 189.73 | 192.56 |
| 6. | Central African Republic | 176.43 | 178.61 | 179.79 | 145.43 | 146.59 |
| 7. | Côte d'Ivoire | 129.32 | 131.96 | 137.88 | 146.53 | 142.75 |
| 8. | Nigeria | 122.04 | 120.59 | 125.48 | 161.54 | 163.25 |
| 9. | Mozambique | 117.09 | 173.34 | 160.61 | 154.46 | 156.9 |
| 10. | Benin | 109.95 | 111.13 | 113.05 | 112.36 | 110.88 |
| 11. | Malawi | 104 | 104.34 | 101.95 | 102.56 | 105.18 |
| 12. | Cameroon | 95.3 | 100.42 | 96.45 | 114.27 | 113.18 |
| 13. | Tanzania | 67.19 | 81.27 | 88.4 | 85 | 75.71 |
| 14. | Indonesia | 68.3 | 56.05 | 56.45 | 56.9 | 54.72 |
| 15. | Uganda | 66.42 | 81.9 | 61.49 | 46.26 | 47.74 |
| 16. | Brazil | 32.28 | 31.46 | 30.36 | 31.14 | 30.8 |
| 17. | Vietnam | 8.85 | 8.33 | 8.76 | 9.78 | 9.35 |
| 18. | India | 3.09 | 3.43 | 3.43 | 4.12 | 4.61 |
| 19. | Thailand | 2.16 | 1.3 | 3.6 | 2.04 | 3.03 |
| 20. | China | 2.11 | 2.13 | 2.1 | 2.13 | 2.17 |

Source: (FAOSTAT, 2024)

In Uganda, cassava stands as the second most consumed staple after cooking bananas followed by maize, sweet potatoes, millet, beans and rice. The consumption levels of cassava are

estimated at 60.76 kg per capita (FAOSTAT, 2024). The root is commonly consumed in its fresh state, dried, or transformed into chips or flour, all of which are utilized in the preparation of porridges, paste, and bread and pancakes (Nakabonge et al., 2018; Cazumbá da Silva et al., 2017). Cassava porridge is an important food used for nourishing infants and serving as a refreshing beverage for individuals in other age brackets (Onyango et al., 2020).

### 2.2.4 Nutrient composition of cassava in comparison to other foods

According to Montagnac et al. (2009), the importance of the nutritional composition of cassava tubers arises from their central role as the primary edible component in developing nations. These roots predominantly provide carbohydrates, yet exhibit lower vitamin and protein levels in comparison to crops like wheat, corn, as well as certain animal and vegetable sources of nutrients (Table 2.3, Montagnac et al., 2009). This implies that communities dependent on cassava, which has ~ 1.3 g/100 g protein need to consume 1000 g of cassava dry weight per day to fulfil this requirement, which is not possible. Therefore, enriching cassava flour with nutritious substances such as house cricket powder can contribute to cassava-based diets meeting the protein requirements for cassava-dependent communities (Alamu et al., 2020).

Table 2.3: Relative nutritional content of cassava when contrasted with alternative food sources

| Food | Water (g) | Energy (kcal) | Energy (kj) | Protein (g) | Total lipid (g) | Ash (g) | Carbohydrate (g) | Dietary fiber (g) | Sugars (g) |
|---|---|---|---|---|---|---|---|---|---|
| Cassava, raw root | 59.68 | 160 | 667 | 1.36 | 0.28 | 0.62 | 38.06 | 1.8 | 1.7 |
| Potato, raw | 79.34 | 77 | 321 | 2.02 | 0.09 | 1.08 | 17.47 | 2.2 | 0.78 |
| **Cereals** | | | | | | | | | |
| Wheat flour, unenriched | 11.92 | 364 | 1523 | 10.33 | 0.98 | 0.47 | 76.31 | 2.7 | 0.27 |
| Bread, wheat | 35.74 | 266 | 1115 | 10.91 | 3.64 | 2.2 | 47.51 | 3.6 | 5.75 |
| Rice, white, unenriched | 12.89 | 360 | 1506 | 6.61 | 0.58 | 0.58 | 79.34 | --- | --- |
| Corn, sweet, white, raw | 75.96 | 86 | 358 | 3.22 | 1.18 | 0.62 | 19.02 | 2.7 | 3.22 |
| Corn, yellow | 10.37 | 365 | 1527 | 9.42 | 4.74 | 1.2 | 74.26 | 7.3 | 0.64 |
| Sorghum | 9.2 | 339 | 1418 | 11.3 | 3.3 | 1.57 | 74.63 | 6.3 | --- |
| **Vegetables (raw)** | | | | | | | | | |
| Green beans | 90.27 | 31 | 129 | 1.82 | 0.12 | 0.66 | 7.13 | 3.4 | 1.4 |
| Carrots | 88.29 | 41 | 173 | 0.93 | 0.24 | 0.97 | 9.58 | 2.8 | 4.74 |
| Spinach | 94 | 14 | 59 | 1.5 | 0.2 | 1.8 | 2.5 | --- | --- |
| Lettuce, green leaf | 95.07 | 15 | 61 | 1.36 | 0.15 | 0.62 | 2.79 | 1.3 | 0.78 |
| Soybeans, green | 67.5 | 147 | 614 | 12.95 | 6.8 | 1.7 | 11.05 | 4.2 | --- |
| **Animal products** | | | | | | | | | |
| Raw egg (white) | 87.57 | 52 | 216 | 10.9 | 0.17 | 0.63 | 0.73 | 0 | 0.71 |
| Cheese, Cheddar | 36.75 | 403 | 1684 | 24.9 | 33.14 | 3.93 | 1.28 | 0 | 0.52 |
| Milk (whole) | 88.32 | 60 | 252 | 3.22 | 3.25 | 0.69 | 4.52 | 0 | 5.26 |
| Raw fish (trout) | 71.42 | 148 | 619 | 20.77 | 6.61 | 1.17 | 0 | 0 | 0 |

**Source:** (Montagnac et al., 2009)

### 2.2.5 Benefits of consuming cassava

Cassava roots contain essential nutrients including thiamine (0.1 mg/100 g), carotenoids (120 μg/100 g), vitamin C (20 mg/100 g), nicotinic acid (0.5 mg/100 g) and riboflavin (0.02 mg/100 g) (Lima et al., 2013; Charles et al., 2005). These roots also present notable proportions of

calcium (15 to 40 mg/100 g) and phosphorus (20 to 50 mg/100 g) (Charles et al., 2005; Lima et al., 2013). Cassava flour is free from gluten, thus it's importance for development of foods for celiac patients whose numbers are increasing worldwide (Rollán et al., 2019)

### 2.2.6 Cassava processing

Cassava roots possess bulkiness and are highly perishable, necessitating their transformation into more shelf stable forms like flour (Dada et al., 2018). Processing extends shelf life and helps reduce the hydrogen cyanide (HCN) levels, thereby ensuring the safety of cassava flour for human consumption (Dada et al., 2018). Within the sub-Saharan Africa region, conventional techniques for cassava processing vary according to geographic area (Dada et al., 2018). In Nigeria, for instance, submerged tuber fermentation a common technique is used to create cassava products e.g. fufu, papuru and gari (Dada et al., 2018), whereas heap fermentation is prevalent in North Western Uganda and Mozambique for cassava flour production (Nanyonjo et al., 2021). Although different Ugandan cassava types are capable of being transformed into flour through a non-fermentation process, certain populations tend to favour cassava products derived from fermented flour (Nanyonjo et al., 2021)

### 2.2.7 Health concerns regarding cassava consumption

According to Liang et al. (2019) and Emmanuel et al. (2012), cassava flour is nutritionally inadequate because of low protein. This deficiency affects a substantial portion of the population in Acholi, Lango, Teso, Bunyoro and Busoga Sub-regions, as well as Arua, Nebbi, and Yumbe, Mukono, Kayunga, and Buikwe districts. Cassava should therefore be consumed in combination with other protein-rich foods such as vegetables, fish, and meat (Khatri & Acharya, 2021).

## 2.3 Entomophagy and house crickets

Entomophagy has been defined by the Food and Agriculture Organization (FAO), as the practice of consuming insects by humans. This practice is observed in various nations across the world, especially in regions of Africa, Asia, and Latin America. There are roughly 2 billion individuals who eat insects (FAO, 2013). Insects are consumed whole or ground into powders or pastes, and discreetly incorporated into other foods. it is therefore acknowledged that insects hold nutritional value as a food source (FAO, 2013). Humans have traditionally included insects in their cuisine, although certain cultures exhibit a level of aversion towards their consumption, especially if not indigenous (FAO, 2013) hence the need to assess the sensory acceptability of composite flours/foods with crickets.

### 2.3.1 Edible insects

More than 1,900 varieties of edible insects constitute a part of human diets globally (Mlcek et al., 2014). The house crickets (*Acheta domesticus*) hold the fourth position in terms of frequency of consumption (Mlcek et al., 2014; FAO, 2013). House crickets, are a species of cricket commonly found in homes and are frequently utilized as a source of food for reptiles, birds, and humans (Van Huis et al., 2013). They are particularly abundant in tropical areas characterized by warm temperatures because their growth is accelerated compared to colder environments (Fuah et al., 2016.). According to Ooninex et al. (2020), cricket farming is recognized for its sustainability and low environmental impact

For equivalent protein production, house crickets require six, four and, two times less feed than cattle, sheep and broiler chickens, respectively (Fuah et al., 2016). Furthermore, their greenhouse gas emissions are low (Fuah et al., 2016) . They also produce less waste, making them a more sustainable protein source (Fuah et al., 2016). House crickets therefore present a promising avenue for conventional protein production which can be harnessed for direct human consumption or indirectly through the incorporation of insect protein extract in processed

foods. In addition, their potential extends to serving as a protein component within various feed formulations (Oonincx et al., 2020).

House crickets can be cultivated using organic waste material such as vegetable remnants or agricultural by-products, which serves to alleviate the environmental pollution linked to waste disposal (Megido et al., 2017). The creation of cricket powder demands fewer resources, rendering it an appealing choice within the realm of sustainable food production systems (Osimani et al., 2018).

### 2.3.2 Processing of edible house crickets for food

According to Liceaga (2021) and Muzzarelli (2011), insect should or may be processed into powder form or derive their components such as lipids, protein, chitin (a long-chain polymer of N-acetylglucosamine) and chitosan (a natural polysaccharide, which is found in the exoskeletons of crickets). When used in powder form, insects can be added to culinary products to help counteract negative perceptions.

### 2.3.3 Preservation and safety issues of house crickets

Crickets are cultivated in environments such as agricultural waste and manure, which have the potential to introduce microbiological risks (van der Fels-Klerx et al., 2018). The rich nutritional composition of house crickets provides an optimal medium for the growth of harmful and spoilage microorganisms, under favourable conditions (Klunder et al., 2012). Pathogenic microorganisms that may be carried by crickets include bacteria (*Campylobacter, Acinetobacter, Staphylococcus,* Enterobacteriaceae, *Bacillus, Escherichia, Pseudomonas, Micrococcus* and *Proteus*). They may also be contaminated by protozoa, viruses and fungi (Klunder et al., 2012). To ensure safety edible insects are subjected to thermal treatment (e.g., roasting and oven drying) and adhere to appropriate storage conditions before introducing them to the market. Roasting is particularly advisable for mitigating microbiological risks;

nonetheless, standardized processing protocols are essential (Turck et al., 2021; Megido et al., 2017).

House crickets may potentially possess chemical pollutants, arising from the presence of these contaminants within the surroundings and may be the medium (van der Fels-Klerx et al., 2018). Such substances comprise heavy metals (lead, cadmium, arsenic, zinc, copper, chromium and mercury), veterinary drug residues (e.g. antifungals, antibiotics and nutritional supplements) and pesticide residues (e.g. malathion, chlorpyrifos, diazinon, cypermethrin, permethrin, deltamethrin, imidacloprid, clothianidin, thiamethoxam, carbaryl, methomyl, methoprene and pyriproxyfen). The principal pathway for chemical exposure occurs through the substrate used for insect cultivation, leading to variation in the types of concerning chemicals. As an illustration, insects cultivated using agricultural residue could be subjected to mycotoxins, whereas those cultivated on manure may face greater exposure to remnants of veterinary medications (van der Fels-Klerx et al., 2018). Consequently, ensuring food items derived from house crickets are safe is of utmost importance.

### 2.3.4 Health benefits of consuming house crickets

Insects offer a valuable source of high-quality protein and essential nutrients, comparable to those present in fish and meat (Udomsil et al., 2019). Their nutritional profile includes; omega-3 fatty acids (alpha-linoleic acid, docosahexaenoic acid and eicosapentaenoic acid), omega-6 fatty acids (linoleic acid), vitamin C, dietary fibre and minerals (magnesium, iron, copper, zinc, selenium, manganese, and phosphorus) (Table 2.4). This makes house crickets particularly valuable as a dietary supplement for malnourished children (Udomsil et al., 2019).

Table 2.4: Nutritional Profile of House Crickets (*Acheta domesticus*)

| Nutrient | Mariod et al. (2017) | Bbosa et al. (2019) | FAO (2013) | Olaniran et al. (2020) | Dada et al. (2018) |
|---|---|---|---|---|---|
| Protein (g/100 g) | 64.38 - 70.75 | 65.00 | 66.00 | 67.50 | 68.20 |
| Fat (g/100 g) | 13.00 - 18.00 | 15.00 | 14.50 | 16.00 | 15.70 |
| **ω-3 Fatty Acids (g/100 g)** | 0.50 | 0.55 | 0.52 | 0.53 | 0.54 |
| ALA (g/100 g) | 0.20 | 0.22 | 0.21 | 0.22 | 0.21 |
| (DHA) (g/100 g) | 0.15 | 0.16 | 0.16 | 0.17 | 0.16 |
| EPA (g/100 g) | 0.15 | 0.17 | 0.15 | 0.14 | 0.17 |
| ω-6 Fatty Acids (Linoleic Acid) (g/100 g) | 2.00 | 2.10 | 2.05 | 2.15 | 2.12 |
| Vitamin C (mg/100 g) | 2.00 | 2.20 | 2.10 | 2.25 | 2.15 |
| Dietary Fiber (g/100 g) | 8.00 | 8.50 | 8.20 | 8.70 | 8.40 |
| Iron (mg/100 g) | 5.00 | 5.50 | 5.20 | 5.30 | 5.40 |
| Phosphorus (mg/100 g) | 120.00 | 125.00 | 123.00 | 127.00 | 124.50 |
| Magnesium (mg/100 g) | 60.00 | 62.00 | 61.00 | 63.00 | 62.50 |
| Selenium (µg/100 g) | 30.00 | 32.00 | 31.00 | 33.00 | 32.50 |
| Manganese (mg/100 g) | 1.00 | 1.10 | 1.05 | 1.15 | 1.12 |
| Zinc (mg/100 g) | 10.00 | 11.00 | 10.50 | 11.20 | 10.80 |
| Copper (mg/100 g) | 0.50 | 0.55 | 0.52 | 0.53 | .0.54 |
| Calcium (mg/100 g) | 75.00 | 80.00 | 78.00 | 82.00 | 79.50 |
| Carbohydrates (g/100 g) | 12.00 | 12.00 | 12.50 | 11.80 | 12.20 |
| Energy (kcal/100 g) | 450.00 | 460.00 | 455.00 | 470.00 | 465.00 |

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

### 2.4 Functional properties of flours

Functional properties refers to the physical, chemical and biological characteristics of flours that affect their performance and behaviour in various applications such as baking, cooking and thickening (Kate et al., 2019). These properties determine how flours interact with other ingredients, respond to processing conditions and influence the final product's texture, structure, appearance and shelf life (Kate et al., 2019).

These properties are subject to the influence of food components, notably proteins, carbohydrates, moisture, fibre, fats and oils, and ash (Kate et al., 2019). The addition of other constituents to the base (flour), the structural composition of these constituents also exert an influence on these properties. Consequently, it becomes imperative to assess the behaviour of novel proteins, fibres, and carbohydrates within specific systems, thereby determining whether

such proteins can effectively substitute or enhance traditional fat, protein, carbohydrate (starch and sugars), and fibre components (Kate et al., 2019).

### 2.4.1 Bulk and tapped density

Bulk density pertains to partitioning of the mass of numerous flour particles by the collective volume they occupy (Kate et al., 2019). Bulk density can change based on how the food substance is managed. For instance, when flour is poured into a cylinder, it exhibits a specific bulk density; however, if the cylinder is subjected to mechanical perturbation, the flour particles tend to relocate and compact more tightly, resulting in an elevated bulk density. Consequently, the volume density for flours is often documented in terms of both "tapped" density and "untapped" (or "freely settled") density. Tapped density signifies the bulk density of flours subsequent to a defined compaction process, often involving agitation of the container (Kate et al., 2019). The granule dimensions and compactness of the flour chiefly influence it, and it holds substantial significance in influencing packaging requisites. In essence, a higher bulk density of flour necessitates more dense packaging material for adequate packaging. Bulk density provides insights into the permeability of a food item, that has implications for packaging design and can aid in influencing the optimal material for packaging (Iwe et al., 2016). Chandra & Samsher (2013) suggested a plausible connection between the initial level of moisture of flours and their bulk density. It was proposed by Chandra & Samsher (2013) that flours possessing high bulk density are well suited for utilization in food preparations, while flours characterized by low bulk density could be advantageous in the creation of supplementary foods.

### 2.4.2 Water holding capacity

Water holding capacity (WHC) describes the quantity of water retained by the flour, which mainly trigger the gelatinization and melting of molecules (Rodríguez-Miranda et al., 2012). Variability in the Water holding capacity of flours can be due to differences in protein content,

the extent to which they interact with water, and also due to their structural properties (Kate et al., 2019). Factors contributing to the water holding capacity of a flour encompass starch (representing approximately 46% of the absorbed water), pentosans (associated with around 23% of total absorbed water) and proteins (accounting for approximately 31% of absorbed water) (Kate et al., 2019). Components like vital wheat gluten (VWG) when incorporated enhance water absorption and imparts greater dough stability. Additionally, the presence of other moisture-retaining elements like fiber, bran hydrocolloids (gums), eggs, among others, can influence water binding (Kate et al., 2019).

### 2.4.3 Oil absorption capacity

The primary process behind the absorption of lipids is primarily associated with the inherent capture of oil plus the entrapment of fat into the polar chains of proteins (Kate et al., 2019). Adding oil to food items enhances mouthfeel and also contributes to the retention of flavours (Kate et al., 2019). An excellent ability to absorb oil highlights the lipophilic characteristics of the flour's ingredients (Kate et al., 2019). Foods rich in protein exhibit a high rate of oil absorption. The capacity of protein in food to absorb oil is attributable to the internal elements, including protein structure, amino acid makeup, and surface polarity or hydrophobic nature (Chandra & Samsher, 2013). The existence of unpolar amino acid side groups in the hydrophobic section enables them to interact with the hydrocarbon side groups of oil in foods and flours, potentially augmenting the oil absorption capacity of flours (Chandra et al., 2015). Flours exhibiting high oil absorption capacity have promise for reinforcing molecular interactions in food structures, particularly for enhancing taste, prolonging shelf life, and preserving flavours, in products like meat and baked goods where oil absorption is advantageous (Chandra et al., 2015). The process underlying oil absorption typically entails capillary interactions within the food matrix, facilitating the retention of absorbed oil (Kate et al., 2019).

### 2.4.4 Swelling capacity

Swelling capacity (SC) refers to the millilitre volume taken up by one gram (1 g) of food material as it swells under specific conditions (Kate et al., 2019). This parameter quantifies the starch's ability to soak up water and expand, serving as an indicator of the associative forces within starch granules. The swelling capacity of flours is subject to influences such as granule size, species variation, manufacturing techniques, or procedural steps, and holds significance as a quality metric in certain food items like bakery products (Iwe et al., 2016; Chandra & Samsher, 2013). It serves as a marker of the noncovalent interactions among starch granule molecules and is closely linked to factors like the α-amylose and amylopectin ratios. High starch content augments the ability of flours to expand, particularly in starch containing higher proportions of the ramified amylopectin (Iwe et al., 2016).

### 2.4.5 Dispersibility

The capacity of flour or starch to reconstitute in water is measured by their dispersibility. A higher dispersibility indicates more effective reconstitution in water, yielding a finer component during mixing (Adebowale et al., 2012).

## 2.5 Pasting properties of flours

The behaviour of starch during heating and cooling in the presence of water is referred to as its "pasting properties," and it is essential for evaluating the texture and quality of foods high in starch (Kate et al., 2019). These properties include parameters such as pasting temperature, peak viscosity, breakdown, setback, and final viscosity. They are typically measured using a rheometer or a viscometer and are important for understanding how starch behaves during cooking and processing, which may affect the final product's stability, consistency, and texture (Kate et al., 2019). Variations in the genetic makeup of cassava determine variability in pasting properties. These differences guide the usage of flour from various types for various purposes (Nuwamanya et al., 2023).

### 2.5.1 Peak viscosity

Peak viscosity (PV) provides insights into the starch or blend's water-retention capability. The peak viscosity is influenced by factors such as temperature, degree of mixing, applied shear stress, and the material's intrinsic characteristics. Higher peak viscosity corresponds to a greater swelling index, while lower peak viscosity suggests increased solubility due to starch degradation or dextrinization (Kate et al., 2019).

## 2.6 Safety and shelf life of composite flours

Flours are vulnerable to rapid moisture absorption, clumping, and undesired colour changes (Ojo & Adeola, 2017). In regions with high humidity exceeding 77%, such as tropical and subtropical zones, exposure to these conditions can foster the growth of mould. The storage stability of flours hinges on the diffusion of air, the migration of moisture, and the water activity level (Ojo & Adeola, 2017). Mould development brings about alterations regarding the product's chemical makeup (Amadi & Adebola, 2008).

The safety and shelf life of enriched composite flour are important considerations to guarantee a product's integrity and quality. Previous research has looked into the microbiological and chemical safety of enriched composite flours (Turck et al., 2022; Dada et al., 2018; Megido et al., 2017; Menon et al., 2015; Lima et al., 2013).

### 2.6.1 Microbiological safety

Microbial safety is a crucial aspect of enriched composite flour. Ensuring appropriate processing and storage conditions are essential to minimise the risk of microbial contamination. Findings have shown that cricket-enriched flours processed using appropriate hygienic practices and stored under suitable conditions can maintain microbiological safety within acceptable limits (Turck et al., 2022). Nevertheless, it's essential to acknowledge that enriched composite flour also depends on the safety of the ingredients used (Klunder et al., 2012).

### 2.6.2 Chemical safety

Chemical contamination of composite flours may be largely due to use of chemically contaminated raw materials and contamination during processing (Saleh et al., 2017). The potential for product contamination increases when house crickets are exposed to agricultural waste, pesticides, and toxic metals during their rearing and processing stages (Nimisha, 2023). Inadequate drying during the processing and storage of cassava and cricket powder can lead to fungal contamination, particularly by species such as *Fusarium, Aspergillus*, and *Penicillium,* that are known to produce harmful mycotoxins (Saleh et al., 2017). Serious health consequences can result from aflatoxins exposure, including both acute (such as aflatoxicosis) and chronic (such as liver cancer, liver damage, immunological suppression, and impaired growth) effects (Wild & Gong, 2010)

### 2.6.3 Product Shelf life

Product shelf life is the amount of time that a product can be kept in a given environment and yet retain its intended quality, safety, and efficacy (Kumar et al, 2019). During its shelf life, a product should remain suitable for consumption or use and should not undergo significant deterioration in quality, performance, or safety. Shelf life of enriched composite flour relies on various factors, including; quality of ingredients, manufacturing techniques, packaging and storage conditions (Dada et al., 2018). Regular quality assessments, including sensory evaluation, microbial testing and chemical analysis, can help monitor the product's freshness and safety to ensure that the cricket-enriched cassava flour remains within acceptable quality standards throughout its shelf life (Turck et al., 2022).

## 2.7 Sensory evaluation

Sensory evaluation holds significant importance within the food industry, serving as a vital tool for tasks such as product development, recipe adjustments, and product assessment (Sharif et al., 2017). Additionally, it has a significant impact in maintaining quality standards and

facilitating effective product marketing (Sharif et al., 2017). Incorporating sensory and consumer testing in the product development phase often enables the cost-effective creation and distribution of products that meet consumer satisfaction, thereby reducing the likelihood of product shortcomings (Singh-ackbarali & Maharaj, 2014). Some of the sensory evaluation methods include acceptability testing and qualitative descriptive analysis. An acceptability test is a test used to assess the palatability, taste, appearance and overall consumer satisfaction of a product (Sharif et al., 2017). It involves gathering feedback from a group of target consumers to determine how well a product meets their sensory preferences through liking and disliking. Acceptability tests play a pivotal role in ensuring that a product meets consumer expectations, aligns with market demands, and maintains high quality standards (Sharif et al., 2017). Qualitative descriptive analysis is a sensory evaluation method used to characterise and describe the sensory attributes of a product using descriptive language (Tomlins et al., 2003). Trained panelists evaluate and articulate the various sensory characteristics like visual appeal flavour, texture, aroma and overall perception. Line scaling is a technique commonly used in qualitative descriptive analysis to quantify the intensity or magnitude of sensory attributes (Navarro et al., 2021). It involves presenting panelists with a set of reference samples or product variations, each representing a specific level of sensory attribute being evaluated, panelists are then asked to rate the samples along a continuous line or scale according to the perceived intensity of the attribute. This method allows for a relative comparison of the sensory attribute's strength or magnitude among the samples (Navarro et al., 2021). The importance of qualitative descriptive analysis lies in its ability to provide detailed sensory information about a product, which is crucial for product development, quality control and consumer understanding.

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Materials

Four improved cassava varieties i.e. MKUMBA, NAROCASS 1, NASSE 3 and NASSE 14 and one local variety i.e. MAGANA were selected based on their agronomical properties (Appendix 1). MKUMBA and NAROCASS 1 fresh roots (*Manihot esculenta* Crantz) at optimal maturity level of 10 and 13 months, respectively, were obtained from the National Crops Resources Research Institute (NaCCRI-NARO) in Namulonge, NASSE 3 at 10 months from Oyengopere village, Kwera subcounty in Dokolo district, NASSE 14 at 12 months from Akakai village in Soroti district and MAGANA at 11 months from Mpyenza village Bugiri district. Following harvest, 100 kg sacks of cassava roots were sent right away to the Kawanda laboratory and processed into cassava flour. House crickets (adults, 12 weeks old) were sourced from MAMIDECOT microfinance (an organization for cricket farmers in greater Masaka region, Uganda). They were transported in cooler boxes (-5$^{o}$C) to Kyambogo University Food Science Laboratory for processing into cricket powder.

## 3.2 Sample preparation and selection

### 3.2.1 Preparation of cassava flour

As soon as the cassava roots arrived at the laboratory, they were processed using the described method in the ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) practical guide series, (2007). The roots underwent sorting to eliminate damaged portions, then cleaning with potable water to get rid of any soil that was attached and prevent introduction of impurities in the following processing stages. Subsequent to peeling, the roots were further cleansed using portable water to ensure their cleanliness. A mechanical grater (GD-CG-3000, Doing Holding-Henan Jinrui Food Engineering Co. Ltd, China), was used to finely grate the peeled cassava roots into chips. The grated cassava chips were placed on clean metallic trays and then dried in an electrical drier (JW-1350ED, Jinwoo Industrial Systems Co. Ltd, South

Korea) at 60 ºC for 14 h (Chimphepo et al., 2021). To create fine cassava flour from the dried chips, a Cross-Beater mill (Glen Mill Corp., Maywood, NJ) with a 0.5 mm screen was used. The flours were packaged in polythene-lined nylon bags and then sealed using fibre thread. The sacks were transported to Kyambogo University Food Science Laboratory for use in product development. The flours were kept on pallets at ambient temperature (18 - 28ºC, Norwegian Meteorological Institute, 2023).

#### 3.2.2 Preparation of cricket powder

*Acheta domesticus* (2 kg) was washed under running tap water. The sample was drained using a plastic colander followed by oven drying (WTB binder, Tuttlingen, Germany) at 105°C for a duration of 17 h. The dried samples was ground using an electric grinder (Medical Research Council (MRC) laboratory grinder, London, UK). The resulting cricket powder was sifted through a sieve with a mesh size of 0.595 mm. Roasted cricket powder was obtained by roasting 2 kg of washed fresh *A. domesticus* samples in an oven (Memmert GmbH Co KG, UM400) at 150°C for 90 min (Halloran et al. 2017) and then ground into cricket powder using the electric grinder. The obtained powder was packaged in sterile zip-lock high-density polypropylene (HDPE) bags and stored at ambient temperature.

#### 3.2.3 Formulation and sensory screening of cassava-cricket powder composites

Using the Design Wizard in Design Expert (version 13) software, an optimal combined design was selected to generate runs for the sensory optimization study. This was chosen due to its flexibility and ability to accommodate custom models, numerical factors, categorical factors, and mixtures of components. The study intended to develop a mixture of cassava flour and cricket powder within the ranges of 70 – 95 % cassava flour and 5–30 % cricket powder. These components were the categorical variables at several levels each, i.e., the cassava flour was from 5 varieties (hence 5 levels) and the cricket powder was made from either pre-heated crickets (referred to as raw in this study) or roasted ones (hence 2 levels).

The D-optimal option of the design was used because it best estimates the effects of the factors involved an aspect best suited for screening studies. The response factors were sensory attributes, i.e., colour, appearance, taste, aroma, flavour, texture, mouthfeel, after-taste, and overall acceptability. The design generated 40 runs (Appendix 2), each with a unique combination of the different levels of each of the factors.

For example, the first run (run 1) had 70% cassava flour (*Mkumba* variety), 30 % cricket powder (roasted type). The combinations were given three letter codes to enable blind coding during sensory evaluation.

## 3.3 Sensory evaluation of cassava-cricket composites

### 3.3.1 Preparation of cassava-cricket stiff porridge for sensory analysis

Cassava-cricket stiff porridge was prepared from the eight composites and then subjected to affective sensory evaluation. Cassava-cricket stiff porridge preparation followed the procedure outlined by Naluwagga & Meron (2022) with certain adjustments such as mingling the slurry at reduced heat after 7 min of mingling. Approximately 500 g flour was added to 1000 mL of boiling water on a gas cooker (Blueflame Gas Cooker O-300J). The blend was allowed to continue boiling for 2 min, and then mingled using a wooden ladle for 7 min. The mingling of the slurry was continued for an additional 3 min at reduced heat. After 10 min, the bread was removed from the saucepan and kept in a vacuum flask to keep hot before serving.

### 3.3.2 Sensory acceptability testing of cassava-cricket stiff porridge

Using a nine-point hedonic scale, the sensory acceptability was evaluated, following the methodology outlined by Wichchukit & Mahony (2014). The scale ranged from 1 to 9, where 1 represented "dislike extremely," 2 indicated "dislike very much," 3 corresponded to "dislike moderately," 4 denoted "dislike slightly," 5 stood for "neither like nor dislike," 6 signified "like slightly," 7 indicated "like moderately," 8 represented "like very much," and 9 indicated "like

extremely”. Forty untrained participants were chosen to offer a compromise between attaining dependable and replicable outcomes and handling the pragmatic limitations of arranging and carrying out the sensory evaluation (Lawless & Heymann, 2010, Meilgaard et al, 2006). Untrained panelists were used because they provide feedback based on their natural, everyday experiences with food, which is valuable for understanding real-world acceptability (Lawless & Heymann, 2010). Before evaluation, the panelists were required to make an informed consent by reviewing and appending their signatures on a consent form. The descriptors' definitions were clarified, after which participants were given instructions to begin assessing the product. Palate cleansing was facilitated with water between samples, and for those panelists who chose not to ingest the samples; receptacles with covers were provided for expectoration. Approximately 10 g of encoded samples with random three digit codes were randomly presented to each panellist for evaluation using a sensory acceptability ballot. The sensory attributes evaluated included colour, taste, aftertaste, flavour, appearance, texture, aroma, mouthfeel and overall acceptability.

Sensory acceptability testing was used to (i) screen the formulated composites, and (ii) select the most acceptable composites among the eight most desirable composites screened by the software. The selected cassava-cricket composite flours (Table 3.1) were presented for qualitative descriptive profiling and other analyses.

Table 3.1: Selected cassava-cricket composite flours after screening

| | | **Composite characteristics** | | | |
|---|---|---|---|---|---|
| SN | Sample | Cassava Flour (%) | Cricket powder (%) | Cassava Variety | Cricket Treatment |
| 1 | MRWF | 90.71 | 9.29 | MAGANA | Raw |
| 2 | MRCF | 89.48 | 10.52 | MAGANA | Roasted |
| 3 | NRWF | 91.64 | 8.36 | NAROCASS1 | Raw |
| 4 | NRCF | 90.51 | 9.49 | NAROCASS1 | Roasted |
| 5 | MGN | 100 | 0 | MAGANA | None |
| 6 | NR | 100 | 0 | NAROCASS1 | None |

Samples 5 = Control flour 1 and sample 6 = Control flour 2

### 3.3.3 Quantitative descriptive profiling of the cassava-cricket composite flours

Qualitative descriptive analysis was executed following the approach by Navarro et al. (2021). The sensory characterisation of cassava-cricket composite flour were carried out in Owen B lecture room at Kyambogo University. The method involved three stages: recruitment, initial screening, and establishment of descriptive language and terms, with the aid of reference material. A questionnaire was used to recruit 40 candidates. Participants were required to make an informed consent by reviewing and appending their signature on a consent form. The pre-selection phase for recruiting candidates included a series of four triangular tests. Nine panelists were then selected following the criterion of an assertion of 65% of the tests. Panelists were trained on the criteria for generating descriptive terms and consensus building around those terms. Assessors were presented with the product to generate descriptors for color and aroma by consensus. During the familiarization phase, the assessors were introduced to the descriptive terms and their corresponding reference materials at a round table during one session. The assessors underwent a training regimen lasting 30 days to familiarise themselves with the developed terms. Subsequently, the assessors carried out a preliminary assessment to confirm their proficiency after training.

### 3.3.4 Sensory shelf life test

The sensory shelf life assessment of cassava-cricket composite flour was conducted following method by Ojo & Adeola, (2017). Each flour sample weighed roughly 100 g and was put within sterile zip-lock high-density polypropylene (HDPE) bags (16 cm × 19 cm), sealed, and then stored on a cupboard shelf under ambient conditions (18 - 28°C, relative humidity of 75%, Norwegian Meteorological Institute, 2023) for a duration of six months. The nine panelists were presented with coded samples randomly to rate the attributes of colour and aroma on a line scale for 6 months at 3 weeks interval.

## 3.4 Proximate analysis of the acceptable cassava-cricket composite flours

### 3.4.1 Determination of moisture content

Moisture content was assessed using method number 925.10 (AOAC, 2012). Approximately 3 g of a well-mixed test portion was accurately weighed (in triplicate) using an analytical balance ( Kern ABJ 120-4NM, Kern & Sohn GmbH, Germany) into cooled and pre-weighed porcelain crucibles, previously heated to 105°C and allowed to cool to room temperature in a desiccator. The crucibles were placed inside a free convection oven (Fisher Scientific Isotemp oven, model 65SF, Fisher Scientific Co. USA) for drying at 105°C for 16 h. After drying, the crucibles were removed from the oven, let to cool in a desiccator, and then accurately weighed to the nearest 0.001 g. The remaining flour residue was designated as total solids, and the reduction in weight was regarded as moisture. Using the original sample weight as a base, the moisture content (W) was calculated as a percentage of the product's weight, using equation 1;

$$W = \frac{M_1 - (M_2 - M_0)}{M_1} \times 100 \quad \text{............ Equation 1}$$

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

### 3.4.2 Determination of ash

Ash content was determined following the procedure outlined in method number 923.03 (AOAC, 2012). A sample weighing approximately 1 g had been precisely measured in triplicate and placed into pre-weighed porcelain crucibles that had been cooled and heated to 105°C, then further cooled to room temperature in a desiccator. The sample-containing crucibles were incineration in a muffle furnace (Fisher Scientific Isotemp Muffle oven, model 186A, Fisher Scientist Co. USA) preheated to 550ºC for a duration of 5 h. After incineration, the crucibles were again left to cool to ambient temperature within a desiccator and then weighed. Subsequently the ash content was computed using equation 2;

Ash content (%) = $\frac{M_2 - M_0}{M_1 - M_0} \times 100\%$ ……………………………………………… Equation 2

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

### 3.4.3 Determination of crude fat

Crude fat was assessed according to method number 920.85 (AOAC, 2012). About 3 g of well ground homogeneous dried test sample was placed into an extraction thimble whose bottom was fitted with a tiny cotton wool piece to prevent sample from sticking on the bottom of the thimble. Into each of the extraction cups of known weight, 50 mL of hexane was added. The Soxhlet extractor (Soxtec System, Hoganäs, Sweden) was then fitted with the extraction cups on the heating plate while the thimbles attached on the magnetic holders. The thimbles were lowered into the solvent and the solvent boiled at 150°C for 1 h. The thimbles were lifted from the solvent and the sample was rinsed by the condensed solvent vapor for 1 h. The tap of the condenser region was then closed to recover the extracted fat from the solvent by retaining the solvent up in the condenser region leaving only the fat in the extraction cups. The cups were then dried in an oven (Fisher Scientific Isotemp oven, model 65SF, Fisher Scientific Co. USA) at 105°C for 30 min to evaporate off any water traces. After drying, the cups were left to cool in a desiccator and re-weighed. Crude fat was computed as per equation 3;

Crude Fat (%) = $\frac{W_2 - W_1}{W} \times 100\%$ ………………………………………………….. Equation 3

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

### 3.4.4 Determination of crude fibre

Crude fibre determination was according to method number 985.29 (AOAC, 2012). Approximately 0.5 g of the sample was measured into fibre crucibles that were subsequently

positioned beneath Dosi- fibre columns (DOSI-FIBER, model 400599, J.P Selecta, South Africa). About 120 mL of dilute sulphuric acid was added to the sample through the columns for the purpose of hydrolysing organic compounds (e.g., protein, carbohydrate) followed by addition of 3 drops of anti-foaming agent (1-octanol). The heating rate was increased to 100% using the heat knob until the solution boiled. After boiling, the heating rate was reduced to 30% and held for 30 min to allow acid hydrolysis to occur. The knobs of the columns were then turned on to filter out the solubilized content of the crucibles into the scrubber unit. The columns were subsequently washed using a small amount of distilled water to eliminate any remaining sulphuric acid after which 120 mL of potassium hydroxide was added for alkaline hydrolysis. The contents of the crucibles were subsequently dried in the forced convection oven (Fisher Scientific Isotemp oven, model 65SF, Fisher Scientific Co. USA) at 105°C for 45 min to evaporate off the moisture. The crucibles were subsequently cooled in a desiccator and weighed. Followed by ignition of crucibles with the dried residue of the sample, in the muffle furnace (Fisher Scientific Isotemp Muffle, model 186A, Fisher Scientist Co. USA) for incineration at 550°C for 3 h. The crucibles were re-weighed after being cooled in a desiccator. Crude fibre content in the samples (dry matter basis) was subsequently computed following the equation 4;

Crude Fibre (%) = $\frac{B_2 - B_1}{B_0} \times 100\%$ ........................................................ Equation 4

$B_o$ = weight of sample; $B_1$ = Weight of crucible plus residue after incineration; $B_2$ = Weight of crucible plus residue before incineration

### 3.4.5 Determination of crude protein

Crude protein determination was done according to method number 920.87 (AOAC, 2012). About 0.5 g of the sample was weighed, W (g) into a digestion flask and 1 g (a tablet) of the Kjeldahl catalyst added to each digestion flask. To the digestion flasks, 15 mL of concentrated

sulphuric acid (98 %) was added and transferred to the digestion apparatus (Kjeltec System HT 2, Foss tecator, Hoganäs, Sweden) and digested at 400°C for 3 h. The flask was let to cool and the digest diluted with 50 mL of distilled water. Into a clean conical flask, 50 mL of boric acid solution (2%) and 3 drops of the mixed indicator were added. The digest in a flask was poured into a distillation flask, 3 drops of the mixed indicator were added and 50 mL of sodium hydroxide solution (50%) was gently added along the sides of the distillation flask. The distillation flask was transferred to the heat source and the distillation apparatus was firmly setup ensuring that the delivery tube was lowered inside the boric acid solution to enable complexation of the liberated ammonia gas with boric acid. After about 10 min of distillation with all the ammonia released and collected, the solution in the collection flask was titrated using standard sulphuric acid solution (0.05 M). The volume, T (mL) of the acid required for complete reaction was recorded. A blank was prepared and titrated, and the volume, B (mL) of sulphuric acid required to reach the end-point was recorded. Using normality (N) of sulphuric acid total nitrogen was determined following equation 5. The nitrogen concentration was multiplied by the conversion factor of 6.25 to generate the crude protein utilizing equation 6.

$$\text{Total nitrogen} = \frac{(T-B)\times N \times 14}{10\times W} \quad \text{Equation 5}$$

$$\%\ \text{Protein} = \text{total nitrogen} \times 6.25 \quad \text{Equation 6}$$

Where, T = Volume of the standard sulphuric acid utilized in the sample titration; B = Volume of the standard sulphuric acid used in the blank titration; N = Normality of the acid in four decimal places; W = weight of the sample used in the determination; 6.25 = is a conversion factor that assumes an average nitrogen-to-protein ratio.

### 3.4.6 Determination of carbohydrate

The difference method number 986.25 was used to compute the carbohydrate content (AOAC, 2012). Total of all measured basic constituents (crude fat, crude protein, crude fibre, ash and moisture) was deducted from 100 % as demonstrated in the equation 7;

% Carbohydrate = 100 – (% moisture + ash + fat + protein + fibre) ……………… Equation 7

## 3.5 Functional properties of the cassava cricket composite flour

### 3.5.1 Dispersibility

The method used to assess dispersibility followed Olapade's (2014) protocol. The flour (10 g) was placed into 100 mL measuring cylinder, followed by the addition of distilled water to the 100 mL mark. Vigorous stirring of the mixture was followed by living it undisturbed for a duration of 3 h. The volume of settled particles was gauged, and this measurement was subtracted from 100 as per equation 8;

% Dispersibility = 100 - volume of settled particles. …………………………….. Equation 8

### 3.5.2 Viscosity

The method for determining viscosity followed the procedure outlined by Ramashia et al (2018). About 10 g of sample was added to 90 mL of distilled water at a temperature of 30°C and permitted to hydrate for a duration of 30 min with intermittent mixing. The viscosity of the resulting slurry was quantified using a Rapid Visco Analyser (RVA 4500, Perten Instruments, Australia) fitted with spindle Q3, rotating at a speed of 100 rpm. The viscosity was recorded in centipoise (cP) units, representing the cold paste viscosity. The slurry was then heated to boiling point (95°C) using a water bath (Patterson Scientific, England) for 20 min, It was cooled to 3°C and cooked using the procedure of Ramashia et al. (2018).

### 3.5.3 Water holding capacity

The employed methodology to assess the water holding capacity (WHC) of the specimen was based on the procedure outlined by Mesías & Morales (2017). In this process, 2 g of respective flour was placed into a pre-weighed centrifuge tube, and then 15 mL of water was added. After 1 min of vortexing, the mixture was allowed to sit at room temperature before being subjected to centrifugation for 15 min at 1,400 g. After centrifugation, any non-absorbed water was carefully drained, and weight of the tube noted. The mass increase per gram of sample was computed, and the water holding capacity determined using the equation 9;

$$WHC = \frac{W_2 - W_1}{W_2} \times 100$$ ………………………………………………………… Equation 9

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

### 3.5.4 Bulk density and tapped density

The Mandge, Sharma, and Dar (2014) method was used to determine bulk density. Approximately 15 g of the sample was weighed without shaking into a 100 mL measuring cylinder. The bulk volume of the flour was then read off the measuring cylinder after the flour had been leveled using a leveling rod. The measuring cylinder was then gently tapped on a level surface for 50 times, and the flour was again levelled. The tapped volume was read off the measuring cylinder. Equations 10 and 11 were used to calculate the bulk density and tapped density, respectively;

$$\text{Bulk density (g/mL)} = \frac{\text{Weight of flour}}{\text{Volume of flour before tapping}}$$ ................................................... Equation 10

$$\text{Tapped density (g/mL)} = \frac{\text{Weight of flour}}{\text{Volume of flour after tapping}}$$ ……………………………… Equation 11

### 3.5.5 Swelling capacity and solubility

The method used for determining swelling capacity was as per the procedure outlined by Kaur et al. (2011). About 0.25 g of flour was weighed into a plastic centrifuge tube and 5 mL of distilled water was added. A shaking water bath (Gupta Pvt Ltd, Ambala Cantt, India) at a temperature of 90°C was used to heat the tube contents for a duration of 30 min, while maintaining constant stirring. The tube was left to cool down to room temperature using cold water. It was then centrifuged at 3,200 rpm for 10 min using a centrifuge (Hettich Zentrifugen centrifuge, model D7200, Tuttlingen, Germany). The resulting supernatant was collected in a pre-weighed dry aluminium dish, after which it was evaporated at 110°C for 24 h. To facilitate the calculation of solubility, the weight of the dried aluminium dishes was taken. The weight of the wet sediment in the centrifuge tube was recorded. The swelling power and solubility were calculated using equations 12 and 13, respectively.

$$\text{Swelling capacity} = \frac{\text{sediment weight}}{\text{dry sample weight}} \quad \text{.................................................. Equation 12}$$

$$\%\ \text{Solubility} = \frac{weight\ of\ soluble\ starch}{weight\ of\ sample} \times 100 \quad \text{................................................Equation 13}$$

### 3.5.6 Oil Absorption capacity

The estimation of oil absorption capacity (OAC) followed procedural steps outlined by Castilho, Fontanari & Batistuti (2010). In this method, 2.0 g of sample was mixed with 10 mL of soybean oil placed in a centrifuge tube and homogenized for 2 min at 1,600 rpm. After draining the non-absorbed oil, the tube was weighed and this was used to compute the percentage of mass increase per gram of sample following equation 14;

$$OAC = \frac{W_{2} - W_{1}}{W_{2}} X100 \quad \text{................................................................ Equation 14}$$

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

### 3.6 Determination of safety of cassava-cricket composite flour

### 3.6.1 Chemical food safety

#### 3.6.1.1 Determination of total aflatoxins

Total aflatoxins was determined following the guidelines outlined in method number 16,050 (ISO, 2003). Approximately 20 g of flour was weighed into a 100 mL amber glass conical flask. To the mixture was added 3 g of sodium chloride and 50 mL of a mixture of methanol and a mixture (30:20). The resulting mixture was shaken in a homogenizer at a speed of 200 rpm for 1 h. After shaking, the suspension was let to settle and then filtered to obtain the first filtrate using Whatman No. 1 filter paper. The filtrate (50 mL) was collected in a conical flask and 10 mL of the filtrate was transferred to a beaker to which 30 mL of phosphate buffer solution was added. Using 0.1 mol/L sodium hydroxide (NaOH), the pH of the solution was adjusted to 7.4. The next step involved passing the sample through an immune-affinity column to acquire the second filtrate, followed by a rinse of the column with 20 mL of ultrapure water. Using 1.5 mL of HPLC grade water-free methanol, the total aflatoxins were subsequently extracted.

A high-performance liquid chromatograph (HPLC) was employed to separate and quantify aflatoxins. The setup consisted of a binary pump, auto sampler, fluorescence detector, reverse phase column C18 (Hypersil ODS, 4.6 × 100 mm, 5 µm), and a column heating chamber. A consistent mixture of water and acetonitrile (75 mL: 25 mL), with a rate of flow set at 1.0 mL/min was as the mobile phase. The fluorescence detector's setup involved an excitation wavelength at 360 nm and an emission wavelength at 423 nm. The time of retention for the elution of each specific aflatoxin were recorded. Standard samples of aflatoxin-B1, aflatoxin-G1, aflatoxin-B2, and aflatoxin-G2 were added to blank flour samples at three different concentration levels to validate the reliability of the method, taking into consideration the background contamination levels of the composite flours.

The mass ($m_t$) of test sample within the portion of the second filtrate subjected to the immunoaffinity column was calculated using equation 15;

$$m_t = m_0 \times \frac{v_2 \times v_4}{v_1 \times v_3} \quad \text{Equation 15}$$

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

The weight proportion of each aflatoxin, represented as, $w_i$ was calculated in micrograms per kilogram of the sample using equation 16;

$$w_i = \frac{v_5 \times m_i}{v_6 \times m_t} \quad \text{Equation 16}$$

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

The weight proportions of the four aflatoxins were summed up to calculate the weight proportion of total aflatoxins.

### 3.6.2 Pesticides residue determination

Pesticide residue determination was done by a multiple pesticide residue analysis method using acetyl acetate extraction. The sample (5 g) was measured and placed into a centrifuge tube, to which 10 mL of MilliQ water and 10 mL of ethyl acetate with 1% acetic acid was added. The amalgamation was vigorously shaken manually and vortexed. Approximately 10 g of sodium sulphate ($Na_2SO_4$) was introduced and mixture shaken vigorously for 10 s. Extraction was done

in an ultrasonic bath for 20 min. The tube was centrifuged for 3 min at 3,200 rpm. After being taken out, the upper phase was filtered into a test tube. The extract (0.5 g sample/mL concentration of methyl acetate) was then analyzed by GC- and LC-MS/MS, Column; Zorbax Eclipse Plus C18 2.1x150 mm and 1.8 µm particle size (Agilent, India) mobile phase A; Water (0.1% formic acid, 5 mM ammonium formate, 2% Methyl alcohol) Mobile phase B; Methanol (0.1% formic acid, 5 mM ammonium formate,2% water), Column temperature: 35ºC, Flow rate: 0.3 ml/min, Injection volume: 5 µL).

### 3.6.3 Microbial safety and quality

#### 3.6.3.1 Detection of *Salmonella typhimurium*

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

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

### 3.6.3.2 Enumeration of *Escherichia coli* (cfu/g)

*Escherichia coli* was enumerated based on method number 16649-2 (ISO, 2001). A sample (10.03 g) was measured to which 90 mL (9 x mass of sample weighed) of the diluent, that is sterile buffered peptone water was added. This initial suspension was homogenised using a stomacher for 2 min. After each dilution, the contents were vortex-mixed (232 Fisher Scientific, USA) for 10 s. Duplicate sterile petri dishes were each inoculated with 1 mL of the initial suspension ($10^{-1}$ dilution) by means of a sterile pipette. Further successive dilutions by a factor of 10 up to $10^{-4}$ were made by pipetting 1 mL samples into 9 mL buffered peptone water and then plated.

About 15 mL of tryptone-bile-glucuronide (TBX) medium, maintained at temperatures between 46.96°C, was added to individual petri dishes. The inoculum was gently combined with the medium by gently swirling the petri dishes, and the medium was allowed to solidify on a cool, level surface. Once fully solidified, the prepared plates were turned upside down and then incubated at 44°C for a duration of 24 h. Control tests were also carried out using *Escherichia coli* as positive control and *Enterococcus faecalis* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was $10^{-1}$ dilution, $10^{-2}$ dilution was also prepared, mixed gently and a pour plate of a loopful of $10^{-2}$ dilution carried out on tryptone-bile-glucuronide and incubated alongside the sample. Pour plates of pipetted 1mL of BPW, approximately 1 mL of unpipetted BPW and tryptone-bile-glucuronide were also prepared to check for sterility of the reagents and apparatus used. After the 24 h of incubation, *Escherichia coli* demonstrating β-glucuronidase activity in each dish containing up to 150 blue colonies was examined and counts taken using a colony counter.

The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated following equation 17;

$$N = \frac{\Sigma C}{V \times [n_1 + (0.1 \times n_2)]d}$$ ............................................................... Equation 17

Where;

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

**3.6.3.3 Enumeration of coagulase-positive *Staphylococcus aureus***

*Staphylococcus aureus* was enumerated according to method number 6888-1 (ISO, 2003). Initial suspension was prepared by measuring a quantity of composite flour (10.10 g) into a sterile stomacher bag, subsequently; 90 mL of buffered peptone water (BPW) was introduced. The initial suspension was then homogenised using a stomacher for 2 min. Further dilutions was prepared up to $10^{-4}$ by pipetting 1mL of previous dilution into 9 mL BPW successively. After each dilution, the contents were vortex-mixed (232 Fisher Scientific, USA) for 10 s. using a sterile pipette, 0.1 mL of the initial suspension ($10^{-1}$ dilution) was transferred, to both of the two pre prepared sterile Baird-Parker agar (15 mL per petridish) plates. The process was repeated for subsequent decimal dilutions. The inoculum was carefully spread rapidly and evenly across the surface of the agar plates, trying not to touch the sides of the dish, using a sterile spreader. The plates were allowed to air dry with their lids secured for approximately 15 minutes at room temperature (20°C) in the laboratory, after which they were inverted and then incubated for 48 h in the incubator maintained 37°C. Control tests were carried using *Staphylococcus aureus* as the positive control and *Escherichia coli* as the negative control. A colony of growth on the control culture slope was touched and inoculated into 9 mL BPW, this is $10^{-1}$ dilution, $10^{-2}$ dilution was prepared, followed by gentle mixing and a spread plate of 1

loopful of the $10^{-2}$ dilution was carried out on Baird-Parker agar and was incubated alongside the sample. The same was repeated for the negative control and uninoculated buffered peptone water. After 48 h of incubation, coagulase-positive *Staphylococcus aureus* on each dish containing a maximum of 300 colonies, including 150 colonies of the typical type (colonies that are black or grey, glossy, and raised with a clear area around them) and/or unusual colonies (shiny black colonies lacking a clear zone) at two successive dilutions were examined and counts taken using a colony counter.

The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated following the equation 17.

**3.6.3.4 Enumeration of Enterobacteriaceae**

Enterobacteriaceae was enumerated according to method number 21528 -2 (ISO, 2017). Initial suspension was prepared by measuring a quantity of composite flour (10.00 g) into a sterile stomacher bag, subsequently; 90 mL of buffered peptone water (BPW) was added. The initial suspension was then homogenized using a stomacher for 2 min. Further dilutions was prepared up to $10^{-4}$ by pipetting 1mL of the previous dilution into 9 mL BPW successively. After each dilution, the contents were vortex-mixed (232 Fisher Scientific, USA) for 10 s. Duplicate sterile petri dishes were inoculated each with 1 mL of the initial suspension ($10^{-1}$ dilution), by means of a sterile pipette. Further successive dilutions in factors of 10 up to $10^{-4}$ were made by pipetting 1 mL samples into 9 mL buffered peptone water and then plated.

Around 15 mL of violet red bile glucose (VRBG) agar, maintained at a temperature of 46.96°C, was put into each petri dish. The medium and inoculum were gently mixed by rotating the petri dishes, and the inoculated medium was allowed to solidify on a cool horizontal surface. Once fully solidified, an additional layer of approximately 5 mL of VRBG agar was added on top, and then cooled to prevent spread of growth and create semi-anaerobic conditions. The

prepared plates were let to solidify, inverted, and thereafter placed in an incubator maintained at 37°C for 24 h. Control tests were also carried out using *Klebsiella aerogenesas* positive control and *Staphylococcus aureus* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was $10^{-1}$ dilution, $10^{-2}$ dilution was also prepared, mixed gently and a pour plate of a loopful of $10^{-2}$ dilution carried out on violet red bile glucose and incubated alongside the sample. The same process was performed for the negative control. Pour plates of pipetted 1ml of BPW, approximately 1 ml of un-pipetted BPW and violet red bile glucose were also prepared to check for sterility of the reagents and apparatus used. After the 24 h of incubation, dishes containing up to 150 colonies with characteristic pink to red or purple (with or without precipitation haloes) colour were selected and counted. Five such colonies were randomly chosen from each dish for sub-culturing onto pre-dried Nutrient agar (NA) by streaking for the biochemical confirmation tests. These plates were then incubated at a temperature of 37°C for a duration of 24 h. Oxidase reaction test was then performed by using a wire loop, to peak a well-isolated colony and streak it onto oxidase disc. The test was classified as negative if the oxidase disc did not exhibit a change in colour to dark blue-purple from white within 10 sec. A fermentation test was also carried out by using wire loop to stab the same selected colonies that produced a negative oxidase test into tubes containing Glucose Oxidation Fermentation medium. The top layer of the medium was an overlay of approximately 1 cm of sterile mineral oil. These tubes were incubated at 37°C for 24 h. The reaction was regarded positive when the yellow colour emerged across the entire content of the tube.

The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated following the equation 17.

## 3.7 Determination of shelf life of cassava-cricket composite flour

### 3.7.1 Chemical shelf life

The chemical shelf life assessment of cassava-cricket composite flour was conducted following the method outlined by Ojo & Adeola (2017). About 100 g of flour was placed within sterile zip-lock high-density polypropylene (HDPE) bags (16 cm × 19 cm), sealed, and then stored in a wooden box at room temperature (18 - 28°C, relative humidity of 75%, Norwegian Meteorological Institute, 2023) for a duration of six months. Samples stored in these conditions were subjected to analysis for moisture content, acid value, and peroxide value at intervals of three weeks throughout the storage period. All experimental procedures were performed in triplicate.

#### 3.7.1.1 Acid value determination

The acid value of a sample was determined according to method number 940.28 (AOAC, 2012). Approximately 30 mL mixture of ethanol and diethyl ether (1:1) was added to 5 g of sample in a centrifuge tube. The tube was hermetically sealed and placed on a rotary stirrer. The mixture was stirred for 1 h at room temperature. It was then centrifuged at 2,000 rpm for 5 min. Using a pipette, the supernatant (20 mL) was transferred to an Erlenmeyer flask, followed by addition of 3 drops of Phenolphthalein. The solution was titrated with 0.07 mol/L ethanolic potassium hydroxide solution using a micro burette graduated in 0.01 ml divisions until a permanent pink colour was formed.

In parallel with the determination, a blank test was carried out using 20 ml of 95% ethanol n place the 20 ml of supernatant liquid.

The acid value in milligrams of potassium hydroxide (KOH) per gram of the flour was calculated using equation 18;

$$Acid\ value(mg\ KOH/g) = \frac{56.11 \text{ x } 0.07 \times (V_S - V_b)}{W}$$ ...................................... Equation 18

Where $V_s$ = titration volume of sample (mL); $V_b$ = titration volume of blank (ml); W = weight of sample used (g); F = factor of 0.07 KOH solution; 56.11 = Molecular weight of KOH; 0.07 = Concentration of KOH (mol/L)

### 3.7.1.2 Peroxide value determination

The peroxide value determination followed the procedure outlined in method number Cd 8-53 (AOCS, 2003). About 5 g of flour was dissolved in a stoppered flask containing a blend of 3 parts acetic acid ($CH_3COOH$) and 2 parts chloroform (30 mL). Subsequently, 0.5 mL of a saturated aqueous potassium iodide (KI) solution was added. The mixture was left standing for 1 min with intermittent shaking, after which 30 mL of distilled water was added. The mixture was titrated with 0.1M sodium thiosulfate solution ($Na_2S_2O_3$), until a pale yellow colour was observed. Starch indicator (0.5 ml) was incorporated, and titration continued until the blue colour just disappeared. A blank was determined in a similar manner. The peroxide value was calculated using equation 19;

$$Peroxide\ value = \frac{(S-B) \times M}{W} \quad \text{......... Equation 19}$$

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

### 3.7.2 Microbial shelf life

The microbial shelf life of cassava-cricket composite flour was assessed using the method by Ojo & Adeola, (2017) with some modifications. Approximately 100 g of flour samples was packed inside sterile zip-lock high-density polypropylene (HDPE) bags (16 cm × 19 cm), sealed and stored at room temperature (18 - 28°C, relative humidity of 75%, Norwegian Meteorological Institute, 2023) in a wooden box for six months. Stored samples were analyzed for total viable count, yeast and moulds and total coliforms at 3 weeks' intervals during storage. All experiments were conducted in triplicate.

#### 3.7.2.1 Determination of total viable count (cfu/g)

Total viable count was determined based on ISO 4833:2013-1 method. Approximately 10 g was measured to which 90 mL of diluent (sterile buffered peptone water) was added. This initial suspension was homogenised using a stomacher for 2 min. After each dilution, the contents were vortex-mixed for 10 s. Duplicate sterile petri dishes were each inoculated, by means of a sterile pipette with 1 mL of the initial suspension ($10^{-1}$ dilution). Further, successive pf factor 10 up to $10^{-7}$ were prepared by pipetting 1 mL samples into 9 mL buffered peptone water and then plated. Approximately 15 mL of sterile plate count agar kept at a temperature of 46.96°C in a water bath was poured into individual petri dishes. The petri dishes were swirled to mix the inoculum with the agar, and the medium was allowed to solidify by placing the dishes on a cool horizontal surface. Once solidified, the prepared plates were inverted and placed inside an incubator (Memmert by Schwabach FRG, Germany, model D-91126) set at a temperature of 30°C for a duration of 72 ± 3 h. Following the specified incubation period, plates containing colonies up to a maximum of 300 were carefully examined, and counts were recorded using a colony counter. The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 17.

#### 3.7.2.2 Enumeration of yeasts and moulds (cfu/g)

Yeast and moulds was enumerated based on the ISO 21527 - 2 method. Approximately 10 g was measured to which 90 mL (9 x mass of sample weighed) of the diluent, that is buffered peptone water was added. This initial suspension was homogenised using a stomacher for 2 min. After each dilution, the contents were vortex-mixed for 10 s. Onto one Dichloran 18% glycerol agar (DG 18) plate, using a sterile pipette, 0.1 mL of the initial suspension ($10^{-1}$ dilution) was inoculated. Further 10-fold serial dilutions up to $10^{-4}$ were prepared by pipetting 1 mL samples into 9 mL buffered peptone water and 0.1 mL plated on DG18 for each dilution

using a sterile pipette each time. For all dilutions duplicate plates were prepared. Using a sterile spreader, the liquid was evenly spread across the agar's surface until full absorption into the medium occurred. The plates were then positioned upright, with lids facing upward, and placed in the incubator for aerobic incubation. (Memmert by Schwabach FRG, Germany, model D-91126) set at 25°C for 5 days. Control tests were carried out using yeasts – *Saccharomyces cerevisiae* and or *Candida albicans*, moulds – *Aspergillus flavus* as positive control and *Escherichia coli* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was $10^{-1}$ dilution, $10^{-2}$ dilution was also prepared, mixed gently and a spread plate of a loopful of $10^{-2}$ dilution carried out on DG 18 and incubated alongside the sample. The same procedure was repeated for the negative control. Spread plates of pipetted 0.1 mL of Buffered Peptone Water, approximately 1 mL of un pipetted Buffered Peptone Water and DG 18 were prepared to check for sterility of the reagents and apparatus used. After 5 days of incubation, the dishes containing up to 150 colonies were selected and counted The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 17.

#### 3.7.2.3 Enumeration of total coliforms

Total coliforms was enumerated based on ISO 4832:2006. A sample (10.03 g) was measured to which 90 mL (9 x mass of sample weighed) of the diluent, that is sterile buffered peptone water was added. This initial suspension was homogenised using a stomacher for 2 min. Following each dilution, the contents were thoroughly vortex-mixed for a duration of 10 s. Using a sterile pipette, 1 mL of the initial suspension ($10^{-1}$ dilution) was transferred to two sterile petri dishes, creating duplicate plates. Subsequently, a series of successive dilutions of factor 10 up to $10^{-5}$ were prepared by transferring 1 mL samples into 9 mL of buffered peptone water, and then these samples were plated. Around 15 mL of molten Crystal violet neutral red

bile lactose agar (VRBL), maintained at a temperature of 46.96°C, was poured into each Petri dish. The inoculum and the medium were carefully mixed by gently rotating the Petri dishes, and the mixture was left to solidify by placing the Petri dishes on a cool, horizontal surface. Once the plates had completely solidified, they were inverted and placed in the incubator at 37°C for 24 h. Control tests were also carried out using *Klebisella aerogenes* as positive control and *Staphylococcus aureus* as the negative control. A colony of growth on the control culture slope was inoculated into 9 mL buffered peptone water (BPW), which was $10^{-1}$ dilution, $10^{-2}$ dilution was also prepared, mixed gently and a pour plate of a loopful of $10^{-2}$ dilution carried out on Crystal violet neutral red bile lactose agar and incubated alongside the sample. The same procedure was repeated for the negative control. Pour plates of pipetted 1ml of BPW, approximately 1 mL of un pipetted BPW and Crystal violet neutral red bile lactose agar were also prepared to check for sterility of the reagents and apparatus used. After 24 h of incubation, the Petri dishes containing with up to 150 colonies of purplish red/pink colonies with a diameter of at least 0.5 mm (sometimes surrounded by a reddish zone of precipitated bile) were selected. The quantity N of microorganisms within the test sample, calculated as a weighted average derived from two consecutive dilutions, was calculated using equation 17.

### 3.8 Statistical analysis

The data were presented as mean ± Standard Deviation (SD). A one-way analysis of variance (ANOVA) was performed, and significant differences between means were determined using the Tukey HSD test at a 95% confidence level. All statistical computations were conducted using the Statistical Package for the Social Sciences (SPSS) software version 20.0.

# CHAPTER FOUR: RESEARCH FINDINGS AND DISCUSSIONS

## 4.1 Sensory evaluation of cassava-cricket composite flour

### 4.1.1 Affective sensory evaluation of cassava-cricket stiff porridge

Following the screening of the cassava-cricket composite flour, eight formulations were obtained (Table 4.1). Findings showed that cassava-cricket stiff porridge containing roasted cricket powder exhibited higher sensory scores for aroma, flavour, taste, mouth feel, overall acceptability and desirability compared to cassava-cricket stiff porridge containing raw cricket powder for a particular cassava variety. The higher aroma and flavour scores can be attributed to the roasting process that can enhance the Maillard reaction, which is a chemical process that generates new flavours and aromas in foods (Starowicz & Zieliński, 2019). The roasting process might also have mitigated any undesirable flavours or aromas present in raw cricket powder (Waszkowiak & Mikołajczak, 2020), resulting in a more pleasant eating experience . Roasted cricket powder could have improved the texture and mouthfeel of the stiff porridge because roasting might have altered the protein structure in cricket powder (Waszkowiak & Mikołajczak, 2020), leading to a smoother and more appealing mouthfeel in the cassava-cricket stiff porridge. The combination of improved aroma, flavour, taste and mouthfeel likely contributed to the higher overall acceptability and desirability scores. The results therefore suggest that using roasted cricket powder in cassava-cricket stiff porridge improved various sensory attributes and overall consumer acceptability compared to using raw cricket powder.

The sensory scores for colour and appearance decreased with increasing cricket powder incorporation level. This can be attributed to cricket powder having a different colour compared to cassava flour, so its incorporation in higher amounts could have altered the overall colour of the stiff porridge.

Table 4.1: Optimised cassava-cricket composite flour formulations

| **Composite characteristics** | | | | | **Composite Sensory attribute and scores** | | | | | | | Desirability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SN | % CSF | % CKP | CSV | CKT | Color | Aroma | Flavor | Taste | Mouth-feel | Appearance | Overall acceptability | |
| 1 | 89.48 | 10.52 | MAGANA | Roasted | 6.180 | 6.506 | 6.773 | 6.154 | 6.317 | 6.197 | 6.322 | 0.715 |
| 2 | 90.73 | 9.27 | NASE 3 | Roasted | 6.321 | 6.146 | 6.429 | 6.258 | 6.401 | 6.332 | 6.632 | 0.702 |
| 3 | 91.38 | 8.63 | NASE 14 | Roasted | 6.393 | 6.531 | 6.245 | 6.014 | 6.512 | 6.401 | 6.647 | 0.697 |
| 4 | 90.51 | 9.49 | NAROCASS1 | Roasted | 6.296 | 6.360 | 6.409 | 5.951 | 5.880 | 6.308 | 6.529 | 0.686 |
| 5 | 92.16 | 7.84 | NASE 14 | Raw | 6.481 | 6.294 | 5.999 | 6.083 | 6.460 | 6.485 | 6.556 | 0.672 |
| 6 | 90.71 | 9.29 | MAGANA | Raw | 6.318 | 6.092 | 6.246 | 5.774 | 6.225 | 6.329 | 6.218 | 0.671 |
| 7 | 91.74 | 8.26 | NASE 3 | Raw | 6.434 | 5.886 | 6.064 | 5.788 | 6.355 | 6.440 | 6.562 | 0.661 |
| 8 | 91.64 | 8.36 | NAROCASS1 | Raw | 6.423 | 6.103 | 6.155 | 5.975 | 5.753 | 6.429 | 6.439 | 0.658 |

CSF = Cassava Flour. CKP = Cricket Powder. CSV = Cassava Variety. CKT = Cricket Treatment.

House cricket powder has a distinctive brown colour (Udomsil et al., 2019) and this colour could become more pronounced as the incorporation levels increase in the stiff porridge potentially affecting the expected colour of traditional cassava stiff porridge. The "yuck" factor (a common psychological response to unconventional food sources, Leong & Lebel, 2020) might have played a role. Some consumers could be hesitant to consume stiff porridge that they know contains insect derived ingredients, regardless of its nutritional value or taste.

Based on the results, it is observed that the eight selected samples of cassava-cricket stiff porridge exhibited some variations in sensory acceptability of the examined attributes (Table 4.2). A notable impact of cricket powder incorporation on the scores for colour was observed ($F_{0.05, 7, 216}$ =3.62, p =0.001), aroma ($F_{0.05, 7, 216}$ = 2.34, p =0.025), aftertaste ($F_{0.05, 7, 216}$ = 2.91, p = 0.006) and overall acceptability ($F_{0.05, 7, 216}$ = 3.37, p = 0.02). Scores for the above-mentioned attributes generally decreased with an increase in cricket powder incorporation indicating that incorporation of cricket powder impacted negatively on these sensory attributes. However, the scores are still above the average sensory score of five implying that the product is sensorialy acceptable. Sample 1 (10.52 % cricket powder) had the lowest mean score and hence acceptability for colour and aroma. There was no significant effect of cricket powder incorporation on flavour ($F_{0.05, 7, 216}$ = 1.36, p = 0.23), taste ($F_{0.05, 7, 216}$ = 1.21, p = 0.30), mouth-feel ($F_{0.05, 7, 216}$ = 1.38, p =0.22), texture ($F_{0.05, 7, 216}$ = 1.42, p = 0.20) and appearance ($F_{0.05, 7, 216}$ = 1.41, p =0.20) scores among the eight composite flours. Cassava cricket stiff porridge containing raw cricket powder exhibited higher mean scores for colour, aroma, appearance and overall acceptability as compared to the cassava cricket stiff porridge containing roasted cricket powder for a particular cassava variety.

Table 4.2: Sensory acceptability of cassava-cricket stiff porridge

| Composite characteristics | | | | | Composite Sensory attribute scores | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SN | % CSF | % CKP | CSV | CKT | Colour | Aroma | Flavour | Taste | Mouth-feel | texture | appearance | aftertaste | Overall acceptability |
| 1 | 89.48 | 10.52 | MAGANA | Roasted | 5.78±1.72$^{b}$ | 5.44±1.70$^{b}$ | 6.33±1.39$^{a}$ | 6.52±1.99$^{a}$ | 6.15±1.59$^{a}$ | 6.52±1.31$^{a}$ | 6.33±1.82$^{a}$ | 6.07±2.07$^{ab}$ | 5.85±1.26$^{ab}$ |
| 2 | 90.73 | 9.27 | NASE 3 | Roasted | 6.07±1.71$^{ab}$ | 5.85±1.90$^{ab}$ | 5.85±1.68$^{a}$ | 5.59±2.22$^{a}$ | 5.81±2.20$^{a}$ | 6.04±2.18$^{a}$ | 5.78±1.81$^{a}$ | 5.63±2.15$^{b}$ | 5.56±1.70$^{b}$ |
| 3 | 91.38 | 8.63 | NASE 14 | Roasted | 6.00±1.92$^{ab}$ | 5.89±1.67$^{ab}$ | 6.15±2.05$^{a}$ | 5.26±1.79$^{a}$ | 6.19±1.86$^{a}$ | 5.96±1.77$^{a}$ | 6.41±1.78$^{a}$ | 6.07±2.02$^{ab}$ | 6.15±1.51$^{ab}$ |
| 4 | 90.51 | 9.49 | NAROCASS1 | Roasted | 5.67±2.06$^{b}$ | 6.07±1.62$^{ab}$ | 5.22±2.34$^{a}$ | 5.33±2.24$^{a}$ | 5.26±2.41$^{a}$ | 5.59±1.85$^{a}$ | 6.11±2.19$^{a}$ | 5.70±2.20$^{ab}$ | 5.44±2.17$^{b}$ |
| 5 | 92.16 | 7.84 | NASE 14 | Raw | 6.81±1.17$^{ab}$ | 6.10±1.89$^{ab}$ | 6.00±1.97$^{a}$ | 5.97±1.99$^{a}$ | 5.87±2.16$^{a}$ | 6.06±1.90$^{a}$ | 5.84±1.79$^{a}$ | 6.77±1.89$^{ab}$ | 6.39±1.61$^{ab}$ |
| 6 | 90.71 | 9.29 | MAGANA | Raw | 6.74±1.51$^{ab}$ | 6.68±1.49$^{ab}$ | 5.74±1.77$^{a}$ | 5.90±2.29$^{a}$ | 6.19±1.91$^{a}$ | 6.32±1.80$^{a}$ | 6.26±1.48$^{a}$ | 6.81±1.47$^{ab}$ | 6.68±1.38$^{ab}$ |
| 7 | 91.74 | 8.26 | NASE 3 | Raw | 6.52±1.24$^{ab}$ | 6.06±1.61$^{ab}$ | 5.77±1.65$^{a}$ | 5.87±1.54$^{a}$ | 6.35±1.54$^{a}$ | 6.26±1.79$^{a}$ | 6.29±1.53$^{a}$ | 6.65±1.66$^{ab}$ | 6.48±1.34$^{ab}$ |
| 8 | 91.64 | 8.36 | NAROCASS1 | Raw | 7.26±1.13$^{a}$ | 7.03±1.68$^{a}$ | 6.58±1.86$^{a}$ | 6.32±1.74$^{a}$ | 6.52±1.69$^{a}$ | 7.00±1.75$^{a}$ | 6.94±1.63$^{a}$ | 7.23±1.84$^{a}$ | 7.03±1.58$^{a}$ |

Each value is a mean of forty determinations. Values with the same alphabet as superscript along the column are not significantly different ($p > 0.05$). CSF = Cassava Flour. CKP = Cricket Powder. CSV = Cassava Variety. CKT = Cricket Treatment.

The higher mean score for colour in the stiff porridge containing raw cricket powder suggests that the natural colour of the raw cricket powder may have blended better with the colour of cassava flour, resulting in a more visually appealing cassava cricket stiff porridge (brownish, Udomsil et al., 2019). Roasting can enhance the Maillard reaction, which is a chemical process that causes browning and colour changes in foods (Starowicz & Zieliński, 2019). This could have affected the cricket powder colour resulting in a stiff porridge with a less visually appealing colour. The higher aroma score for the stiff porridge containing raw cricket powder indicated that the natural aroma of the cricket powder was better preserved when used in its raw state. The roasting process applied to the cricket powder could have influenced the aroma of the cassava cricket stiff porridge. This could have resulted in changes to the volatile compounds (Starowicz & Zieliński, 2019) responsible for aroma, potentially affecting the overall aroma of the stiff porridge. The combined effect of better colour, aroma and appearance likely contributed to the higher overall consumer acceptability.

Mean scores for sample 8 (8.36 % raw cricket powder) were significantly higher compared to all the other samples and hence highest sensory acceptability for all the nine evaluated sensory attributes. Samples, MAGANA with 10.52 % roasted cricket powder, NAROCASS1 with 9.49 % roasted cricket powder, MAGANA with 9.26 % raw cricket powder and NAROCASS1 with 8.36 % raw cricket powder were therefore selected for further analysis because they had high cricket powder incorporation levels coupled with better agronomical properties of the cassava variety used.

### 4.1.2 Qualitative descriptive profile of cassava-cricket composite flours

Qualitative descriptive analysis was carried out using 9 trained panelists who developed sensory descriptors for cassava cricket composite flours as indicated in Table 4.3.

Table 4.3: Qualitative sensory descriptors for cassava-cricket composite flours

| | | Descriptions | |
|---|---|---|---|
| SN | Sample | Aroma | colour |
| 1 | MRWF | Faint silver fish aroma | Greyish white |
| 2 | MRCF | Faint silver fish aroma | Greyish white |
| 3 | NRWF | Faint silver fish aroma | Greyish white |
| 4 | NRCF | Faint silver fish aroma | Greyish white |
| 5 | MGN | Undercoat paint aroma | Azam wheat flour color |
| 6 | NR | Undercoat paint aroma | Azam wheat flour color |

MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA: 10.52% Roasted cricket powder)

**4.2 Proximate composition of selected cassava-cricket composite flours**

The selected cassava cricket composite flours underwent analysis for crude protein, fat, ash, fibre, carbohydrates and moisture content as displayed in Table 4.4.

Table 4.4: Proximate composition of the selected cassava-cricket composite flours

| | | Moisture and proximate components (%) | | | | | |
|---|---|---|---|---|---|---|---|
| SN | Sample | Protein | Fat | Ash | Fibre | Carbohydrate | Moisture |
| 1 | MRWF | $6.46±0.30^{a}$ | $2.77±0.03^{a}$ | $2.04±0.06^{d}$ | $2.98±0.83^{a}$ | $85.75±0.61^{b}$ | $5.09±0.91^{ab}$ |
| 2 | MRCF | $6.87±0.20^{a}$ | $2.41±0.07^{b}$ | $2.39±0.07^{abc}$ | $2.37±0.49^{a}$ | $85.96±0.40^{b}$ | $5.49±0.43^{ab}$ |
| 3 | NRWF | $6.87±0.37^{a}$ | $2.38±0.02^{b}$ | $2.18±0.16^{cd}$ | $2.63±0.26^{a}$ | $85.94±0.44^{b}$ | $4.45±0.05^{b}$ |
| 4 | NRCF | $6.81±0.18^{a}$ | $2.30±0.02^{b}$ | $2.57±0.08^{a}$ | $2.85±0.72^{a}$ | $85.46±0.86^{b}$ | $5.93±0.33^{a}$ |
| 5 | MGN | $1.11±0.10^{b}$ | $0.71±0.06^{c}$ | $2.22±0.06^{bcd}$ | $3.23±1.51^{a}$ | $92.73±1.45^{a}$ | $6.34±0.20^{a}$ |
| 6 | NR | $1.05±0.00^{b}$ | $0.74±0.02^{c}$ | $2.04±0.06^{ab}$ | $2.61±0.42^{a}$ | $93.17±0.45^{a}$ | $5.18±0.43^{b}$ |

Values are means of three separate determinations. Average values with the same alphabet as superscript in the same column are not significantly different from one another ($p > 0.05$). MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48 % MAGANA: 10.52 % Roasted cricket powder)

### 4.2.1 Crude Protein content

The incorporation of cricket powder increased the protein content from 1.08% in the cassava to 6.64% in the composite flour (Table 4.4). All cassava-cricket composite flours showed significantly higher protein contents with increasing level of cricket powder incorporation ($p < 0.05$). The inclusion of the protein-rich cricket powder is responsible for the composite flours' higher protein content. This increase makes up around 28% of the adult recommended dietary requirement of 0.83 g per kg body weight per day (Food and Agriculture Organization of the United Nations, 2011). These results are consistent with those of Bawa et al. (2020) and Terry et al. (2017) who reported that cricket powder led to an increase in the protein content of the final product.

### 4.2.2 Fat content

With increasing levels of cricket powder incorporation, all cassava-cricket composite flours exhibited a notable rise in fat content ($p < 0.05$) from 0.71 – 0.74% in cassava flours to 2.30 – 2.77% in the cassava-cricket powder composites. The increase in fat content can be ascribed to the inclusion of fat rich-cricket powder. These findings are in agreement with those documented by Simeon et al. (2022) who reported that house cricket powder addition increased fat content of the formulated complementary food.

### 4.2.3 Ash content

Ash content increased significantly ($p < 0.05$) as the amount of cricket powder incorporation increased. This finding is similar to study findings by (Eddy et al., 2007) that indicated that ash content increased with the level of cricket powder incorporation. The increase could be due to the cricket powder's high chitin and chitosan content (Otieno et al., 2021). Chitin and chitosan molecules can bind with various minerals such as calcium, iron, magnesium, zinc, copper, phosphorus, and manganese to form complexes such as calcium chitin complexes responsible for the structural integrity of the insect's exoskeleton (Muzzarelli, 2011). The increased ash

content in the composite flours signifies the augmentation in mineral content (Otieno et al., 2021) of the composite flour, which suggests that adding cricket powder to cassava flour could increase its nutritious value. The results obtained concur with those reported by Rawdkuen (2022) who reported increase in ash content of whole wheat bread enriched with cricket powder with increasing addition of cricket powder.

### 4.2.4 Fibre content

No significant difference ($F_{0.05, 5, 12} = 0.42$, $p = 0.83$) was observed in the fibre content of the cassava-cricket composite flours. The fibre content varied from 2.37% to 2.98%. This can be ascribed to the inherently low fibre content of cricket powder (Otieno et al., 2021).

### 4.2.5 Carbohydrate content

The carbohydrate content considerably decreased ($p < 0.05$) with increasing level of incorporation of cricket powder. The observed decrease in carbohydrate content is attributed to the dilution effect. This effect occurs when a less concentrated material, such as cricket powder with low carbohydrate content, is mixed with a more concentrated one, such as high-carbohydrate cassava flour, thereby reducing the overall concentration of carbohydrates in the blend (Nkhabutlane et al., 2020).The results are consistent with those of Simeon et al (2022) who reported decrease in the supplemental diet consisting of cricket powder and millet flour.

### 4.2.6 Moisture content

In the findings of this study, moisture content ranged from 4.45% - 6.34%, with sample 3 (8.36% raw cricket powder) having the lowest moisture content (4.45%) and sample 5 (100% MAGANA) having the highest moisture content (6.34%). Moisture generally decreased with increasing fraction of cricket powder. The differences in sample moisture content may be linked to variances in the composition of the formulations, processing techniques or drying methods used. It's also conceivable that the variations in moisture content might arise from

variation in the cassava varieties as has been postulated in a study conducted in Ghana (Eriksson, 2013). This study's results are consistent with those of Chukwu & Abdullahi (2015) who reported the moisture content of cassava flour to range from 3.50 - 6.31%.

### 4.3 Functional properties of the selected cassava cricket composite flours

When formulating new flour products by mixing two or more ingredients, it is important to evaluate the functional properties of the blends. Although the behaviour of individual components is important, interactions among them could affect the functional attributes of the blends. The functional properties of cassava and cassava-cricket composite flours (water holding capacity, oil absorption capacity, bulk density, tapped density, swelling capacity, solubility and dispersibility) are presented in Table 4.5.

Table 4.5: Functional properties of the selected cassava-cricket composite flours

| | | **Functional Properties** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SN | Sample | WHC (g/100g) | OAC (g/100g) | Bulk Density (g/cm$^3$) | Tapped Density (g/cm$^3$) | Swelling Capacity (g/g) | Solubility (%) | Dispersibility |
| 1 | MRWF | 20.40±0.58$^{bc}$ | 17.54±1.12$^{b}$ | 0.37±0.01$^{a}$ | 0.57±0.01$^{a}$ | 6.72±0.42$^{a}$ | 5.15±0.56$^{c}$ | 42.00±0.00$^{a}$ |
| 2 | MRCF | 19.43±0.26$^{c}$ | 16.61±1.70$^{bc}$ | 0.37±0.02$^{a}$ | 0.60±0.07$^{a}$ | 5.61±0.23$^{b}$ | 5.46±0.16$^{c}$ | 42.00±0.00$^{a}$ |
| 3 | NRWF | 23.57±0.41$^{a}$ | 23.99±0.61$^{a}$ | 0.33±0.02$^{b}$ | 0.53±0.01$^{a}$ | 5.71±0.38$^{ab}$ | 4.20±1.20$^{c}$ | 37.33±1.15$^{c}$ |
| 4 | NRCF | 19.94±0.45$^{bc}$ | 14.53±0.80$^{c}$ | 0.35±0.01$^{ab}$ | 0.56±0.00$^{a}$ | 6.57±0.60$^{ab}$ | 1.52±0.18$^{d}$ | 38.00±0.00$^{c}$ |
| 5 | MGN | 20.76±1.49$^{bc}$ | 15.98±0.34$^{bc}$ | 0.38±0.01$^{a}$ | 0.60±0.01$^{a}$ | 4.59±0.19$^{c}$ | 24.89±1.26$^{b}$ | 43.33±1.15$^{a}$ |
| 6 | NR | 21.83±0.33$^{ab}$ | 15.35±0.94$^{bc}$ | 0.37±0.01$^{a}$ | 0.55±0.01$^{a}$ | 3.99±0.20$^{c}$ | 19.37±0.90$^{a}$ | 40.00±0.00$^{b}$ |

Each value is an average of three determinations. Average value with the same alphabet as superscript in the same column are not significantly different from one another ($p < 0.05$). MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), WHC = Water Holding Capacity, OAC = Oil Absorption Capacity.

#### 4.3.1 Water holding capacity

Water holding capacity (WHC) in this study varied from 19.43 in sample 3 (with 8.36% raw cricket powder) to 23.57 g/100 g in sample 4 (with 9.49% roasted cricket powder). The water

holding capacity values for the cassava-cricket composite flours did not show a significant difference ($F_{0.05, 5} = 13.13$, $p < 0.05$) from those of their respective NAROCASS1 and MAGANA control flours (Table 4.5). Irrespective of the variety, WHC was generally lower for composites with roasted cricket powder (samples 2 & 4) with higher incorporation levels, and higher for those with raw cricket powder with lower incorporation levels. This could be attributed to a combination of factors such as changes in protein structure due to protein denaturation (Hatamian et al., 2020; Waszkowiak & Mikołajczak, 2020) during the roasting process of cricket powder, which resulted into a reduction in the water-binding molecules in the composite. This implies that these composite flours cannot be used in food applications in which water holding is of importance.

### 4.3.2 Oil absorption capacity

The oil absorption capacity ranged from 14.53 to 23.99 g/100 g with composite 3 (8.36 % Raw cricket powder) exhibiting the highest value and composite 4 (9.49 % Roasted cricket powder) having the lowest value ($F_{0.05,5} = 34.03$, $p < 0.05$). Protein is a key factor that influences oil absorption due to the combination of hydrophilic and hydrophobic properties (Zhu, Zhou, & Qian, 2020). Therefore, the increased oil absorption capacity can be ascribed to the heightened protein content as cricket powder incorporation levels increase. The low oil absorption capacity for sample 4 could be due to protein structural alterations brought on by roasting, which might have reduced the protein's ability to bind oil as effectively as in the raw form (Zhu, Zhou, & Qian, 2020).

### 4.3.3 Bulk density and tapped density

The bulk density and tapped density values of the cassava-cricket composite flours were generally not significantly different ($p = 0.005$ and $p = 0.010$ respectively) from their respective control flours except the bulk density for composite flour 3 (0.33 g/cm$^3$). This indicates that

the bulk density and tapped density of cassava flour are not affected by addition of cricket powder.

#### 4.3.4 Swelling capacity and solubility

The swelling capacity ranged from 3.99 to 6.72 g/g with control 2 (100% NAROCASS1) exhibiting the lowest swelling power (3.99 g/g) and composite 1 (9.29% Raw cricket powder) exhibiting the highest (6.72 g/g) value (Table 4.5). The swelling capacity values of the cassava-cricket composite flours were significantly higher ($F_{0.05, 5} = 25.70$, $p < 0.05$) than those of the respective control flours. Enrichment of cassava flour with cricket powder increases its swelling capacity irrespective of the cassava variety and the cricket powder treatment. The observed differences in swelling capacity can be explained by the varying levels of amylose and non-starch constituents, as highlighted in the study by Chisenga et al. (2019). The addition of cricket powder replaced some of the starch content in cassava flour with proteins, lipids, and other nutrients found in crickets thus decreasing the overall starch content in the composite mixture (Smith & Johnson, 2020). Proteins have a high water-binding capacity primarily because of the hydrophilic amino acid residues that can interact with water molecules through hydrogen bonding (Damodaran, Parkin, & Fennema, 2017). These hydrophilic residues, such as serine and threonine containing hydroxyl (-OH), glutamine containing carboxyl (-COOH), and lysine containing amine ($-NH_2$) groups, are able to form hydrogen bonds with water. The interaction between these polar residues on the protein surface and water molecules leads to the absorption and retention of water within the protein structure. The presence of water-binding proteins and fibres, along with reduced amylose gel formation, leads to an increased ability of the composite flour to swell (Smith & Johnson, 2020).

On the other hand solubility values ranged from 1.52% to 24.89% with control 1 (100% MAGANA) having the highest solubility and composite 4 having the lowest solubility. There was a significant difference ($F_{0.05, 5} = 484.60$, $p < 0.05$) among the solubility values of the

cassava-cricket composite flours and their respective control flours. Solubility decreased with increasing level of cricket powder incorporation due to the high chitin and chitosan, protein and fat content of the cricket powder (Otieno et al., 2021). Cricket powder is rich in protein, and fat, which have low solubility in water. When added to cassava flour, the overall protein and fat contents increased, leading to lower solubility due to reduced dispersion and interaction with water molecules.

### 4.3.5 Dispersibility

Dispersibility refers to the capacity of flour or starch to reconstitute when mixed with water (Adebowale et al., 2012). The dispersibility significantly decreased ($F_{0.05, 5} = 39.20$, $p < 0.05$) with increasing fraction of the cricket powder for a particular cassava variety (Table 4.5). This could be attributed to cricket powder particles' tendency to aggregate or clump together (Hirsch et al., 2019). These aggregates could be larger and more resistant to breaking apart as the amount of cricket powder increases leading to uneven distribution within the mixture. The reduced dispersibility of the composite flours suggests that the samples do not reconstitute well in water and do not yield a fine mixture during the mixing process (Adebowale et al., 2012).

## 4.4 Pasting properties of cassava-cricket composite flour

Flour pasting properties are important in understanding the heating/cooking and processing quality of flour. The peak viscosity was highest for NR (7,679 cP) and lowest for MRWF (4,088 cP) as indicated in Figure 4.1. The controls (NR and MGN) were observed to have higher peak viscosity values than their respective composite flours. Composites NRCF and MRCF containing roasted cricket powder had higher peak viscosity values than NRWF and MRWF. The observed differences may result from fluctuations in the starch's swelling power and the starch granules' rate of disruption (Imoisi et al., 2020). However the peak viscosity in this research was within the range of 4,989 – 8,367 cP reported for improved cassava varieties (Nuwamanya et al., 2023) and higher than 3,036 – 4,139 cP for local varieties (Nuwamanya et

al., 2010). This implies that the composite flours may be suited for products where cassava flour is used as an ingredient.

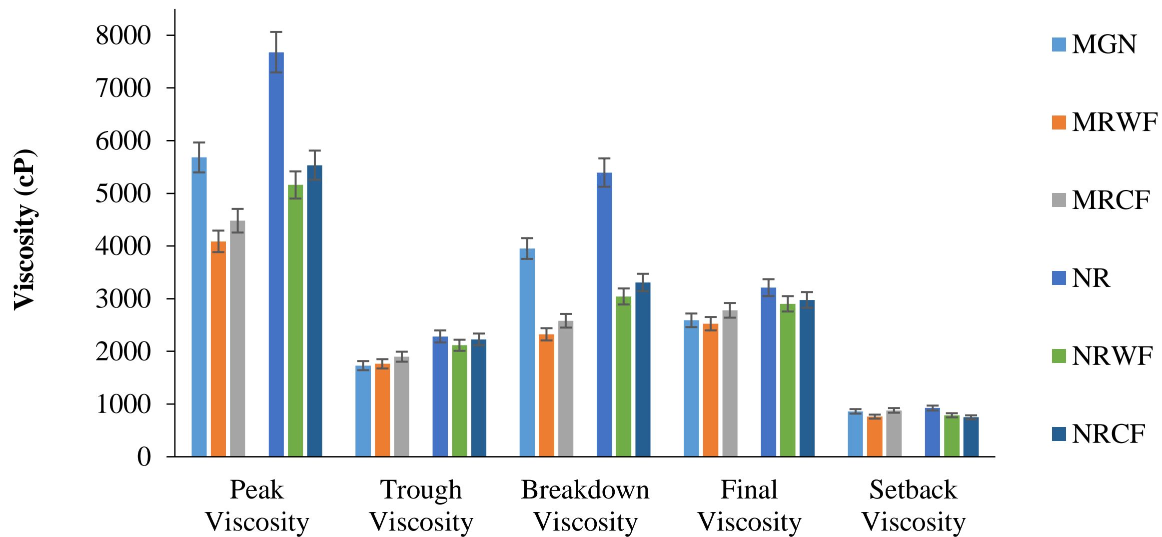


Figure 4.1: Pasting properties of cassava-cricket composite flours. MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71 % MAGANA: 9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA: 10.52% Roasted cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference

The trough viscosity of cassava-cricket composite flours did not vary significantly ($p < 0.05$) and ranged from 1,729 to 2,285 cP (Figure 4.1). These values were within the trough viscosity range (808.1 – 2,666 cP) reported for cassava varieties grown in Uganda (Nuwamanya et al., 2023). Trough viscosity refers to the lowest viscosity observed after the peak viscosity, indicating that the starch granules within the flour paste remain intact over a consistent temperature interval, time, and applied shear force (Bakare et al., 2016). This implies that cassava-cricket composite flours can behave in the same way as cassava flour in baking and other applications.

The final viscosity of cassava-cricket composite flours did not significantly differ from that of the control flours as presented in Figure 4.1. Sample NR had the highest final viscosity (3,209.7 cP) whereas sample MRWF had the lowest final viscosity (2,525.7 cP). The final viscosity of

flour signifies its capability to create a thick paste or gel upon cooking, and it also reflects the paste's ability to withstand shear stress during stirring (Imoisi et al., 2020). Therefore, the cassava cricket composite flours could have the same ability to form a viscous paste during processing like their respective control flours.

Setback viscosity refers to the starch's propensity to undergo retrogradation and associate upon cooling (Wang et al., 2015). Setback viscosity did not vary significantly across samples with NR at 925 cP having the highest setback viscosity and NRCF at 749 cP having the lowest setback viscosity (Figure 4.1). The observed set back viscosity is consistent with what was documented (245 – 1,410 cP) by Nuwamanya et al. (2023) for Ugandan elite varieties. A higher setback viscosity value is indicative of reduced retrogradation during the cooling process of products crafted from the flour (Ikegwu et al., 2010). High set back viscosity is important in products such as creamy soups and gravies that require maintenance of a thick and stable consistency upon cooling and desserts like custards, puddlings, and fillings, where a smooth, creamy texture that holds its shape after cooking and cooling is important (Ikegwu et al., 2010). Baked goods like bread, cakes and pastries benefit from low setback viscosity (Aidoo et al., 2022) because it results in products with lighter textures, finer crumb structures, and improved dough handling during mixing and shaping. Therefore, the cassava cricket composite flours have the potential to create an improved flour paste suitable for utilization in the confectionery industry.

Breakdown viscosity gauges the vulnerability of swollen starch granules to fracture in situations of elevated temperatures and ongoing shear, offering insight into the stability of the resulting paste (Akanbi et al., 2009). A Higher breakdown viscosity corresponds to a higher propensity of the expanded starch granules to rupture when subjected to elevated temperatures and sustained shearing, a sign of reduced paste stability (Akanbi et al., 2009). The breakdown viscosity significantly decreased ($p < 0.05$) from 3,952 to 5,394.3 cP for cassava flours to

2,324.3 to 3,043.3 cP for cassava-cricket composite flours (Figure4.1). This suggests that the composite flours exhibit resistance to breakdown when subjected to heating, making them suitable for use in food products that undergo high-temperature processing. Cassava cricket composite flours containing roasted cricket powder had higher breakdown viscosity values than their corresponding flours containing raw cricket powder. This could be because roasting cricket powder could lead to protein aggregation or cross-linking (Waszkowiak & Mikołajczak, 2020), potentially forming a network structure that interacts more strongly with cassava starch. This could contribute to increased break down viscosity as the network resists disintegration during heating.

The term "peak time" refers to the number of minutes it takes for the flour to attain its maximum viscosity, representing the duration required for the flour to fully develop (Adebowale et al., 2005). The peak time ranged from 3.8 to 4.6 min with the highest observed for sample NRCF while the lowest observed for sample NR (Figure 4.2). MAGANA and its composite flours had the same peak time whereas Narocass1 had a lower peak time than its composites. Flour with low peak time forms pastes more readily in contrast to that with high peak time (Nuwamanya et al., 2023). This property affects the cooking properties of the flour since it relates to the amount of energy required for paste formation. the findings of this study are similar to those of Nuwamanya et al. (2023) who reported peak time of 3.5 to 4.4 min for Ugandan elite (improved) cassava varieties. Low peak time for cassava flour and cassava flour composites is indicative of the ability to cook faster. Therefore, these flours would be preferred for home applications and industrial processing without increments in cooking energy.

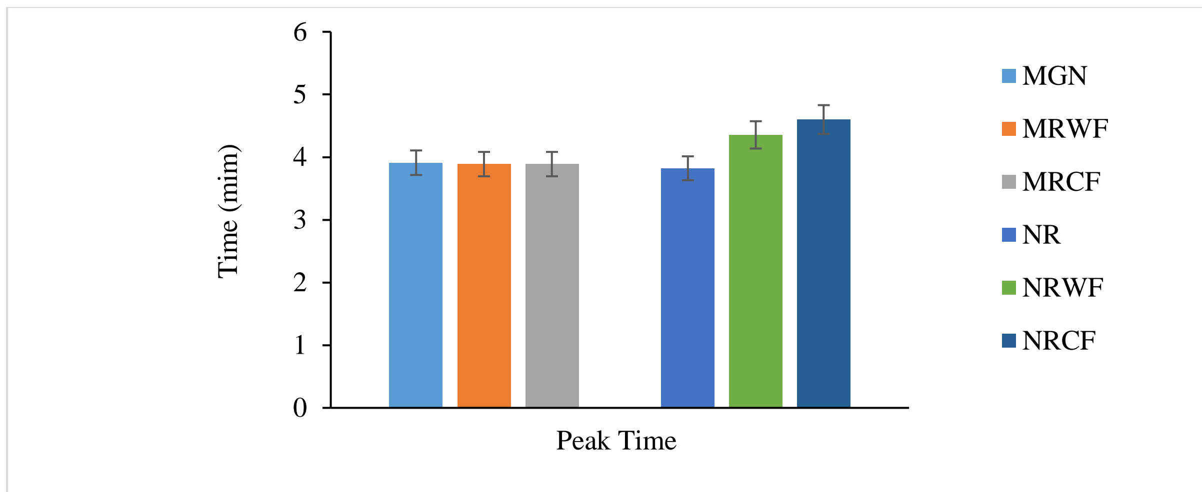

Figure 4.2: Peak time of cassava-cricket composite flours. MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29 % Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

The pasting temperature is the minimum temperature required for cooking or gelatinizing the flour (dos Santos et al., 2022). The starch's ability to soak up water and expand is primarily impacted by the pasting temperature (dos Santos et al., 2022). The pasting temperature ranged from 69.3 to 72.9°C with the highest observed for MRWF while the lowest was observed for NRWF (Figure 4.3). The pasting temperature was in agreement with that reported in past research (dos Santos et al., 2022; Mikidadi et al., 2023). It was however lower than the upper limit of 75°C that has been specified in the East African standard (EAS) 740, 2010. This implies that inclusion of cricket powder doesn’t significantly influence the cooking temperature beyond acceptable limits.

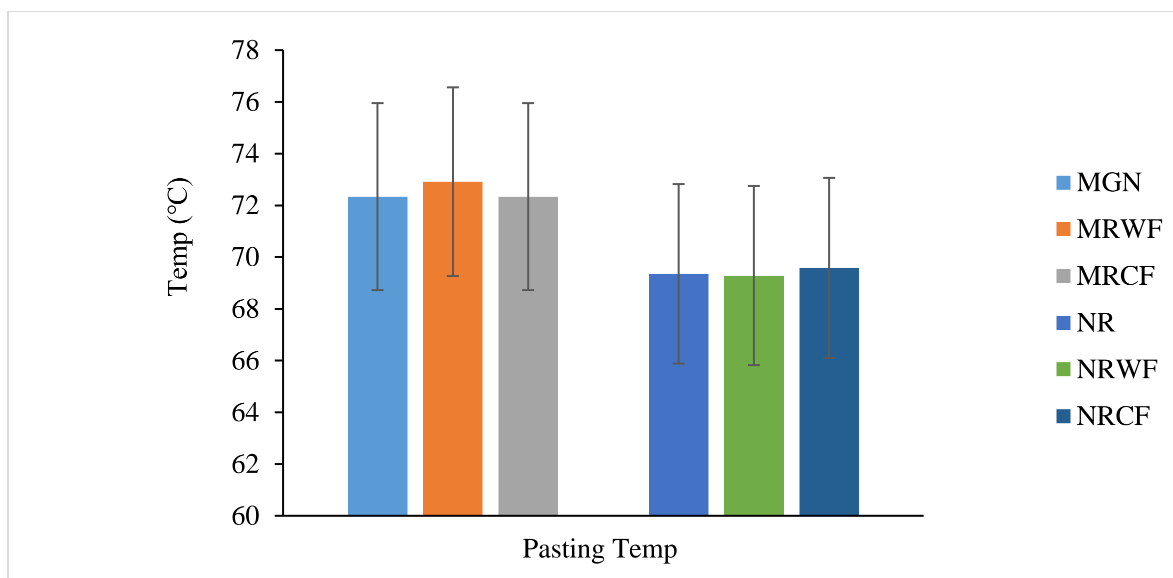


Figure 4.3: Pasting temperature of cassava-cricket composite flours. MGN (100 % MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

## 4.5 Quality and safety of cassava-cricket composite flour

### 4.5.1 Microbial quality and safety of cassava-cricket composite flour

The incorporation of cricket powder into cassava flour resulted in a significant increase ($P < 0.05$) in the microbial counts of the cassava-cricket composite flours (Table 4.6).

Table 4.6 – Microbial quality (log cfu/g) of the selected cassava-cricket composite flours

| **Parameter** | MRWF | MRCF | NRWF | NRCF | MGN | NR |
|---|---|---|---|---|---|---|
| Total plate count | 6.62±0.15$^{a}$ | 6.35±0.01$^{a}$ | 6.43±0.09$^{a}$ | 5.54±0.05$^{b}$ | 4.50±0.04$^{c}$ | 4.45±0.03$^{c}$ |
| Total coliforms | 5.27±0.05$^{a}$ | 5.07±0.10$^{a}$ | 5.03±0.11$^{a}$ | 4.28±0.06$^{b}$ | 3.95±0.01$^{c}$ | 3.78±0.03$^{c}$ |
| Yeast and moulds | 3.36±0.05$^{a}$ | 3.45±0.03$^{a}$ | 3.06±0.08$^{bc}$ | 3.45±0.08$^{a}$ | 3.32±0.06$^{ab}$ | 2.84±0.09$^{c}$ |
| *Enterobacteriaceae* | <10 | <10 | <10 | <10 | <10 | <10 |
| *E. coli* | <10 | <10 | <10 | <10 | <10 | <10 |
| *S. aureus* | <10 | <10 | <10 | <10 | <10 | <10 |
| *S. typhimurium* (/25g) | ND | ND | ND | ND | ND | ND |

MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1: 9.49 % Roasted cricket powder), MRCF (89.48 % MAGANA:10.52 % Roasted cricket powder). Each value is a mean of duplicate determinations. Mean value with the same alphabet as a superscript on the same column are not significantly different from one another ($p > 0.05$). ND: Not Detected

The total plate count (TPC) ranged from 5.54 to 6.43 log cfu/g for the cassava cricket composite flours and 4.45 to 4.50 log cfu/g for the control flours. The ranges for the cassava cricket composite flours were above the limit of 5.00 log cfu/g specified in the East African Standard EAS 779:2012. The TPC for the control flours was within the acceptable limits. The increased PTC may be attributed to the incorporation of cricket powder which strongly favours microbial growth (Klunder et al., 2012).

The total coliform counts observed in both the control flours and the cassava-cricket composite flours varied between 3.78 and 5.27 log cfu/g. According to the Standard EAS 779:2012, cassava flour should not contain any coliforms. The presence of coliforms in food products is an indicator of the presence of conditions conducive to the growth and proliferation of microorganisms (Odetunde et al., 2014) However, all the flour samples showed significant coliform contamination, which suggests inadequate hygiene practices and potential faecal contamination during the processing stages since a commercial mill was used. However flours are not eaten and yet the observed pasting temperature (69.3 – 72.9°C) for cassava flour and its composites is above 65°C (WHO, 2020) above which coliforms can be killed.

The yeast and mould count ranged from 2.84 to 3.45 log cfu/g. This range was below the maximum limit of 4.00 log cfu/g specified in East African standard EAS 782:2019 for composite flours. The low yeast and mould counts may be attributed to the fact that flours were packaged in airtight high-density polypropylene (PDPE) bags thus protecting them from absorption of water and spores of various species of yeast and moulds, which are heavily suspended in air, especially in untidy and unhygienic environments. According to Odetunde et al., (2014) spore-forming yeasts and moulds can readily come into contact with foods that are openly exhibited. The low yeast and moulds counts can also be attributed to the fact that cassava chips were dried using an electric drier immediately after chipping which fastened the drying process of cassava chips thus limiting mould growth.

*Staphylococcus aureus*, *Enterobacteriaceae, Salmonella typhimurium* and *Eschechia coli* were found to be absent in all the flours. These findings are in agreement with the limits of the Codex Standard 176-1989 (CAC, 2013) and East African Standard EAS 779:2012 that require absence of *Enterobacteriaceae, Salmonella typhimurium* and *Eschechia coli* and a limit of 2.00 log cfu/g of *Staphylococcus aureus.*

### 4.5.2 Chemical quality of the cassava-cricket composite flours

The cassava-cricket composite flours and their control flours were analysed for quality (acid and peroxide values) and findings are presented in Table4.7.

Table 4.7 - Acid and peroxide values of cassava-cricket composite flours

| **Composite characteristics** | | | | | **Chemical parameter** | |
|---|---|---|---|---|---|---|
| Sample | Cassava Flour (%) | Cricket powder (%) | Cassava Variety | Cricket Treatment | Acid value (mg KOH/g) | Peroxide value (meq/kg) |
| 1 | 90.71 | 9.29 | MAGANA | Raw | $1.74\pm0.14^{ab}$ | $1.70\pm0.84^{ab}$ |
| 2 | 89.48 | 10.52 | MAGANA | Roasted | $2.30\pm0.05^{ab}$ | $4.83\pm1.82^{a}$ |
| 3 | 91.64 | 8.36 | NAROCASS1 | Raw | $1.86\pm0.42^{ab}$ | $2.93\pm1.27^{ab}$ |
| 4 | 90.51 | 9.49 | NAROCASS1 | Roasted | $2.81\pm1.12^{a}$ | $2.16\pm0.01^{ab}$ |
| Control 1 | 100 | 0 | MAGANA | None | $1.20\pm0.16^{ab}$ | $1.46\pm0.11^{b}$ |
| Control 2 | 100 | 0 | NAROCASS1 | None | $1.13\pm0.03^{b}$ | $1.54\pm0.50^{b}$ |

Each value is an average of three separate determinations. Average values with the same alphabet as superscript on the same column are not significantly different from one another ($p > 0.05$)

The findings indicate that there existed generally no significant difference ($p > 0.05$) between the peroxide value of control flours (1.46 to 1.54 meq/kg) and that of the composite flours (1.70 to 4.83 meq $O_2$/kg) except sample 2 containing 10.52 % roasted cricket powder whose peroxide value was significantly higher ($p = 0.02$) than that of its respective control. This may be ascribed to the higher cricket powder content, which contributes to higher fat content consequently leading to greater susceptibility to oxidation. Lipid oxidation rates are directly correlated with temperature (Gichau et al., 2019) therefore, the roasting process of the cricket

powder that involved high temperature (150 °C) may have had an impact on the higher peroxide value. The peroxide value for all the sample where below the permissible limit (15 meq $O_2$ /kg) specified in Codex Alimentarius standard CODEX STAN 19-1981, Rev 2-1999.

The acid value serves as a standard measure to assess the level of lipid rancidity and is crucial for evaluating the quality of the fat contained in the food item (Park et al., 2018). There was no significant difference between the acid value of the control flours (1.13 to 1.20 mg KOH/g) and the composite flour (1.74 to 2.81 mg KOH/g) with the exception of sample 4 containing 9.49% roasted cricket powder whose acid value was considerably more ($P = 0.39$) than the respective control flour (control 2). The acid value for samples exceeded the East African Standard EAS 782:2019 limit of 0.8 mg KOH/g composite flour. The elevated acid value may be attributed to the increased surface area exposed to oxygen and the heat generated during the milling of cassava and house crickets. Comparable findings were noted in the investigation of Gulkirpik et al., (2021) in which high acid value (3.13 mg KOH/g) of soybean flour was observed.

### 4.5.3 Chemical safety of the cassava-cricket composite flours

The findings indicate that all aflatoxin and pesticide levels were below the detection level of 0.02 mg/Kg and 0.01 mg/Kg respectively (Table 4.8). Chiona et al. (2014) found comparable outcomes, noting that none of the examined cassava flour samples from Malawi exhibited contamination with aflatoxins (< 0.02 mg/Kg) and Muzanila et al. (2000) who recorded no aflatoxin contamination (< 0.02 mg/Kg) was observed in cassava flour samples from Tanzania villages.

Table 4.8 – Aflatoxin and pesticides levels in the selected cassava cricket composite flours

| Sample | MRWF | MRCF | NRWF | NRCF | MGN | NR |
|---|---|---|---|---|---|---|
| Analytes | mg/Kg | mg/Kg | mg/Kg | mg/Kg | mg/Kg | mg/Kg |
| | | | **Aflatoxin** | | | |
| Aflatoxin B1 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| Aflatoxin B2 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| Aflatoxin G1 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| Aflatoxin G2 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| | | | **Pesticide residues** | | | |
| Acephate | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| BHC-alpha | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Hexachlorobenzene | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| BHC-beta | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Diazinon | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| BHC-delta | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Malathion | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Anthraquinone | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Chlorpyrifos | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Procymidone | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Prothiofos | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| DDT-p,p' | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Ethion | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Cyhalothrin (Lambda) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Tebuthylazine | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Fenitrothion | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

Each value is a mean of triplicate determinations. MGN (100% MAGANA), NR (100% NAROCASS1), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder), MRWF (90.71% MAGANA:9.29% Raw cricket powder), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), MRCF (89.48% MAGANA: 10.52% Roasted cricket powder)

The contamination levels recorded in this research were lower than the maximum permissible limit for aflatoxins set by the Codex Alimentarius Commission (CAC), which is 0.01 mg/kg in flours (Codex Alimentarius Committee, 2019). This implies that the cassava-cricket composite flours are free from aflatoxins and therefore safe for human consumption. Pesticide residues were analysed since cricket powder was being incorporated yet cricket feeds could be exposed to pesticides (Kolakowski et al., 2021). The findings of this study are in line with those obtained by Kolakowski et al. (2021) who reported absence of pesticide residues (< 0.01 mg/Kg) in six types of consumable insects originating from Uganda and Kenya. Therefore, the cassava-cricket composite flours are free from the chemicals for which were analysed.

## 4.6 Shelf life of cassava-cricket composite flour

Determining shelf life is a crucial aspect of food production. Shelf life refers to the duration during which products maintain an acceptable level of sensory quality and safety (Gichau et al., 2019). The assessment of shelf life for cassava-cricket composite flour was conducted over a span of 24 weeks (6 months) at ambient temperature.

### 4.6.1 Moisture content

In a shelf life study, moisture content is a crucial factor to monitor because it has a direct effect on the quality, stability, and safety of the food product over time (Kumar, Sahoo, Sirohi, & Raj, 2019). Moisture content influences microbial growth, various chemical reactions such as lipid oxidation and enzymatic activities that can degrade food quality. The level of moisture of cassava-cricket composite flour generally increased from 4.5 to 6.1 g/100 g in week 0 to 5.5 to 7.1 g/100 g in week 24 (Figure 4.2)

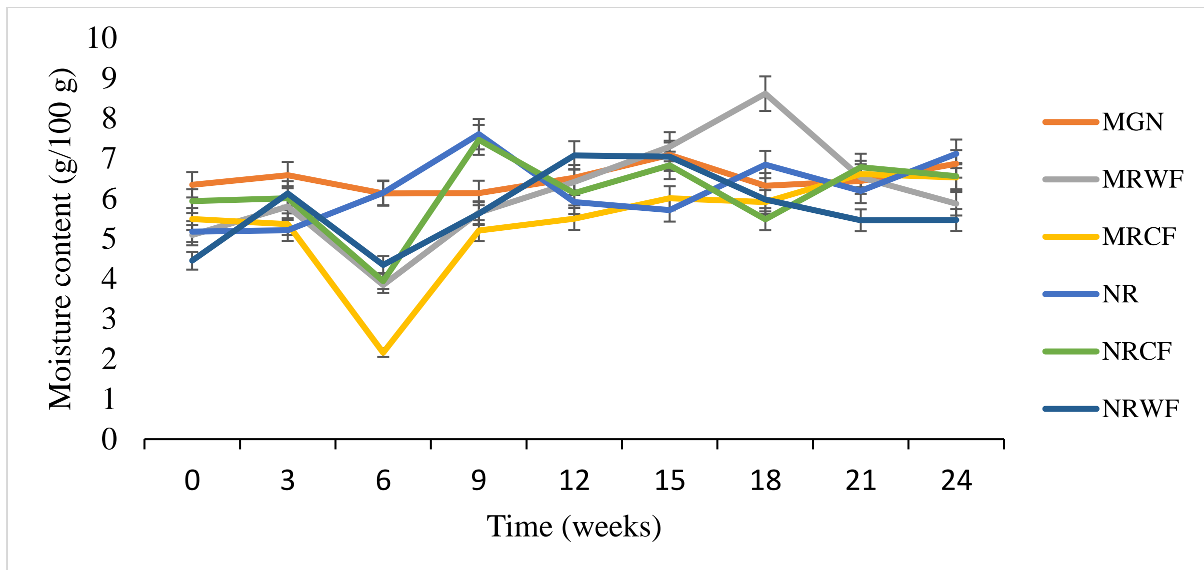

Figure 4.4.: Changes in moisture content of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

For example, the lowest observed moisture content was 2.2 g/100 g for MRCF at week 6 which would be expected at week one, whereas the highest observed moisture content was 8.0 g/100 g for MRWF at week 18 which would have been expected at week 24. These inconsistencies could be because of a variety of factors, such as measurement errors, and uneven changes in humidity, temperature and air saturation in the storage room (Saif & Ahmed, 2015). The changes in moisture content were low and the highest observed moisture content was below the allowed maximum limit of 14 g/100 g in the East African standard EAS 782:2019 for composite flours. This can be attributed to the high density polypropylene (HDPE) packaging material used that is known to have significant moisture barrier properties (Kirwan et al., 2011). These results are consistent with findings by Nkhabutlane et al. (2020) who reported that cassava-cricket blends packaged in HDPE packaging exhibited minimal change in moisture content over time. The low moisture content of the flours implies that no excess moisture can enhance microbial growth, spoilage and a resultant reduction in shelf life. By remaining below the specified limit, the cassava cricket composite flour maintains its overall quality.

### 4.6.2 Peroxide value

The peroxide value is used in monitoring oxidative degradation of fats and oils (Gordon, 2004). The results of Figure 4.5 indicate that the peroxide value of the cassava cricket composite flour and the controls generally increased from 1.7 - 4.5 meq $O_2$/kg to 8.2 – 14.4 meq $O_2$/kg over 24 weeks

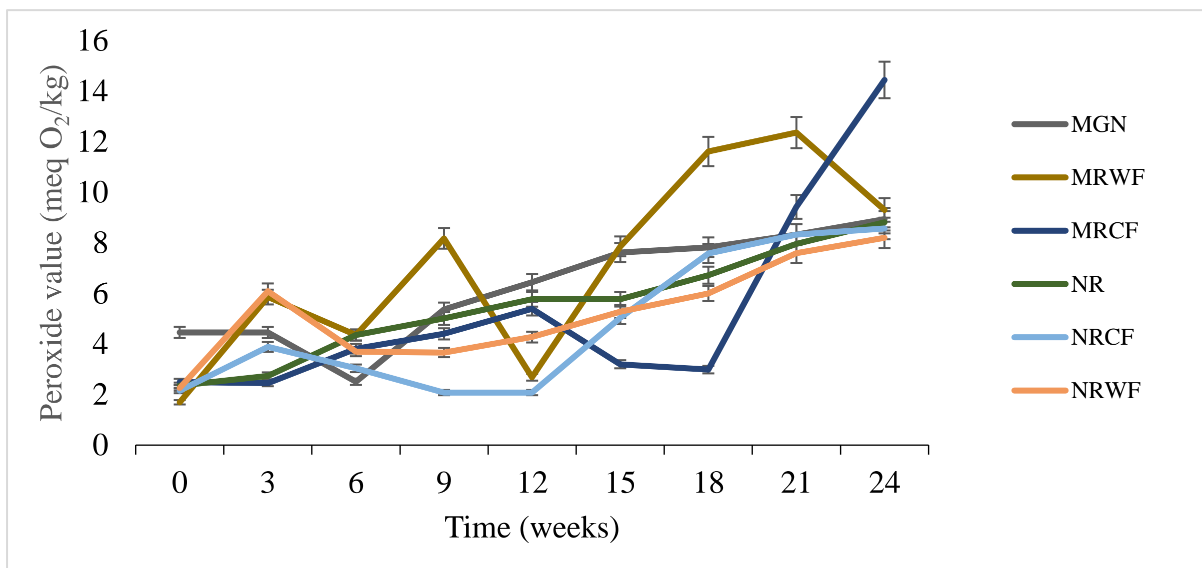


Figure 4.5: Changes in peroxide value of cassava-cricket composite flour with time. MGN (100 % MAGANA), MRWF (90.71 % MAGANA: 9.29 % Raw cricket powder), MRCF (89.48 % MAGANA: 10.52 % Roasted cricket powder), NR (100 % Narocass1), NRCF (90.51 % Narocass1: 9.49 % Roasted cricket powder), NRWF (91.64 % Narocass1: 8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

According to Udomsil et al. (2019), cricket powder contains approximately 4.14 g/100 g monounsaturated fatty acids (MUFA), 1.46 g/100 g polyunsaturated fatty acids (PUFA), 0.07 g/100 g omega-3 fatty acids and 1.13 g/100 g omega-6 fatty acids. The presence of unsaturated fatty acids in cricket powder increases the likelihood of oxidative stress, which can lead to lipid peroxidation (Parker & Reilly, 2020). Incorporation of cricket powder resulted into increased fat content (0.7 to 2.7 g/100 g) which could have resulted into increased MUFA and PUFA leading to formation of peroxides in the flour. The highest peroxide value (14.4 meq $O_2$ /kg at week 24) was observed for sample MRCF while the lowest peroxide value (1.7 meq $O_2$/kg) was observed for MRWF. The elevated peroxide value for MRCF sample after 24 weeks may

be due to the higher cricket powder content (10.52%) than all other formulations, which led to a higher fat content consequently leading to greater oxidation rates. An increased peroxide value is a critical indicator of lipid oxidation, signalling a decline in the quality, nutritional value, and safety of food products (Gichau et al., 2019). After 24 weeks, the observed peroxide value for cassava-cricket composite flours was below the Codex Alimentarius standard (CODEX STAN 19-1981, Rev 2-1999) limit of 15 meq $O_2$/ kg, therefore all the composite flours were shelf stable at 24 weeks with regard to the level of peroxides.

### 4.6.3 Acid value

Findings indicate that the acid value of cassava and its composite flours generally increased from the start with samples NR (100% NAROCASS1) and NRCF (89.48% MAGANA:10.52% Roasted cricket powder) exhibiting the lowest (1.0 mgKOH/g) and highest (2.8 mgKOH/g) values at week 1 (Figure 4.6). After 24 weeks samples MGN (100% MAGANA) and MRWF (90.71% MAGANA:9.29% Raw cricket powder) exhibited the lowest (1.6 mgKOH/g) and highest (3.1 mgKOH/g) acid values.

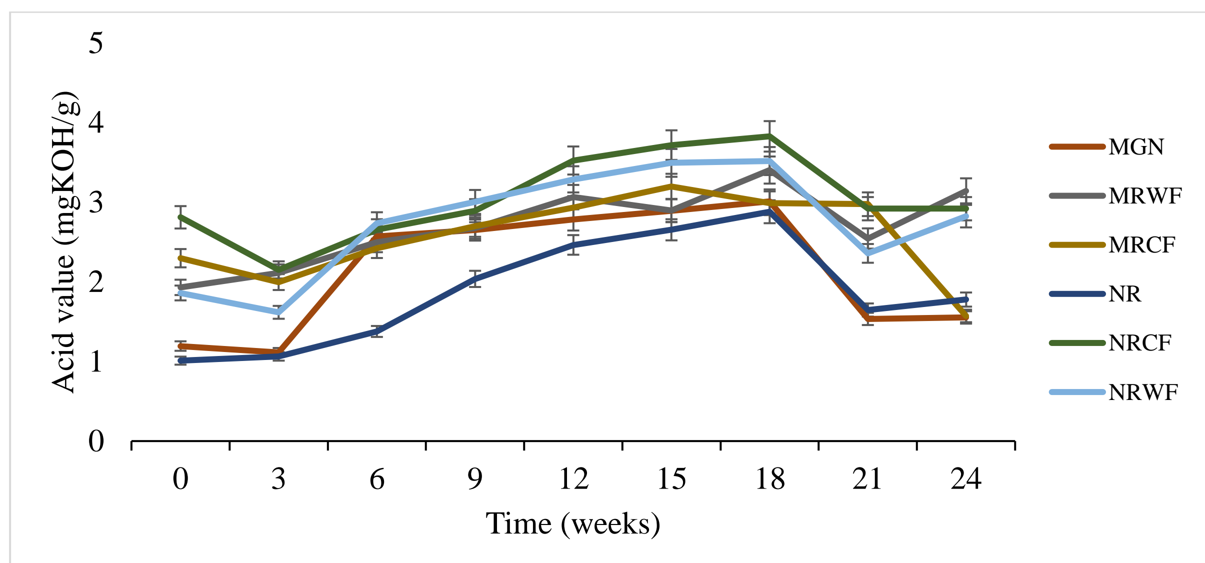


Figure 4.6: Changes in acid value of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52 % Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1:9.49 % Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

The acid value serves as a measure of the quantity of free fatty acids within a sample, offering insights into the extent of hydrolytic rancidity or deterioration of fats and oils (Gulkirpik et al., 2021). Initially and during storage, the acid value for samples exceeded the maximum limit of 0.8 mgKOH/g for composite flours (EAS 782:2019). The initial high acid value could be attributed to the exposure to oxygen and heat during the cassava milling process and house crickets grinding. When fats and oils are exposed to oxygen, they undergo oxidative reactions. Oxygen reacts with unsaturated fatty acid double bonds, initiating the oxidation process (López & Barros, 2019). Heat can promote triglyceride hydrolysis to produce free fatty acids thus increasing the acid value. The increase in acid value over the storage period is a sign of increase in free fatty acids due to lipid hydrolysis (Farag & Al-Obaidi, 2014). Increased free fatty acids may provide substrate for peroxidation, particularly under elevated temperatures and in the presence of oxygen. However, since the observed peroxide values throughout the storage period remained below the Codex Alimentarius standard (CODEX STAN 19-1981, Rev 2-1999) limit of 15 meq $O_2$/kg, it implies that the conditions necessary for significant peroxidation of free fatty acids were not present thus rendering the product stable.

### 4.6.4 Total plate count

Findings indicate that the total plate count of cassava-cricket composite flours generally decreased over 24 weeks storage period for each formulation (Figure 4.7). The observed highest count at week 1 was 6.6 log cfu/g for sample 90.71 % MRWF, while the lowest count observed was 3.2 log cfu/g for NR at week 24. The observed microbial counts at week 24 are generally below the maximum limit of 5.0 log cfu/g for composite flours by East African standard EAS 782: 2019. The decrease in total plate count implies a reduction in the overall microbial population within the cassava cricket composite flours over the observed period. The study's low moisture content may have contributed to the total plate counts overall decrease since this could have limited the amount of moisture available for microbial multiplication.

The implications of these results are positive in terms of product stability and safety. A decrease in total plate count can signify a reduction the risk of microbial spoilage, potentially leading to an extended shelf life.

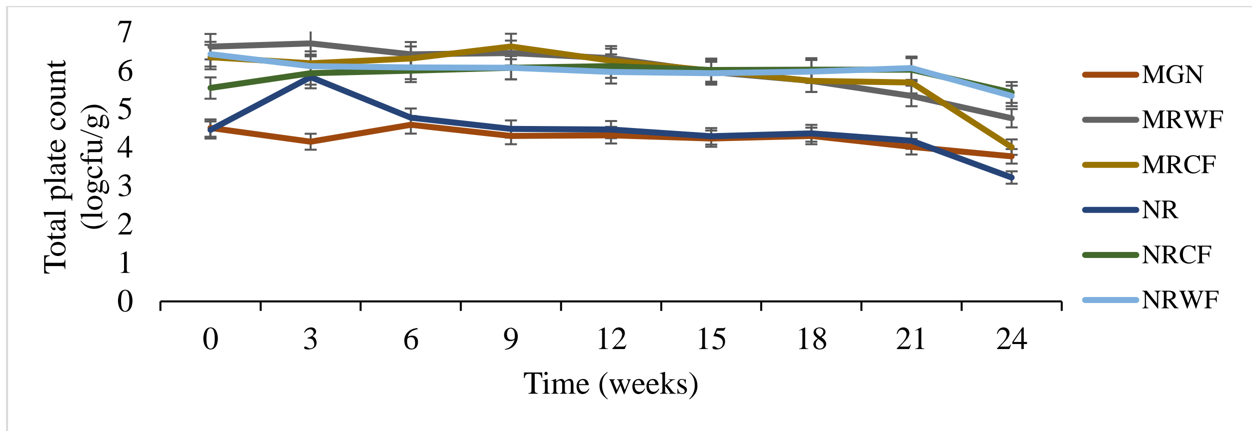


Figure 4.7: Changes in total plate count of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

### 4.6.5 Yeast and moulds

Findings indicate that the yeast and moulds ranged from 2.5 to 3.7 log cfu/g for cassava flour and its composites (Figure 4.8). Microorganisms require moisture to grow and since the flours had a moisture content lower than 14 g/100 g (EAS 782:2019); this could have limited availability of water necessary for microbial proliferation. The consistent yeast and moulds in these samples suggest a degree of microbial stability within the composite flour formulations. This stability can contribute to product quality and safety minimizing the risk of microbial spoilage.

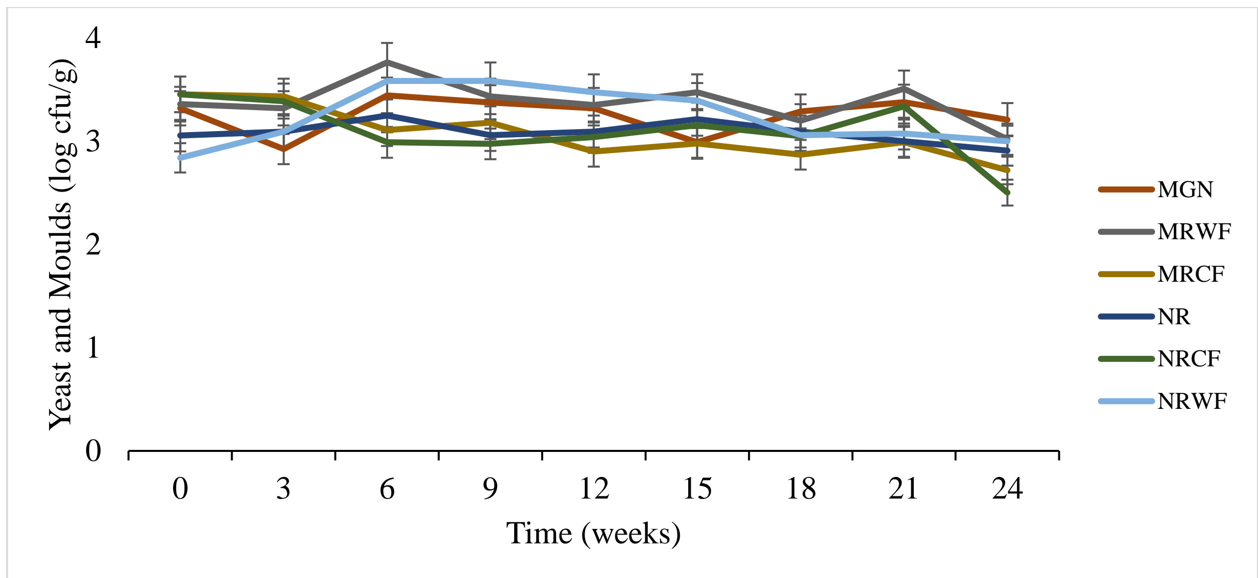

Figure 4.8: Changes in yeast and moulds counts of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

### 4.6.6 Changes in colour

Findings indicate that the sensory scores were generally consistent over the period of the study (Figure 4.9). Exception is with sample MRCF whose sensory score initially decreased from 6.4 in week 1 to 3.7 in week 6. This deviation could be attributed to non-uniform mixing of the sample that possibly made the shelf life study sample to have a higher proportion of cricket powder than others. This is because the pure cassava flour samples show an almost consistent trend. This implies that similar to pure cassava, incorporation of cricket powder does not affect the colour of cassava-cricket composites with time.

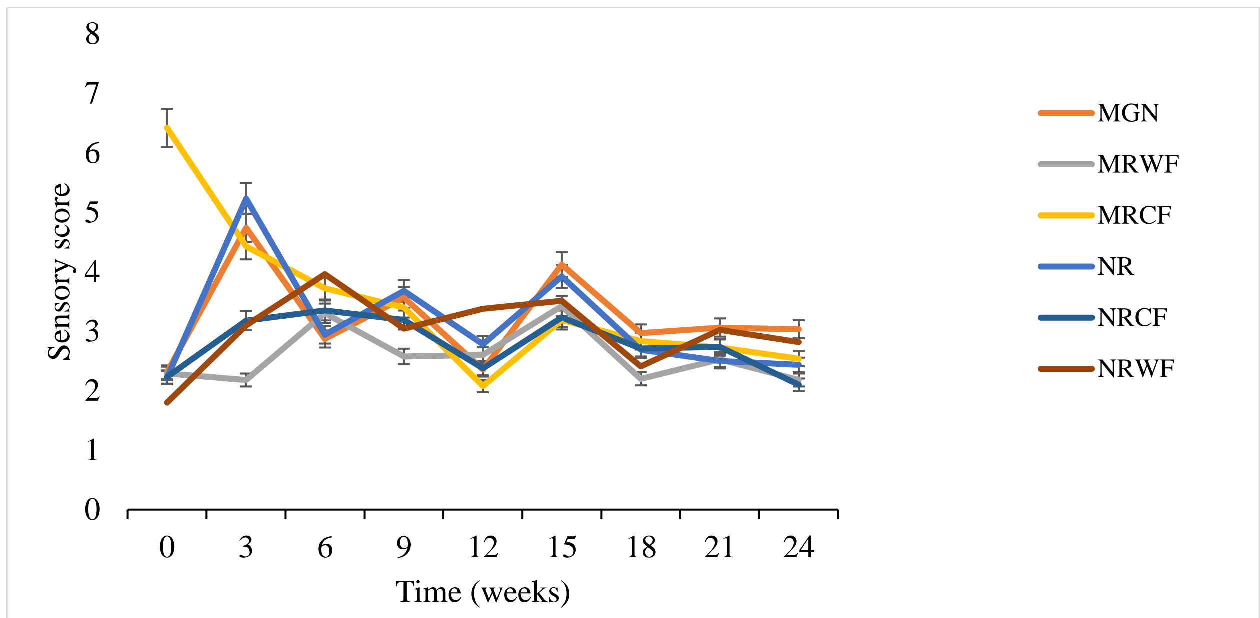

Figure 4.9: Changes in colour of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1:9.49% Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars show no significant difference.

### 4.6.7 Changes in aroma

Findings indicate general decrease from 2.5 – 6.5 in week 1 to 2.0 – 3.8 at week 12 followed by an increase before another general decrease to 2.0 – 3.1 in week 24 (Figure 4.10). Exception is with samples NRWF and MRWF whose sensory scores increased from 4.5 and 5.9 to 9.2 and 9 respectively in week 9 before sharply decreasing to 2.9 and 4 respectively. Samples NRCF, MRCF and MRCF also show a similar deviation from a rather constant trend in weeks 15 and 18. These deviations could be attributed to non-uniform mixing of the samples that possibly made some shelf life study samples have a higher proportions of cricket powder than others. This is because the pure cassava flour samples show an almost constant trend. The latter observations are similar to study findings which found no difference in the smell/aroma of cassava flour varieties over time (Lima et al., 2013).

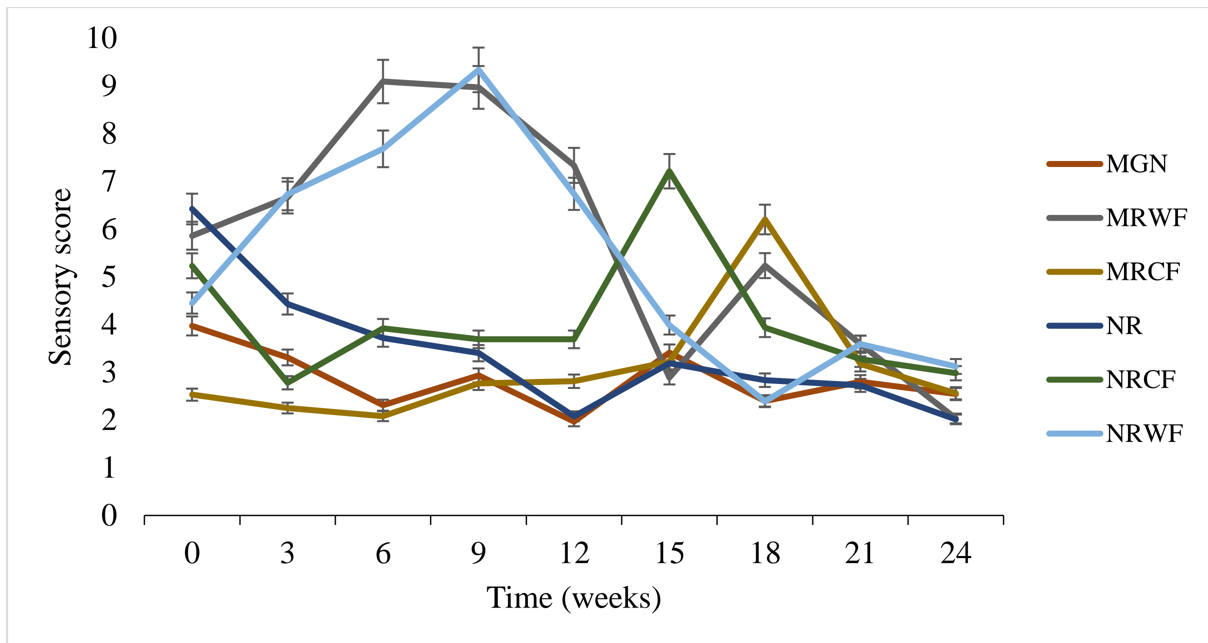


Figure 4.10: Changes in aroma of cassava-cricket composite flour with time. MGN (100% MAGANA), MRWF (90.71% MAGANA:9.29% Raw cricket powder), MRCF (89.48% MAGANA:10.52% Roasted cricket powder), NR (100% NAROCASS1), NRCF (90.51% NAROCASS1: 9.49% Roasted cricket powder), NRWF (91.64% NAROCASS1:8.36% Raw cricket powder). Points are means of three independent observations and error bars are ± standard errors of means ($p < 0.05$). Overlapping error bars indicate no significant difference.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

The incorporation of up to 10.52% cricket (*Acheta domesticus*) powder into cassava flour significantly enhanced its nutritional profile, particularly in terms of protein and fat content, while maintaining acceptable sensory properties, safety, and shelf life. This suggests that cricket powder can serve as a viable ingredient for improving the nutritional value of cassava-based foods.

The study identified that 8.36% cricket powder incorporation was sensorialy acceptable, with significant effects on colour, aroma, and overall acceptability compared to pure cassava flour.

The sensorialy acceptable cassava-cricket composite flour exhibited a significant increase in protein content by 5.54% and fat content by 2.21%. Functional properties, such as oil absorption capacity and swelling capacity, were enhanced by the addition of cricket powder.

The composite flour met safety standards based on Codex Alimentarius and East African Standards, showing absence of Salmonella typhimurium, Staphylococcus aureus, or aflatoxins. Over a 24-week storage period, microbial counts and spoilage indicators (total plate count, acid value, peroxide value) remained within acceptable limits, indicating good shelf stability.

## 5.2 Recommendations

Cricket powder should be incorporated into cassava flour products to improve the nutritional content, particularly in protein-deficient populations.

Further research can explore its use in different food matrices and sensitizing potential consumers should be sensitized about the nutritional benefits of edible insects

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# APPENDICES

**Appendix 1:** Selected Cassava Varieties for the Study

| TITLE OF PAPER OR REPORT | AUTHOR AND YEAR OF PUBLICATION | VARIETIES CONSIDERED | RESULTS | REASONS |
|---|---|---|---|---|
| Development and Dissemination of Improved Cassava Varieties in Uganda: Analysis of Adoption Rates, Variety Attributes and Speed of Adoption | (Abele, 2007) | NASE 1, NASE 2, NASE 3, NASE 4, NASE 10 and NASE 12 | NASE 3 most adopted variety | Superiority in desirable attributes ie high resistance to diseases (especially to CMD), high storage root yields and short maturity period. Famers reported that it has high dry matter content and high market demand |
| Effects of Improved Cassava Varieties on Farmers' Income in Northern Agro-ecological Zone, Uganda | (Akongo et al., 2021) | NAROCAS1, , NASE14 and NASE19 | NAROCAS1 with high profitability | High yield of 37.3 tons per hectare, high profit generation and and disease resistance |
| Evaluation of Newly Released Cassava Varieties for Yield Performance, Reactions to Cassava Diseases and Farmers' Preference in Adjumani District of Uganda | (Abaca et al., 2021) | Local variety (Alifasia), NAROCASS 2, NASE 14, NASE 19, and NAROCASS 1 | NAROCASS1 | High yield of 41.71 t/Ha |
| | | | Order of farmers' preference was NASE 19 (40.96%), NAROCASS 1 (24.86%), NAROCASS 2 (15.82.28%), NASE 14 (15.54%), and a Local cassava—Alifasia (2.83%) | Yields, planting material characteristics, tolerance to CBSD, dry matter content, root size and shape, plant vigour to smoothen weeds, early maturity, experience in growing that particular, variety, sweetness, susceptibility to CMB. |

**Appendix 2: Cassava-cricket blend formulations as generated by design expert**

| Run | Code | Cassava Flour | Cricket powder | Variety | Cricket Roast |
|---|---|---|---|---|---|
| 1 | 133 | 76.25 | 23.75 | NAROCASS1 | Raw |
| 2 | 120 | 95 | 5 | MAGANA | Roasted |
| 3 | 114 | 82.5 | 17.5 | MUKUMBA | Raw |
| 4 | 101 | 95 | 5 | NASE 14 | Roasted |
| 5 | 112 | 95 | 5 | MUKUMBA | Roasted |
| 6 | 119 | 76.25 | 23.75 | NASE 14 | Raw |
| 7 | 103 | 88.75 | 11.25 | NAROCASS1 | Raw |
| 8 | 102 | 82.5 | 17.5 | NAROCASS1 | Roasted |
| 9 | 141 | 95 | 5 | MUKUMBA | Raw |
| 10 | 115 | 70 | 30 | MAGANA | Raw |
| 11 | 134 | 95 | 5 | NASE 3 | Raw |
| 12 | 134 | 95 | 5 | MAGANA | Raw |
| 13 | 133 | 70 | 30 | MUKUMBA | Raw |
| 14 | 136 | 70 | 30 | MAGANA | Roasted |
| 15 | 140 | 82.5 | 17.5 | NASE 14 | Roasted |
| 16 | 111 | 82.5 | 17.5 | MUKUMBA | Raw |
| 17 | 121 | 95 | 5 | NASE 14 | Roasted |
| 18 | 106 | 70 | 30 | MUKUMBA | Roasted |
| 19 | 107 | 82.5 | 17.5 | MUKUMBA | Roasted |
| 20 | 127 | 82.5 | 17.5 | MAGANA | Raw |
| 21 | 136 | 70 | 30 | MUKUMBA | Roasted |
| 22 | 143 | 95 | 5 | NAROCASS1 | Raw |
| 23 | 100 | 70 | 30 | NASE 3 | Roasted |
| 24 | 121 | 82.5 | 17.5 | NASE 3 | Raw |
| 25 | 128 | 88.75 | 11.25 | NASE 14 | Raw |
| 26 | 124 | 70 | 30 | NASE 3 | Raw |
| 27 | 111 | 76.25 | 23.75 | NASE 3 | Raw |
| 28 | 122 | 70 | 30 | NAROCASS1 | Raw |
| 29 | 125 | 70 | 30 | NASE 14 | Raw |
| 30 | 104 | 70 | 30 | NAROCASS1 | Roasted |
| 31 | 119 | 95 | 5 | NASE 3 | Roasted |
| 32 | 138 | 82.5 | 17.5 | NASE 3 | Roasted |
| 35 | 116 | 95 | 5 | NAROCASS1 | Roasted |
| 36 | 121 | 82.5 | 17.5 | NAROCASS1 | Raw |
| 37 | 125 | 82.5 | 17.5 | NASE 14 | Raw |
| 38 | 137 | 95 | 5 | NASE 14 | Raw |
| 39 | 105 | 82.5 | 17.5 | MAGANA | Roasted |
| 40 | 122 | 70 | 30 | NASE 14 | Roasted |

**Appendix 3: Image of house crickets (*Acheta domesticus*)**