DEVELOPMENT OF FINGER MILLET-AMARANTH BASED WEANING PORRIDGE FLOUR ENRICHED WITH EDIBLE CRICKET (*Scapsipedus icipe*)

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DECLARATION

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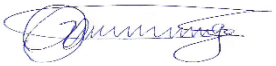
Signature  Date: 29/07/2022

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DEDICATION

To my lovely children Jayden Kabaka, Rowan Baraka, Michelle Cheptoo and Maya Angela, you are my inspiration. To my parents, Samuel Kimaiyo Barngetuny and Elizabeth Cherono Saina, who never saw this adventure.

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ACRONYMS AND ABBREVIATIONS

ALA	Alpha-linolenic acid
AOAC	Association of Official Analytical Chemists
CF	Composite flour
CFU	Colony-forming units
DHA	Docosapentaenoic acid
EPA	Eicosapentaenoic Acid
FAO	Food and Agriculture Organization of the United Nations
FAME	Fatty acids methyl ester
FM	Finger millet
GC-MS	Gas Chromatography-Mass Spectrometry
Icipe	International Centre of Insect Physiology and Ecology
KDHS	Kenya Demographic and Health Survey
PDA	Potato dextrose agar
PEM	Protein-energy malnutrition
PUFA	Polyunsaturated fatty acid
RDA	Recommended Dietary Allowance
SDG	Sustainable Development Goals
SFA	Saturated fatty acid
SSA	Sub–Saharan Africa
UNICEF	United Nations Children's Fund
UPLC	Ultra-performance liquid chromatography
WHO	World Health Organization

ABSTRACT

Globally, there is growing interest to integrate nutrient–dense foods such as insect flour into food products to achieve nutritional goals and address food insecurity. Though cereal–based porridge is widely consumed in many sub–Saharan African countries, there is a lack of information on its enrichment with edible cricket, *Scapsipedus icipe*. The objective of this study was to develop and determine the nutritional composition, anti–nutrient content, sensory acceptability, microbial safety and storage stability of porridge flour formulations enriched with edible cricket. Porridge was prepared from the flour formulations with a cricket content of 0%, 10%, 15% and 20% (w/w). A sensory evaluation pretest, indicating 10% cricket-finger millet-amaranth flour as the most desirable porridge, informed the basis of using 10% cricket, 60% finger millet, and 30% amaranth for the preparation of four porridge flour samples, using traditional processing methods: germination, fermentation and roasting. Untreated formulation and an existing finger millet-based commercial porridge flour served as control. Cricket enriched formulations had high protein (2–folds), crude fat (3.4–4–folds) and energy (1.1–1.2–folds) compared to the commercial flour. Processing by germination and fermentation resulted in high phytic acid degradation (67% and 33% respectively) and improved mineral bioavailability. The iron content of the formulated flours ranged from 8.6–19.5 mg/100 g with the germinated sample having the highest content (19.5 mg/100 g). Zinc content was in the range of 3.1–3.7 mg/100 g while the range obtained for calcium was from 234.9 mg–278.6 mg/100 g. The commercial flour recorded zinc and calcium contents of 1.86mg/100g and 312.7mg/100 g respectively. Cricket enriched formulations had significantly ($p < 0.05$) higher content of vitamin B_{12}, vitamin B_5, vitamin B_6, nicotinamide and thiamine when compared to the commercial flour. A total of 44 fatty acids methyl esters (FAMEs) were detected in the porridge flour oil extract using Gas Chromatography coupled to Mass Spectrometry (GC–

MS). Of the 24 saturated fatty acids (SFAs) detected, Methyl hexadecanoate (palmitic acid) contributed the highest proportion followed by Methyl octadecanoate (stearic acid) across the flour samples. In addition, Methyl 9E–octadecenoate (oleic acid) was the predominant monounsaturated fatty acid (MUFA) whereas Methyl 9Z, 12Z–octadecadienoate (linoleic acid, LA) contributed the highest proportion of the polyunsaturated fatty acids (PUFAs). Alpha–linolenic acid (ALA) was detected in all the cricket-enriched samples while docosapentaenoic acid (DHA) was only present in the fermented sample. Fermentation process caused a significant ($p < 0.05$) increase in the levels of PUFAs (30%) and MUFAs (14%) and a decrease in the SFAs (3%) while roasting process caused a significant ($p < 0.05$) increase in both MUFAs and SFAs by 27 and 10%, respectively. Total flavonoids were reduced during germination (42%) and roasting (10%) but increased during fermentation (13%), while tannin content decreased during germination (29%). Panelist–based sensory evaluation revealed significant differences ($p < 0.05$) among the porridge samples. Results suggest cricket formulations at 10, 15 and 20% were all acceptable with significant variations. 10% cricket formulation had the highest scores for all attributes. On the effect of processing, roasted and fermented samples had the highest sensory scores compared to the germinated porridge sample with the least overall acceptability score. The total viable plate count for the formulations ranged from 2.4 to 4.1 $\log_{10}$ CFU/g, whereas mold and yeast count was in the range of 1.4 to 1.7 $\log_{10}$ CFU/g. Roasted flour formulation had low counts of bacteria, yeasts and mold and low moisture content. The flour formulations packaged in paper bags exhibited higher variations in terms of microbial loads and moisture content as compared to those packaged in aluminium bags. This observation shows that enrichment combined with proper processing may improve the nutritional quality of cereal–based foods and reduce the levels of anti-nutrients. High sensory

rating and low microbial count confirm that cricket flour can be used as an effective functional ingredient to enrich porridge flour.

CHAPTER ONE: INTRODUCTION

1.1. Background to the study

Malnutrition is a serious health concern in children under the age of five in sub–Saharan African (SSA) countries (Akombi *et al*., 2017; WHO/UNICEF, 2019). Notably, the most common forms of malnutrition reported in these countries are protein–energy malnutrition (PEM) and deficiencies of important micronutrients including iron, zinc, and vitamin A (Stevens *et al*., 2013; WHO, 2020). Kenya tops the list of 20 countries that account for 80% of the world's malnourished children (Bryce *et al*., 2006) where stunting, wasting, and underweight in children below five years have been estimated at 26, 4, and 11%, respectively (KDHS, 2014). Malnutrition in infancy and early childhood affects physical growth and cognitive behavior leading to delays in mental and motor development, as well as increased morbidity and mortality (Akombi *et al*., 2017). Low socio–economic status is considered the key underlying cause where the children lack food or survive on diets of low nutritional quality, coupled with improper feeding practices, inadequate care and high rates of infections (Akombi *et al*., 2017; Anigo *et al*., 2010). Besides, commercially fortified weaning foods or animal proteins remain unaffordable to most households due to high costs (Muhimbula *et al*., 2011). Therefore, improving the quality of complementary foods through food-to-food fortification with edible insects is essential in the realization of Sustainable Development Goal (SDG) 2 of Zero hunger which aims at sustainable and improved food and nutritional security and agricultural sustainability (United Nations, 2018).

Porridge is an important traditional beverage in many African countries (Onyango *et al*., 2004). This porridge is mainly prepared from sorghum, finger millet, cassava and maize flour and serves as an important complementary food for children as well as a refreshing drink for adults (Onyango & Wanjala, 2018; Wanjala *et al*., 2016). Despite these staples being rich in carbohydrates, their

energy and nutrient densities are extremely low (Dewey, 2013). These cereals may also be less nutritious owing to the presence of anti–nutritional factors which form insoluble complexes with protein and key minerals primarily zinc, iron, calcium, and magnesium, thereby leading to poor uptake of these nutrient components (Zhang *et al*., 2020).

The African continent is blessed with a rich diversity of food crops, most of which have received little or no attention in terms of research and development of policy frameworks that could promote their effective commercial and industrial utilization. Grain amaranth (*Amaranthus* spp.) and finger millets are some of such neglected and underutilized species that could be used to produce porridge products to serve as important traditional beverages and complementary food for adults and children, respectively, in Africa (Mmari *et al*., 2017; Onyango *et al*., 2000). Finger millet grain contains high amounts of proteins and minerals compared to other staple cereals (Saleh *et al*., 2013; Singh & Raghuvanshi, 2012). On the other hand, amaranth grain is a pseudo–cereal richer in quality protein, lipids and micronutrients (Njoki *et al*., 2015). Amaranth (*Amaranthus* spp.) is an indigenous African leafy vegetable grown in at least fifty tropical countries and consumed by several million people (Békés *et al*., 2017; Ochieng *et al*., 2019) for many nutritional reasons. Farmers in sub-Saharan Africa cultivate amaranth either for its leaves or for its grain (Ochieng *et al*., 2019). The leaves are rich in vitamin C and pro-vitamin A as well as in iron, zinc, and calcium (Yang *et al*.,2009). The grains are also rich in quality protein, lysine, and calcium and are consumed directly or used to fortify maize flour (Njoki *et al*., 2015; Macharia *et al*.,2011). However, these grains contain high content of anti–nutrients mostly in the form of phytic acid (Pastor & Ačanski, 2018; Shibairo *et al*., 2014). These anti-nutrients can be reduced to improve the nutritional quality through traditional food processing methods including soaking, germination, fermentation, roasting, and milling (Saleh *et al*., 2013).

Globally, there has been a lot of effort in promoting edible insects as a potential solution to food and nutritional insecurity. Current research has established that edible insects contain adequate amounts of protein, unsaturated fatty acids, and essential micronutrients (Cheseto *et al*., 2020; Kinyuru *et al*., 2013; Rumpold & Schluter, 2013). Crickets, in particular, are an economically viable source of essential amino acids, fatty acids, vitamins and minerals chiefly zinc and iron (Magara *et al*., 2021). Besides, crickets are easy and relatively cheap to rear for commercial and subsistence use owing to their high feed conversion efficiency and reproductive potential (Oonincx *et al*., 2015).

Enriching cereal–based foods with edible insects may improve the nutritional value of staple foods (Ayieko *et al*., 2010; Kinyuru *et al*., 2015; Osimani *et al*., 2018). Enrichment combined with proper processing techniques can provide nutrient–dense food suitable for complementary feeding. However, for a stable intake and legislative purposes, insect–based foods must meet other essential standards such as food safety and sensory acceptability. Acceptance of edible insects as human food remains an obstacle in promoting entomophagy, and this is majorly associated with disgust sensitivity or food neophobia (Megido *et al*., 2016). Choice of insect–based products as part of the consumers' regular diet can be related to a number of factors not limited to visual appearance, taste and good quality while overlooking the general environmental sustainability and/or protein quality (House, 2016). The current study, therefore, evaluates the differences among formulations of finger–millet–amaranth porridge flour enriched with cricket and the effect of processing methods on nutritional properties, anti–nutritional factors, sensory acceptability and storage stability.

1.2. Statement of the problem

High levels of Protein Energy Malnutrition (PEM) and hidden hunger are major challenges amongst children below five years in developing countries such as Kenya. In these countries, high starch and less nutrient–dense food such as cereals and tubers are customarily used for complementary feeding. These staples are an excellent source of nutritional energy but are considered low in various essential amino acids and micronutrients necessary for healthy growth (Dewey, 2013; Onweluzo & Nnamuchi, 2009; Tizazu *et al*., 2010), they also contain high anti–nutrient content which inhibit the bioavailability of essential minerals and lower protein digestibility. Feeding young children on these cereals and tubers exposes them to malnutrition. Furthermore, improper feeding practices, coupled with inadequate care and high rates of infections are other underlying factors associated with child malnutrition (Akombi *et al*., 2017; Anigo *et al*., 2010). On the aspect of food safety, contaminated complementary foods are associated with diarrhea and malnutrition among infants and young children, hence the need to ensure that complementary foods do not pose a risk of gastrointestinal diseases (Rahman *et al*., 2016). Therefore, the enrichment of cereal–based porridge flour using edible insects combined with proper processing techniques would result in a nutrient–dense food product suitable for weaning. Additionally, there is inadequate information on the utilization of edible insects such as crickets in complementary foods.

1.3. Justification of the study

Edible insects are widely acknowledged to contain adequate amounts of protein, unsaturated fatty acids, and essential micronutrients, thereby meeting the nutritional requirements for young children. Crickets are economically viable sources of essential amino acids and minerals like zinc,

calcium, and iron. Suggestively, fortification of traditional staple food like finger millet–based porridge flour is a more effective way of alleviating malnutrition among infants and young children. Furthermore, suitable traditional processing methods such as fermentation, roasting and germination would reduce the anti–nutrient content hence improving nutrient bioavailability. The adoption of well–formulated finger millet–amaranth–cricket food would be impetus in curbing malnutrition among vulnerable groups, particularly young children under the age of five.

The study would also improve feeding practices, diversification of eating habits and promote the culture of consuming edible insects. In addition, the rearing and marketing of edible crickets could also provide an alternative means of income to many families hence improving their livelihoods.

1.4. Objectives

1.4.1. Overall objective

The overall objective of this study was to develop and evaluate finger millet–amaranth-based porridge flour enriched with edible cricket.

1.4.2. Specific objectives

i. To determine the nutrient and anti–nutrient composition of composite flours formulated from finger millet, amaranth, and cricket flours.

ii. To determine the sensory acceptability of the porridges made from finger millet, amaranth and cricket composite flours.

iii. To determine the microbial safety of the composite flours made from finger millet, amaranth and cricket.

iv. To establish the shelf stability of the formulated composite flours made from finger millet, amaranth and cricket.

1.5. Hypotheses

i. Composite flours formulated from finger millet, amaranth and cricket do not have the same nutrition value as commonly consumed porridge flour.

ii. Enrichment of finger millet-amaranth flour with cricket powder has no effect on the sensory acceptability of its porridge.

iii. The microbial load of the composite flour formulated from finger millet, amaranth and cricket is not within the recommended limit.

iv. The composite flour formulated from finger millet, amaranth and cricket is not shelf stable under conventional storage conditions.

CHAPTER TWO: LITERATURE REVIEW

2.1. Entomophagy culture

Entomophagy is defined as the practice of eating insects as food. This practice, though perceived as a non–tradition, is practiced across the globe, especially in Asia, Africa, and Latin America (Van Huis, 2013; Fontaneto *et al.*, 2011). Around 2100 species of edible insects have been documented and more than two billion people eat insects as part of their diet (Daylan *et al*., 2017; Jongema 2017; Van Huis, 2013). Insect orders such as Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera, and Orthoptera are the most commonly consumed insect species (Payne *et al*., 2016). Locusts, grasshoppers, termites, caterpillars, worms, palm weevils, and crickets constitute the most renowned edible insects. Africa harbors a large variety of edible insects, having more than 470 insect species currently recognized as edible (Kelemu *et al*., 2015). In sub–Saharan Africa, there are approximately 246 edible insect species (Van Huis, 2003) comprising majorly of lake flies, termites, crickets, black ants, and grasshoppers (Ayieko *et al*., 2010; Kinyuru *et al*., 2012). Crickets (Figure 2.1) are the most commonly consumed insects and are regarded as complete protein sources (Magara *et al*., 2021). However, for human consumption or animal feed, a large number of insects need to be harvested from nature. The collected insects cannot satisfy the demand and therefore, commercial mass rearing is a sustainable solution (Van Huis & Oonincx, 2017).

Figure 2.1: Edible cricket (*Scapsipedus icipe*) and its preparations for human consumption.

2.2. Nutritional aspect of edible insects

2.2.1. Protein composition

Insects have large amounts of essential amino acids (46–96%) which are readily digestible (Ramos–Elorduy *et al.*, 1997; Rumpold & Schluter, 2013). Insects from the order Lepidoptera followed by Coleoptera, have been reported to contain high amounts of crude protein while the order Hymenoptera has the least. According to Xiaoming *et al*. (2010), protein contents of edible insects differ from 13–77% of dry weight, which varies considerably between and within the insect orders. Insect proteins are relatively higher than proteins found in most plants and other animal proteins (Teffo *et al*., 2007; Xiaoming *et al*., 2010). Some of the essential amino acids contained in edible insects include tyrosine, lysine, tryptophan, phenylalanine, and threonine (Rumpold & Schluter, 2013). For crickets, the crude protein level is about 65% (Table 2.1). Therefore, the inclusion of edible cricket in staple cereal diets could be of high benefit in complementing the essential amino acids.

Table 2.1: Overall nutritional composition of crickets (*Acheta domesticus*), finger millet and amaranth grain.

Parameters	Nutrient values (dry weight)		
	Acheta domesticus [a, b, c]	**Finger millet** [d, e, f]	**Amaranth grain** [g]
Proximate composition (g/100g)			
Moisture	8.1	7.5–13.1	9.74
Protein	62.4–71.09	5.6–12.7	14.44
Carbohydrate	9.7	59.0–83.3	66.28
Dietary fiber	10.20	15.0–22.0	4.27
Total fat	9.80–22.80	1.3–1.8	7.09
Total ash	5.10–9.10	–	3.18
Minerals (mg/100g)			
Calcium	314.77–1290.00	162.0–358.0	578.24
Manganese	2.97–5.87	17.61–48.3	–
Phosphorous	33.13–957.79	130–250	–
Potassium	979.75–1126.2	420–490	729.69
Zinc	2.18–21.79	0.92–2.55	5.33
Iron	5.18–19.68	3.3–20	35.02
Copper	0.85–2.94	0.47	0.92
Sodium	435–850.2	49.00	94.54
Magnesium	–	78–201	653.27

Sources: [a] (Rumpold & Schluter, 2013) [b] (Ayieko *et al*., 2016) [c] (Magara *et al*., 2021), [d](Singh & Raghuvanshi, 2012) [e] (Ramashia *et al*., 2019), [f] (Devi *et al*., 2014), [g] (Njoki *et al*., 2015).

2.2.2. Fat content

Fatty acids found in edible insects are important for brain development in humans (Van Huis *et al*., 2013). The amount of fat varies from 2–77% on a dry weight basis and is much more in the larval stages than in adult stages (Kouřimská & Adámková, 2016; Rumpold & Schluter, 2013; Womeni *et al*., 2009), although, the fat profile is highly dependent on their foodstuff. Edible insects are rich in unsaturated fatty acids (Van Huis, 2016) ranging from, 10–44%, monounsaturated acids, 2–49%, and polyunsaturated fatty acids, 19–74% (PUFAs) (Fontaneto *et al*., 2011). Larvae, worms, and caterpillars have the highest fat content than those found in grasshoppers, locusts, and crickets (Kouřimská & Adámková, 2016).

2.2.3. Fiber content

Insects contain a large amount of crude fiber in the form of chitin (Anankware *et al.*, 2015). Chitin contains prebiotic properties that selectively encourage the growth of beneficial intestinal bacteria, thus promoting good health (Stull *et al*., 2018). However, protein digestibility improves with the removal of chitin (Finke, 2007). Bednářová *et al*. (2013) demonstrated that African migratory locust contains the maximum content (27%) of chitin whereas the Jamaican field cricket contains the smallest amount of chitin (8%). In-ground house cricket, chitin ranges from 4.3–7.1% while chitosan ranges from 2.4–5.8% on a dry weight basis (Ibitoye *et al*., 2018).

2.2.4. Micronutrient content

Micronutrients from these insects include minerals like iron, zinc, potassium, magnesium, calcium and a range of vitamins that can easily be assimilated by humans (Payne *et al*., 2016) and therefore better supplementation of micronutrient inadequacies in most third-world countries (Christensen *et al*., 2006). According to (Sirimungkararat *et al*., 2010) the iron and calcium contents in edible insects are higher than those in beef, pork, and chicken. Crickets also contain vitamins such as thiamine, vitamin B12 and Riboflavin (Table 2.1) (Bukkens, 2016; Xiaoming *et al*., 2010).

2.3. Consumer acceptance of edible insects

Food neophobia refers to extreme fear or dislike of tasting unfamiliar foods and has proven to be one of the biggest barriers to entomophagy (Megido *et al*., 2016). Visual appearance, taste and good quality are some of the widely considered attributes by consumers in selecting insect-based products as part of their meal (House, 2016). Habits of not eating insects are mostly attributable to cultural inclinations or the impression that insects are unpalatable and not on sensory experiences

(Hamerman, 2016). According to Wendin *et al*. (2016), the consent for consuming food supplemented by protein powder from insects was higher than the approval of consuming food supplemented with whole insects. Suggestively, foods, where insects are incorporated in an invisible way, could be more acceptable to consumers (Stoops *et al*., 2017). In addition, creating benefit awareness as well as maintaining good taste, attractiveness and accessibility can improve insect–eating behaviour.

2.4. Methods of processing edible insects

The process of processing food aims at increasing the shelf–life and providing a safe product at optimum quality. It is important to execute a cost–effective post–harvest handling and management system that adheres to food safety standards (Melgar–Lalanne *et al*., 2019) and also maintains the nutritional importance of the product. The system must also ensure that the other essential quality aspects (e.g., chemical composition, texture, colour, size, taste) remain intact (Rumpold & Schluter, 2013). Different approaches such as blanching, drying, and milling have been used in the processing of these insects. Blanching involves pretreating edible insects to lower microbial counts and inactivate degradative enzymes. It lowers the number of mesophilic bacteria, yeast, and moulds (Klunder *et al*., 2012; Megido *et al*., 2017). However, it is ineffective against the mesophilic bacterial spores and it may affect the chemical and nutrient composition of the products (Niamnuy *et al*., 2008).

Drying is commonly used to reduce water activity ($aw < 0.65$) thereby restricting microbial and enzymatic degradative reactions (Schlüter *et al*., 2017). However, drying may affect protein digestibility, lipid oxidation, vitamin content, and color. For commercial products, hot–air oven drying is preferred as it has minimal changes on the final quality of the product (Azzollini *et al*.,

2016). Blanching, followed by drying or refrigerated storage results in safe produce (Vandeweyer *et al.*, 2017). In addition, grinding has also been often during processing insects for food. However, grinding insects with high–fat content is often difficult.

2.5. Production and consumption of finger millet and amaranth grains

Cereals are the most important source of food worldwide (Oghbaei & Prakash, 2016). The most cultivated cereals are wheat, rice and corn. However, in many African countries as well as in Asia, millet grains are considered alternative cereals for human consumption and traditional food and beverages in form of pieces of bread, porridges, and snack foods (Saleh *et al.*, 2013). They comprise a variety of species that can be grown in arid and tropical regions. Millets can be grouped into six small-grained cereal crops *viz.* finger millet (*Eleusine coracana*), barnyard millet (*Echinochloa* spp.), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), and little millet (*Panicum sumatrense*) (Gupta *et al.*, 2017). Finger millet (FM) (*Eleusine coracana)*in particular is one of the economically important cultivated millet and serves as a staple food in most African and Asian countries (Saleh *et al.*, 2013). The crop is drought–tolerant and is mostly grown in semi–arid areas with limited rainfall. FM is also easy to grow and suffers fewer diseases and pest infestation (Devi *et al.*, 2014; Gull *et al.*, 2014). They have unique characteristic and excellent nutritional properties and their consumption have resulted in numerous health benefits including lowering risks of cancer, tumour, celiac disease, cardiovascular disease, promoting digestion and is a recommended food for patients with diabetes and gastrointestinal illness (Gupta *et al.*, 2017; Saleh *et al.*, 2013). Millets primarily finger millet are critical diets for expectant and breastfeeding mothers, and children because they supply the required energy, proteins, lipids, and macronutrients that are essential to prevent protein-energy-

malnutrition (PEM) (Akinsola *et al* 2017; Gupta *et al*., 2017). Prior to consumption, millet grains are prepared using traditional processing methods such as decorticating, germination, fermentation, roasting, flaking, and grinding. This method simply makes the millet palatable as well as improves its nutritional values and sensory properties (Saleh *et al*., 2013). Finger millet-based flours can be blended with other flours to improve the nutritional and functional properties and come up with good blends for making different types of food (Rathod *et al*., 2018). For making porridge, finger millet-based composite flours are preferred over unblended flours because it gives superior sensory and nutritional properties (Tumwine *et al*., 2019; Wanjala *et al*., 2016).

Grain amaranth is categorized as a pseudo–cereal from a dicotyledonous plant native to America. It is an ancient crop that largely contributed to the diets of the Maya and Aztec people (Békés *et al*., 2017). Three main species are cultivated for grain production and include *A. hypochondriacus, A. cruentus* and *A. caudatus* (Pastor & Ačanski, 2018). The crop is a highly reproductive plant and disease–tolerant requiring less rainfall and moderate soil fertility. The leaves from the crop can also be consumed as a vegetable which is a common practice in the humid tropics of Africa and Asia. Despite amaranth being widely cultivated in Africa as vegetables, their grains have been documented to contain excellent nutritional properties and have been milled and blended with flours of other cereal staples to improve their overall acceptability (Okoth *et al*., 2017; Zebdewos *et al*., 2015).

2.6. Nutrient and anti-nutrient content of millet and amaranth grains

For any food, the nutritional composition is very critical in maintaining the overall health of humans. Nutrient-rich food is essentially required for growth and development as well as increasing the overall potential of human genetic makeup. Compared to other major cereals like wheat and rice, millets are easy to cultivate and have high nutritive value including phytochemicals

(dietary fiber and polyphenols), micronutrients and essential amino acids with relatively high levels of methionine but lack lysine and threonine (Gupta *et al* 2017; Saleh *et al*., 2013). Millet grains are rich in starch, minerals, dietary fibers, and antioxidants (Saleh *et al*., 2013). Saleh *et al* (2013) recounted that finger millet encompasses about 92.5, 63.2, 7.8, 3.6, 2.8, and 2.1% of dry matter, starch, crude fat, crude protein, crude fiber and ash, respectively. Among the minerals found in abundance in finger millet are calcium, manganese, phosphorus, iron, copper, chromium, magnesium, molybdenum, zinc and selenium (Gull *et al*., 2014; Gupta *et al*., 2017). The grains are particularly rich in calcium (344 mg/100 g) as compared to all other millets (Singh & Sarita, 2016). Finger millet is also rich in amino acids, specifically, it has high higher methionine which is essentially absent in other cereals (Gupta *et al*., 2017).

Porridge from finger millet is often used as a weaning food since it is considered gluten-free, non–acid-forming and easily digestible cereal food (Singh & Raghuvanshi, 2012). However, it also contains a high content of anti–nutrients like phytates, polyphenols, tannins and enzyme inhibitors that can inhibit the bioavailability of nutrients (Singh & Sarita, 2016).

Amaranth grains have excellent nutritional qualities and are rich in protein (about 14%), carbohydrates (66.28%) and fat (7.09%) (Békés *et al.*, 2017; Njoki *et al.*, 2015). The protein in amaranth is more balanced compared to other cereals as it contains high content of lysine which is often limiting in most cereal crops, but is still limited in leucine and threonine (Gorinstein *et al*., 2002; Mburu *et al*., 2012). They are also a good source of dietary fiber and contain higher levels of iron, zinc, calcium and potassium (Lamothe *et al*., 2015; Njoki *et al*., 2015). Just like FM, amaranth is also a gluten-free grain making it a suitable option for complementary feeding. However, it also contains anti–nutrients mostly in the form of phytic acid hence the need for

appropriate processing techniques that can lower the anti–nutrients and increase nutrient bioavailability (Pastor & Ačanski, 2018).

2.7. Processing methods and their effect on the nutritional value of millet and amaranth grains

Prior to consumption, cereal grains are usually processed to render them palatable as well as improve their nutritional values and sensory properties. Cereal grains are processed using traditional processing methods such as decorticating, germination, fermentation, roasting, flaking, and grinding (Saleh *et al*., 2013). Processing methods can also improve the bioavailability of micronutrients and reduce the levels of ant nutrients, including phytates (Shibairo *et al*., 2014).

Methods such as milling change the grain's overall chemical composition. Differential milling reduces polyphenols and phytic acid in plant-based food and improved the digestibility of protein and starch (Oghbaei & Prakash, 2016). An increase in milling time decreases the contents of protein, fat, ash and fibers, however, sieving reduces the nutrient, and ant nutrient contents but improves their bioaccessibility (Saleh *et al*., 2013).

Germinating millet grains reduces the dry weight, crude protein, fat content, starch content and ant nutritional factors while increasing their total sugars and free amino acids like lysine and tryptophan, and mineral bioavailability including iron, calcium, and zinc (Akinsola *et al*., 2017; Saleh *et al*., 2013). Germinating millet grains improve the protein content by 14 to 26% and starch content by 86 to 112% (Saleh *et al*., 2013). Therefore, germination combined with other processing methods has the potential to significantly improve the nutritional quality of traditional cereal food.

Roasting affects the properties of food including reducing moisture content, vitamins, mineral contents and anti-nutritional factors (lection, protease, and trypsin inhibitors)(Agume *et al*., 2017). It also changes protein through pyrolysis, lowers reducing sugars and enhances the browning of food to improve its flavor, aroma and overall acceptability (Akinsola *et al*., 2017).

Fermenting cereal grains reduces their levels of anti-nutrients (total polyphenols and phytic acid content) and improves the bioavailability of protein, fiber, fat and starch (Abdelhaleem *et al*., 2008; Akinsola *et al*., 2017). During the fermentation process, proteins are converted to amino acids, and starches are transformed into simple sugars while increasing riboflavin and niacin contents (Akinsola *et al*., 2017). It also improves tryptophan, and vitamin B2 contents but reduces lysine, arginine, glycine, vitamin A, flavonoid, and tannin contents (Saleh *et al*., 2013).

Soaking cereal grains reduces their levels of anti-nutrients through phytate degradation while reducing phosphorus, calcium, and iron (Saleh *et al*., 2013). Therefore, processing methods have been shown to significantly affect the chemical composition of the cereal food, however, the effect may vary with the type of the cereal food.

2.8. Microbial safety of composite flours and storage stability

Microbial load in various food enriched with edible insects may vary according to processing methods, storage time and conditions, packaging materials, insect species and the formulations (Adesina, 2021; Mwangi *et al*., 2019). Mwangi *et al.* (2019) found that the microbial loads in individual products based on locusts, grasshoppers and malted finger millet as well as in composite flours fluctuated between 10^6–10^8 CFU/g for total viable counts (TVC), 10^5–10^8 CFU/g for coliforms, 10^4–10^7 CFU/g for Lactic acid bacteria (LAB), and $1.7–2.5 \times 10^7$ for yeasts and moulds. However, the microbial load especially in composite flour fortified with edible crickets remain

least explored. Adesina (2021) packaged pearl millet extruded snacks in aluminium and polyethylene bags and evaluated the microbial loads at 7 days intervals for two months at freezing, refrigeration and room temperature. He found a higher microbial population in polythene packages than in aluminium packages. Also, the highest microbial population and frequency of occurrence were evident when samples were stored at 28 and 37°C. Additionally, within two months of storage, microbial loads remained low (less than 10^6 CFU/g) and were within the maximum permissible level. Polythene paper increases the pH and moisture content compared to the aluminium package which was ascribed to microbial activities (Adesina, 2021). Similarly, Geetha *et al*. (2019) observed that storage of millet-based composite flour increases moisture content and free fatty acids (FFA) as well as microbial population and occurrence. However, a similar study evaluating the extended shelf stability on microbial loads and overall physical characteristics of composite cereal flour such as finger millet and amaranth fortified with edible crickets is needed.

2.9. Sensory acceptability of composite flour products

Other than the intrinsic properties of composite flour, their processing methods critically affect their nutrient content, nutrient quality, nutrient availability, shelf life, flavour, aroma, palatability and bulkiness. The blending of flours not only improves the nutritional value of composite flours but also helps in developing desirable sensory properties and masking undesirable odours and tastes (Gebretsadikan *et al*., 2015). For instance, the addition of amaranth flour in cereal staples increases their sensory test and acceptability (Zebdewos *et al*., 2015). Although the addition of insect flour into staple cereal flours significantly improves their nutritional quality, especially the protein content, their proportion in the composite flour should be regulated. In a study done by Kinyuru *et al*. (2009), wheat buns enriched with 5% termite concentration were more acceptable

when compared to those with 20% termite concentration. Awobusuyi *et al*. (2020) also reported high acceptability of sorghum-insect meal biscuits as compared to biscuits without insect meal. Preparation processing such as roasting improves the flavour and aroma of the porridge (Akinsola *et al*., 2017).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Collection of raw materials

Grain amaranth *Amaranthus spp.*, finger millet *Eleusine coracan L.* and a commonly–used commercial porridge flour (CPF) were procured from a local market in Nairobi, Kenya. Crickets (*Scapsipedus icipe)* were obtained from the Animal Rearing and Containment Unit (ARCU) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya (Appendix 1)

3.2. Preparation of raw materials

3.2.1. Preparation of crickets

Frozen cricket samples were allowed to thaw overnight at 5°C refrigeration temperature. The samples were then washed thrice in fresh clean tap water at 18°C to remove dirt, drained and appropriately heat–treated in hot water at 100°C for 5 minutes for efficient sterilization. Thereafter, the crickets were oven–dried (WTB binder, Tuttlingen, Germany) at 60°C for 24 hours, milled using an electric grinder [Medical Research Council (MRC) laboratory grinder, London, UK] and sieved through a 0.595-mm aperture sieving mesh. The resulting powder was packaged in a sterile zip–lock polyethylene bag and stored at 4°C for subsequent composite flour formulation.

3.2.2. Processing of finger millet and amaranth grains

Finger millet and amaranth grains were each divided into a batch of 500 g in four replications. The first batch was germinated according to the method described by Onyango *et al.* (2013). Briefly, the grains were cleaned and steeped in tap water for 24 hours at 25 °C and germinated for 72 hours at 25°C in the dark, while being moistened and turned at 12–hour intervals. Germination was halted by oven-drying at 50°C for 12 hours. The dried germinated grains were then removed and milled

using an electric grinder (MRC laboratory grinder, London, UK). The second batch was roasted according to the modified method (Zhang *et al*., 2010). The grains were spread in a uniform, thin layer on an oven tray and roasted in a preheated oven at 120°C for 20 min. The grains were allowed to cool to room temperature before milling. The third batch was milled and fermented according to the method described by Onyango *et al*. (2000) with slight modifications. The composite flour was mixed with tap water that had been boiled, then cooled to 45°C, at a ratio of 2:3 (flour to water). The slurries were fermented spontaneously in round-bottomed flasks placed in a water bath (Blue M Electric Company, IL 60406, USA) at 45°C. After 24 hours the fermented slurries were inoculated into freshly prepared slurries before fermenting at 45°C for 24 hours. The samples were then spread on trays and dried in an oven at 50°C for 24 hours and ground in an electric grinder (MRC laboratory grinder, London, UK). The fourth batch was unprocessed grains treated according to farmers' field practices of open sun drying and storage, using appropriate technology to ensure availability throughout the year (Masarirambi *et al*., 2010). Thereafter, the grains were milled and prepared for further processing into porridge products. The fifth batch was considered as the "control treatment" and consisted of the CPF, which had undergone approved commercial standards of processing. All the samples were ensured to have a moisture content of <12.0%.

3.3. Composite flours formulation

Composite flour formulations were developed and optimized using the Linear Programming Excel solver 2010 version based on minimum RDA for Protein (13 g), energy (500 kcal), calcium (500 mg), iron (7 mg) and zinc (4.1 mg) for children aged of 1–3 years (WHO/FAO, 2002, 2004). Composite flours were prepared from blends of finger millet: amaranth: cricket and labeled as follows [composite flour (CF)] CF1 (56:44:0) CF2 (50:40:10), CF3 (47:38:15) and CF4 (45:35:20) as shown in Table 3.1 and used as samples for flavor optimization.

Table 3.1: Formulations for CF1, CF2, CF3 and CF4 composite flours (%).

Formulation	Composition (%)		
	Cricket	Finger millet	Amaranth
CF1	0	56	44
CF2	10.0	50	40
CF3	15.0	47	38
CF4	20.0	45	35

Composite flour 1(CF1), Composite flour 2(CF2), Composite flour 3(CF3), Composite flour 4(CF4)

3.4 Experimental design

This study was set up as a completely randomized design (CRD) whereby raw materials for composite flour formulation were randomly collected and processed into flour. The experimental design was conducted at two levels. In the first set of experiments, flavor optimization (sensory evaluation) was carried out using 7–point hedonic scales with 40 panelists randomly selected from the Food Science Department at the University of Nairobi. This was to ascertain the level of cricket which is acceptable without giving off–flavors as the basis of flavor optimization. Porridge prepared from the composite flour 2 (CF2) was the most desirable porridge and formed the basis of the preparation of four porridge samples. In the second experiment, formulation CF2 was prepared into four complementary flours using traditional processing methods: germination, fermentation and roasting. Four porridge flour formulations were prepared and coded as follows, fermented finger millet–amaranth + cricket (FFM–AC), germinated finger millet–amaranth + cricket (GFM–AC), roasted finger millet–amaranth + cricket (RFM–AC) and untreated finger millet–amaranth + cricket (UFM–AC) which served as the control for treatments.

3.5. Assessing the chemical composition

3.5.1. Analysis of proximate composition

Proximate analysis for the flour was carried out following the Association of Official Analytical Chemists (AOAC) methods (AOAC, 2019). Moisture content was determined in an air oven adjusted to 105°C (method 925.10). The Kjeldahl method (method 978.04) was used to assess the crude protein (N × 6.25). Fat content was extracted using petroleum ether in a Soxhlet extractor (method 930.09). Ash content was determined by gravimetric (method 930.05). Crude fiber was determined by acid digestion and loss of ignition (method 930.10). Carbohydrate contents were determined as the difference (CHO%= [100 – protein% – fat% – crude fiber% – ash %]). The energy value was computed by multiplying the Atwater factors of 4, 9, and 4 with protein%, fat% and carbohydrate% contents, respectively. All proximate analyses were carried out in triplicates.

3.5.2. Determination of mineral composition

Mineral composition was established by the ICP OES quantitation method as follows. Exactly 0.5 g of each sample was digested with concentrated HNO_3 (8 mL) and 30% H_2O_2 (2 mL) and left overnight in a fume chamber. Samples were then digested in a temperature–controlled block digester (Model TE007–A, TECNAL, São Paulo, SP, Brazil) following these conditions; 75°C for 30 min, 120°C for 20 min, 180°C for 20 mins and 200°C for 10 min. The resulting solutions were cooled and transferred to 25 mL falcon tubes before being diluted with 2% Nitric acid. Mineral compositions were assessed using an inductively coupled plasma optical emission spectrometer (ICP–OES) (Model Optima 2100 DV Perkin Elmer, Massachusetts, USA) and analyzed using Winlab 32 software. The following operational conditions were used: radiofrequency power (1.45 kW), auxiliary gas flow rate (1.5 L min^{-1}), plasma gas flow rate (15.0 L min^{-1}), nebuliser gas flow

rate (0.7 L min^{-1}), sample flow rate (1.5 L min^{-1}), source equilibrium time (10 s) and delay time (10 s). Signal intensity measurements of the analytes in all samples solutions were carried out at wavelengths (nm) as follows: Mg: 285.213, Fe: 259.939, Mn: 257.61, Ca: 317.933, P: 213.617, Mo: 202.031, K: 766.49, Al: 396.153, Cu: 224.7, Co: 228.616, Zn: 213.857. ICP–OES quantification was done using a multi–element standard solution (TraceCERT) CatNo.43843 (Sigma–Aldrich, USA). The calibration standards were prepared by titrating the standard solution in 2% (v/v) nitric acid to obtain the working ranges required (400–4000 µg L^{-1}). The correlation coefficient obtained was ≥ 0.999 (Fe–0.999, Zn–0.999, Mn–0.999, Ca–0.999, P–0.999, Mg–0.999, Co–0.999, Mo–0.999, K–0.999, Al–0.999,). Calibration was performed using Winlab 32 software (Perkin Elmer, USA). All determinations were carried out in triplicates.

3.5.3. Fatty acids determination

Oil extraction from the formulated porridge flours was achieved based on the modified previous method (Folch *et al*., 1957). Samples, 5 g each, were weighed into 50 mL falcon tubes and diluted with10 mL of dichloromethane (DCM) and methanol (MeOH) (2:1) mix. The mixtures were vortexed for 1 min followed by sonication for 10 mins and centrifuging at 4200 rpm for 10 mins. The supernatants were carefully filtered (Whatman filter paper grade 1; Diameter 90 mm) into clean 250 mL round-bottomed flasks and solvent evaporated *in vacuo* to yield approximately 200 mg of oil.

Compositions of fatty acids (FAs) in the oil extract from formulated porridge flours were examined as fatty acid methyl esters (FAMEs) according to modified previous methods (Cheseto *et al*., 2020). Approximately 1 mL of sodium methoxide solution (100 mg/ mL) was added to 100 mg of recovered oil extract, vortexed for 1 min followed by sonication for 10 min. The resulting mixture

was placed in a water bath (70°C) for 1h and the reaction was halted by adding 100 μL of deionized water followed by vortexing for another 1 min. To extract the FAMEs, 1 mL of gas chromatography (GC)-grade hexane (Sigma–Aldrich, St. Louis, MO, USA) was added to the mixture followed by 20 min centrifugation at 14,000 rpm. The resulting hexane layer (upper) was dried over anhydrous Na_2SO_4 and analysis was performed using an Agilent GC–MS on a 7890A gas chromatograph connected to a 5975 C mass selective detector (Agilent Technologies Inc., Santa Clara, CA). GC was fitted with a (5%–phenyl)–methylpolysiloxane (HP5 MS) low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm (J&W, Folsom, CA, USA). The sample injection volume was 1 µL. Helium acted as the carrier gas at flow rates of 1.25 mL/min. Oven temperature was programmed between 35°C to 285°C, with the initial and final temperature kept for 5 min and 20.4 min respectively, and a rising rate of 10°C min^{-1}. Workstation software was used to control the operation of GC–MS. Ion trap mass selective detector was maintained at an ion source temperature of 230°C and quad temperature of 180°C. The mass detector was run in electron impact (EI) mode (70 eV). Fragment ions were determined in the full scan mode at over 40–550 m/z mass range. The filament was delayed for 3.3 min. Authentic Standard methyl octadecenoate (0.2–125 ng/μL) serial dilutions prepared from octadecanoic acid (≥ 95 % purity) (Sigma-Aldrich, St. Louis, MO) was analyzed using GC-MS in full scan mode. GC-MS generated a linear calibration graph of peak area versus concentration with the equation; [$y = 5E + 07x + 2E + 07$] with R^2 of 0.9997. This regression equation was used for the external quantification of the different FAs from the samples.

A Hewlett-Packard (HP Z220 SFF intel xeon) workstation with ChemStation B.02.02. Acquisition software was used. Chemstation integration parameters was calibrated as follows: initial threshold = 3, initial peak width = 0.010, initial area reject = 1, and shoulder detection = on. These

calibrations yielded the mass spectrum for the peaks. Identification of compounds was based on comparing the generated mass spectra and retention times with those of authentic standards and reference spectra in library–MS databases: National Institute of Standards and Technology (NIST) 05, 08, and 11. Determination of the FAMES and sterols in all the processed samples were made in triplicates.

3.5.4. Determination of water–soluble vitamins

Determination of water–soluble vitamins was conducted according to a previously described method (Thermo Fisher Scientific, 2010). For sample preparation, 100 mg of flour sample was suspended in 25 mL of distilled water in a 50 mL falcon tube. The mixture was ultra–sonicated for 15 min and the solution filtered through 0.2 µm filters into UPLC vials. The chromatographic analysis was performed on a Liquid chromatographic system with Diode Array Detector (LC–30AC with Nexera column oven CTO–30A, Shimadzu, Tokyo, Japan). A Phenomenex C18 Column Synergi 100 mm ×3.00 mm, 2.6 µm polar (Phenomenex, Torrance, CA, USA) at 30°C was used. The mobile phase consisted of two phases, A: 25mM phosphate buffer. Mobile phase B: 7:3 v/v Acetonitrile–Mobile phase A. Total run time was set at 12 min with a flow rate of 0.4 mL/min. Stock solutions of 1.0 mg/ml were prepared by dissolving the individual water–soluble vitamin standards in distilled water except for Vitamin B_2 in (5mM potassium hydroxide) and Vitamin B_9 in (20mM potassium hydrogen carbonate). Four calibration standards at a concentration of 2, 5, 10 and 15 µg/mL were prepared from the mixed stock solutions. The retention times (mins) for the vitamins were as follows: Vitamin C–1.596, Vitamin B_1–1.922, Nicotinic acid–2.228, Vitamin B_6–3.496, Nicotinamide–5.050, Vitamin B_5–6.772, Vitamin B_9–8.236, Vitamin B_{12}– 8.936 and Vitamin B_2–9.224. R^2 was 0.996 or greater. All determinations were carried out in triplicates.

3.5.5. Determination of fat-soluble vitamins

Determination of fat-soluble vitamins was carried out according to a method previously described by Bhatnagar-Panwar *et al*. (2015) with modifications. Briefly, 6 mL of ethanol with 0.1% (BHT)) was added to 500 mg of the flour sample and homogenized. To the resulting mixture, 120 µL of potassium hydroxide 80% (w/v) was added and vortexed for 1 min followed by incubation at 80 °C for 5 min. Cooling was done by placing the test tubes in ice and 4 mL deionized water added to achieve better phase separation followed by vortexing for another 1 min. For extraction, 5 mL HPLC-grade hexane (Sigma–Aldrich, St. Louis, MO, USA) was added to the mixture followed by 5 min centrifugation at 3,000 rpm. The resulting hexane layer (upper) was transferred into a separate test tube, the pellet was re-extracted twice more using hexane and the upper phases were collected and pooled. Drying was done under nitrogen gas flow to complete dryness and the residue was reconstituted in 1 ml of methanol: tetrahydrofuran (85:15 v/v), vortexed and sonicated for 30 seconds and filtered into HPLC sample vials. Analysis was performed using reverse-phase HPLC (Shimadzu Nexera UPLC system) linked to SPD -M2A detector. The UPLC was fitted with a YMC C30, carotenoid column (3µm, 150X3.0 mm, YMC Wilmington, NC). The mobile phase consisted of two phases A: methanol/tert-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water) and B: methanol/tert-butyl methyl ether/water (8:90:2, v/v/v, with 1% ammonium acetate in the water). The injection volume was 10 µL with a total flow rate of 0.4 ml/min. The retention times for retinol, α and γ-tocopherol were 2.74, 5.40 and 6.29 respectively. Compounds presenting the eluting sample were monitored at 290 nm. Peaks were identified by their retention time and absorption spectra were compared to those of known standards (Sigma Chemicals). Sample concentrations were calculated by comparing the peak area of samples to the peak area of the standards.

3.5.6. Assessment of phytic acid, tannins, and flavonoids

Phytic acid contents were assessed using K–PHYT Phytic Acid (Phytate)/Total Phosphorus kit (Megazyme Int. Ireland Ltd, Wicklow, Ireland) following the manufacturer instructions (Megazyme, 2017). Flour sample (1 g) was added to 20 mL of 0.8 M HCl and mixed by shaking at 25°C for 24 hours and 1.5 mL of the resulting extract was centrifuged at 13000 rpm for 10 min. The supernatant (0.5 mL) was neutralized by addition of 0.5 mL of 0.8 M NaOH. The neutralized sample was subjected to the enzymatic dephosphorylation reaction and the total phosphorous and inorganic phosphate were quantified. In the meantime, 0.5 mL of the neutralized sample was used to quantify the inorganic phosphate. Following the enzymatic treatment, total phosphorous and inorganic phosphate of the samples were subjected to colorimetric development to yield estimates of the phosphorous content. This kit estimates inorganic phosphate released from the extracted flour sample after treatment with phytase and alkaline phosphatase. The content of free inorganic phosphate was estimated from samples not treated with phytase. The above experiment was replicated three times.

Total tannin content was assessed by Folin–Denis method using a microplate reader as outlined by Saxena *et al*. (2013). Results were expressed in mg tannic acid equivalents (TAE)/100 g of sample. The total flavonoid content was analyzed using the Aluminium Chloride colorimetric procedure according to the method by Zhishen *et al*. (1999) Results were expressed in mg catechin equivalents (CEQ)/100g of dry sample. All determinations were carried out in triplicates.

3.5.7. Determination of mineral bioavailability

Mineral bioavailability (zinc, iron and calcium) was expressed as phytate/mineral molar ratio (Norhaizan & Nor Faizadatul Ain, 2009). Moles of phytic acid were calculated by dividing the

recorded value of phytic acid by its atomic weight (660) while the moles of minerals (zinc, iron and calcium) were determined by dividing the recorded values by the individual molecular weight of the respective compounds i.e., Zn = 65, Fe = 56, and Ca = 40.

3.6. Microbiological analysis

Microbiological analysis was carried out according to procedures by AOAC (1990) with modifications. 1 g of the porridge flour sample was suspended in 9 mL 0.85% aseptic normal saline (NS). Afterward, the mixture was vortexed for 2 min. A series of ten-fold dilutions were prepared using 0.85% NS and used for microbial plate count. For total viable counts (TVC), 100 µL of the last five dilution series were spread–plated on Plate Count Agar (Merck, Darmstadt, Germany) in Petri dish in 3 replications. Inoculated plates were incubated for 48 hours at 37±2°C. The number of bacterial colonies was counted microscopically at × 400 magnification and expressed as log colony–forming units (CFU)/g. Enumeration of yeasts and moulds was done using the above–described method with the following modifications. Potato Dextrose agar (Oxoid, Basingstoke, Hampshire, England.) containing 0.025 g/L antibacterial agent (chloramphenicol) was used as culture media and incubated at 25±2°C for 3–5 days. Kenya Bureau of Standards (KEBS) limits on the microbial quality of insect-enriched foods were used as indicators of food safety which include total viable bacterial counts of $< 10^5$ and total yeast and mould count of $< 10^2$. The different porridge flour formulations were then packaged in aluminium and paper bags and stored at ambient conditions for shelf–life analysis.

3.7. Porridge preparation and sensory evaluation

The porridges were prepared using the method described by Onyango *et al*. (2020). Approximately 40 g of each porridge flour sample was mixed with 200 mL of cold water into a slurry. The resulting slurry was added in 640 mL boiling water (100^{o}C) over a hot plate and stirred continuously for 5 min. The porridge was allowed to cook for 5–10 min. It was cooled to about 40^{o}C then coded for sensory evaluation. For flavor optimization, porridge prepared from each composite formulation were assigned codes as follows: CF1 (56% finger millet: 44% amaranth: 0% cricket), CF2 (50% finger millet: 40% amaranth: 10% cricket), CF3 (47% finger millet: 38% amaranth: 15% cricket) and CF4 (45% finger millet: 35% amaranth: 20% cricket).

Sensory analysis of the prepared porridges and overall consumer preference was assessed using a 7–point hedonic scale ranging from 7 (like extremely) to 1 (dislike extremely). Panelists were required to assess the colour, aroma, consistency texture, taste and overall acceptability of the porridge using a scale of 1–7 (Appendix 2). The panel comprised 40 (32 females and 8 males, non-smokers, age 23-50 years) panelists who were familiar with sensory evaluation of food from the Food Science Department, University of Nairobi. During sample evaluation, panelists were served 20 mL porridge in identical plastic cups labeled with 3–digit codes. The samples were served randomly to panelists with 5 min break between each sample. Clean water was provided for mouth rinsing before and between sample evaluations. For sensory evaluation based on the effect of processing, the above procedure was repeated with 100 untrained panelists (mothers and caregivers, aged 20-66 years) and the porridge samples were coded as follows: T1= roasted finger millet–amaranth + cricket (RFM–AC), T2= unprocessed finger millet–amaranth + cricket (UFM–AC), T3= fermented finger millet–amaranth + cricket (FFM–AC) and T4= germinated finger millet–amaranth + cricket (GFM–AC).

3.8. Shelf–life determination

Shelf–life stability of formulated composite flours was monitored for six months. Flour samples were packaged in kraft paper and aluminium bags and stored at ambient storage conditions. Microbial quality, moisture content and acid value were monitored at three–month interval. Moisture content and microbial load were determined using previously outlined methods.

To evaluate acid levels, a 5 g sample was placed in a 200 mL conical flask and then dissolved with 40ml of the solvent mixture benzene: ethanol. The mixture was then titrated against 0.1 M alcoholic potassium hydroxide solution with phenolphthalein indicator until the appearance of pink color. Acid value was determined as follows:

$$Acid\ value = (Initial\ titre - Final\ titre) \times \frac{5.611}{Weight\ of\ the\ samples}$$

Acid values obtained were used to calculate the free fatty acid (FFA) content using the following formula:

$$\text{FFA}\ (\%) = \frac{Acid\ value}{1.99}$$

3.9. Data analysis

Datasets of proximate compositions, mineral contents, fatty acids and anti–nutritional data were subjected to analysis of variance (ANOVA). Datasets on microbial counts and shelf–life studies were analyzed using a generalized linear model. Sensory datasets were analyzed using multiple analysis of variance (MANOVA) and principal component analysis (PCA). Mean separation was performed using the *lsmeans* package (Lenth, 2015) with the Tukey test. These analyses were performed in R software (R Core Team, 2020).

CHAPTER FOUR: RESULTS

4.1. Proximate composition

Five porridge flour samples used in this study are presented in Figure 4.1. Proximate composition and energy values of the five porridge flour samples are presented in Table 4.1**.** Samples varied significantly ($p < 0.05$) in their proximate values except on ash content. All flour samples enriched with cricket had significantly ($p < 0.05$) higher protein (2–folds), fat (3.4–4–folds) and energy content (1.1–1.2–folds) when compared to commercial porridge flour. Germinated sample presented the highest protein content (16.12 g/100 g) followed by the unprocessed sample (15.90 g/100 g), whereas the fat content of the fermented product (7.22 g/100 g) was significantly ($p < 0.05$) lower than that of other formulated products (8.08 g to 8.31 g/100 g) but higher when compared to commercial porridge flour (2.41 g/100 g). The total carbohydrates in formulated porridge flour ranged from 68.31 g/100 g to 71.88 g/100 g, which were significantly lower than in commercial porridge flour (81.94 g/100 g). The highest crude fiber content was recorded in the germinated sample (5.34 mg/100 g) whereas the lowest was recorded in the fermented sample (3.33 mg/100 g).

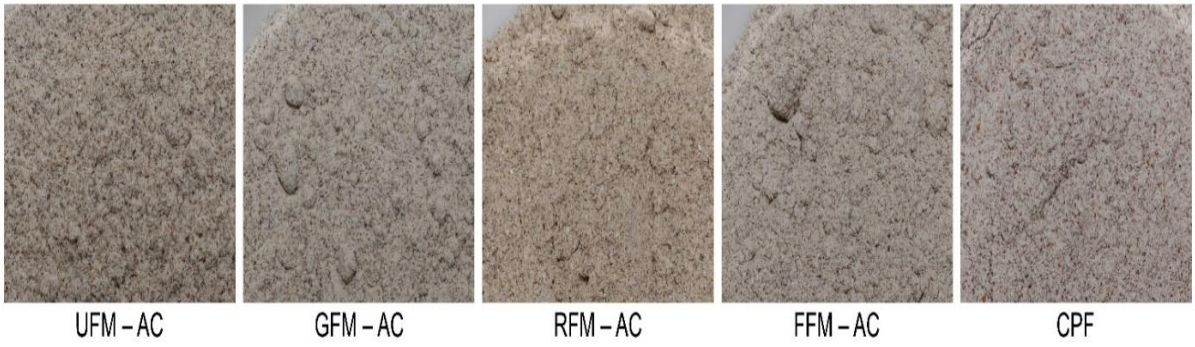

Figure 4.1: Porridge flour samples. FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth+ cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth+ cricket. CPM = Commercial porridge flour.

Table 4.1: Proximate and energy values of porridge flour on dry weight basis (dwb).

	Proximate composition						
[a]Products	[1]Moisture (%)	Ash (g/100g)	Fiber (g/100g)	Protein (g/100g)	Fat (g/100g)	CHO (g/100g)	Energy (kcal/100g)
FFM–AC	4.84 ± 0.25[b]	2.22 ± 0.15[a]	3.33± 0.29[a]	15.34±0.17[b]	7.22 ± 0.06[b]	71.88 ±0.29[c]	413.92± 0.80[c]
RFM–AC	3.02 ± 0.22[a]	2.76 ± 0.05[ab]	3.88 ± 0.04[b]	15.54±0.16[bc]	8.08 ± 0.28[c]	69.74 ± 0.13[b]	413.83± 1.44[c]
GFM–AC	5.92 ± 0.42[c]	2.88 ± 0.48[b]	5.34 ± 0.02[d]	16.12±0.15[d]	8.19 ± 0.09[c]	67.46 ± 0.39[a]	408.12± 2.27[b]
UFM–AC	7.44 ± 0.26[d]	2.83± 0.09[ab]	4.64 ± 0.11[c]	15.90±0.28[cd]	8.31 ± 0.38[c]	68.31 ±0.71[a]	411.68±1.71[bc]
CPM	11.76 ± 0.25[e]	2.57 ± 0.04[ab]	4.79 ± 0.28[c]	8.55 ±0.16[a]	2.14± 0.16[a]	81.94 ± 0.26[d]	381.28 ±0.35[a]

[a]Products: FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour. **[1]** Moisture content not based on dry weight. Values are mean (± standard deviation) while different superscript letters within columns are significantly different at $p < 0.05$ according to the Tukey test.

4.2. Fatty acids

The results of the fatty acids methyl esters (FAMEs) in different porridge flour samples are presented in Table 4.2. Of the 44 fatty acids detected, the proportion of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) present in porridge flour samples were 23, 54 and 23%, respectively. More FAMEs (3-4-fold) were detected in the cricket-enriched porridge flours than in the commercial porridge flour. Additionally, variations of FAMEs across the different porridge products are illustrated in Figure 4.2. All cricket-enriched porridge flour samples had higher amounts of SFAs, MUFAs, and PUFAs compared to the commercial porridge flour. No PUFAs were detected in commercial porridge flour.

Table 4.2: Compositions of fatty acids (µg/g of oil) of porridge flour samples analyzed using Gas Chromatography coupled to Mass Spectrometry (GC-MS).

Peak No.	tR (min)	Compound name	ω-n(Δn)	FFM-AC	GFM-AC	CPF	RFM-AC	UFM-AC
1	18.96	Methyl Dodecanoate	C12:0	0.67 ± 0.09	0.27 ± 0.01	–	0.58 ± 0.05	0.35 ± 0.02
2	19.72	Methyl 11-Methyldodecanoate	Iso-methyl-C12:0	0.05 ± 0.01	0.06 ± 0.00	–	–	–
3	20.12	Methyl Tridecanoate	C13:0	0.12 ± 0.01	0.06 ± 0.00	–	0.09 ± 0.01	0.06 ± 0.00
4	20.82	Methyl 12-Methyltridecanoate	Iso-methyl-C13:0	0.21 ± 0.02	0.12 ± 0.00	–	0.07 ± 0.01	0.13 ± 0.01
5	21.22	Methyl Tetradecanoate	C14:0	14.72 ± 0.84	6.09 ± 0.10	0.85 ± 0.04	12.04 ± 1.21	8.26 ± 0.97
6	21.78	Methyl 4-Methyldodecanoate	Iso-methyl-C12:0	0.72 ± 0.07	0.44 ± 0.00	–	–	–
7	22.00	Methyl 13-Methyltetradecanoate	Iso-methyl-C14:0	3.64 ± 0.21	1.98 ± 0.01	–	1.42 ± 0.17	0.89 ± 0.17
8	22.00	Methyl 12-Methyltetradecanoate	Iso-methyl-C14:0	0.85 ± 0.04	0.54 ± 0.00	–	1.84 ± 0.10	1.29 ± 0.19
9	22.29	Methyl Pentadecanoate	C15:0	3.79 ± 0.28	1.61 ± 0.00	0.38 ± 0.04	3.20 ± 0.48	2.93 ± 0.56
10	22.74	Methyl 5,9,13-Trimethyltetradecanoate	Iso-trimethyl-C14:0	0.45 ± 0.05	0.00 ± 0.00	–	–	–
11	22.94	Methyl 14-Methylpentadecanoate	Iso-methyl-C15:0	1.22 ± 0.10	0.85 ± 0.01	–	1.24 ± 0.07	0.94 ± 0.06
12	23.37	Methyl Hexadecanoate	C16:0	200.94 ± 6.55	255.32 ± 7.40	32.22 ± 1.37	559.63 ± 50.55	319.23 ± 28.46
13	23.93	Methyl 15-Methylhexadecanoate	Iso-methyl-C16:0	3.89 ± 0.38	2.90 ± 0.06	–	3.09 ± 0.42	3.15 ± 0.39

(tR Retention time, Mean±standard deviation). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, ALA = α -Linolenic acid, EPA= Eicosapentaenoic acid, DHA = Docosapentaenoic acid, α-ESA= alpha Eleostearic acid.). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

Table 4.3: Cont'

Peak No.	tR (min)	Compound name	ω-n(Δn)	FFM-AC	GFM-AC	CPF	RFM-AC	UFM-AC
14	24.02	Methyl 14-Methylhexadecanoate	Iso-methyl-C16:0	11.82 ± 0.94	5.85 ± 0.12	1.30 ± 0.13	12.80 ± 1.11	6.23 ± 0.88
15	24.29	Methyl Heptadecanoate	C17:0	9.92 ± 0.71	3.08 ± 0.05	1.11 ± 0.22	7.40 ± 0.68	4.58 ± 0.91
16	24.69	Methyl 14-Methylheptadecanoate	Iso-methyl-C17:0	1.40 ± 0.18	1.43 ± 0.02	–	2.99 ± 0.24	1.66 ± 0.20
17	25.25	Methyl Octadecanoate	C18:0	170.54 ± 2.98	78.14 ± 1.41	13.02 ± 0.51	36.71 ± 2.68	114.00 ± 23.09
18	26.12	Methyl Nonadecanoate	C19:0	2.08 ± 0.14	1.46 ± 0.05	–	1.92 ± 0.06	1.44 ± 0.30
19	26.98	Methyl Eicosanoate	C20:0	13.07 ± 0.15	11.07 ± 0.00	3.05 ± 0.06	26.99 ± 1.49	16.48 ± 2.30
20	27.58	Methyl 18-Methyleicosanoate	Iso-methyl-C20:0	4.90 ± 0.78	3.45 ± 0.00	–	5.11 ± 0.76	2.80 ± 0.17
21	27.80	Methyl Heneicosanoate	C21:0	2.30 ± 0.23	3.34 ± 0.30	–	3.75 ± 0.66	1.79 ± 0.10
22	28.59	Methyl Docosanoate	C22:0	5.35 ± 0.13	6.30 ± 0.13	2.47 ± 0.02	9.01 ± 0.45	6.92 ± 0.85
23	29.37	Methyl Tricosanoate	C23:0	3.95 ± 0.09	3.93 ± 0.01	–	4.63 ± 0.39	2.89 ± 0.21
24	30.13	Methyl Tetracosanoate	C24:0	9.45 ± 0.54	6.07 ± 0.04	–	10.98 ± 0.48	4.43 ± 1.23

(tR Retention time, Mean±standard deviation). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, ALA = α - Linolenic acid, EPA= Eicosapentaenoic acid, DHA = Docosapentaenoic acid, α-ESA= alpha Eleostearic acid.). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

Table 4.4: Cont'

Peak No.	tR (min)	Compound name	ω-n(Δn)	FFM-AC	GFM-AC	CPF	RFM-AC	UFM-AC
		∑ SFA				–		
25	20.95	Methyl 11Z-Tetradecenoate	C14:1 (n-3)	0.52 ± 0.01	–	–	0.93 ± 0.09	–
26	21.08	Methyl 9Z-Tetradecenoate	C14:1 (n-3)	0.89 ± 0.09	–	–	0.67 ± 0.07	–
27	23.12	Methyl 9Z-Hexadecenoate	C16:1 (n-7	55.80 ± 1.46	18.50 ± 0.16	2.24 ± 0.26	67.70 ± 1.84	31.80 ± 2.43
28	24.09	Methyl 10Z-Heptadecenoate	C17:1 (n-7)	7.42 ± 1.10	1.37 ± 0.03	–	1.75 ± 0.16	0.81 ± 0.08
29	25.00	Methyl 11-Octadecenoate	C18:1(n-9)	3.69 ± 0.18	3.48 ± 0.11	–	2.83 ± 0.19	3.72 ± 0.12
30	25.07	Methyl 9E-Octadecenoate	C18:1(n-9)	729 ± 42	631 ± 13	112 ± 7	1149 ± 94	333 ± 41
31	25.88	Methyl 10-Nonadecenoate	C19:1 (n-9)	2.69 ± 0.14	3.81 ± 0.17	––	8.99 ± 0.45	4.55 ± 0.46
32	26.77	Methyl 11Z-Eicosenoate	C20:1(n-9)	8.63 ± 0.55	8.52 ± 0.16	2.94 ± 0.14	19.96 ± 0.57	11.31 ± 2.20
33	28.41	Methyl 11-Docosenoate	C22:1(n-11)	4.23 ± 0.20	–	–	3.27 ± 0.30	–
34	29.95	Methyl 15Z-Tetracosenoate	C24:1(n-9)	3.31 ± 0.16	–	–	–	–
		∑ MUFA				–		
35	24.74	Methyl 9Z,12Z-Octadecadienoate	C18:2(n-6)	74.70 ± 4.02	38.38 ± 2.09	–	16.69 ± 1.10	22.62 ± 2.07
36	24.76	methyl 6Z,9Z,12Z-Octadecatrienoate	C18:3(n-3)	4.60 ± 0.43	3.48 ± 0.40	–	1.67 ± 0.15	2.80 ± 0.03
37	25.41	Methyl 7,12-Octadecadienoate	C18:2(n-7)	8.87 ± 0.66	11.07 ± 0.94	–	6.55 ± 0.96	5.22 ± 0.27
38	25.79	Methyl 9Z,11E-Octadecadienoate	C18:2(n-7)	2.39 ± 0.09	2.60 ± 0.42	–	1.62 ± 0.21	1.35 ± 0.01

(tR Retention time, Mean±standard deviation). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, ALA = α - Linolenic acid, EPA= Eicosapentaenoic acid, DHA = Docosapentaenoic acid, α-ESA= alpha Eleostearic acid.). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

Table 4.5: Cont'

Peak No.	tR (min)	Compound name	ω-n(Δn)	FFM-AC	GFM-AC	CPF	RFM-AC	UFM-AC
39	26.24	Methyl 9Z,11E,13E-Octadecatrienoate (α-ESA)	C18:3(n-3)	1.03 ± 0.04	0.92 ± 0.10	–	0.57 ± 0.00	0.74 ± 0.03
40	26.26	Methyl 9Z,12Z,15Z-Octadecatrienoate (ALA)	C18:3(n-3)	7.01 ± 0.23	7.10 ± 0.61	–	3.79 ± 0.01	5.07 ± 0.02
41	26.44	Methyl 5Z,8Z,11Z,14Z-Eicosatetraenoate (AA)	C20:4(n-6)	1.55 ± 0.09	2.76 ± 0.06	–	1.89 ± 0.04	1.95 ± 0.19
42	26.50	Methyl 5Z,8Z,11Z,14Z,17Z-Eicosapentaenoate (EPA)	C20:5(n-3)	13.07 ± 0.77	4.44 ± 0.02	–	–	–
43	26.64	Methyl 8,11,14,17-Eicosatetraenoate (AA)	C20:4(n-6)	2.040 ± 0.10	–	–	–	–
44	28.07	Methyl 4Z,7Z,10Z,13Z,16Z,19Z-Docosahexaenoate (DHA)	C22:6(n-3)	2.40 ± 0.34	–	–	–	–
		∑ PUFA		117.66 ± 3.17	70.76 ± 1.30	–	32.68 ± 1.72	39.73 ± 2.16
		∑ n-6 PUFA		89.55 ± 4.42	54.82 ± 0.92	–	26.65 ± 1.68	31.13 ± 2.15
		∑ n-3 PUFA		28.11 ± 0.75	15.94 ± 0.99	–	6.03 ± 0.15	8.60 0.01
		∑ n-6/n-3		3.2	3.4	–	4.4	3.6
		∑ ALA+EPA+DHA		27.08 ± 0.70	15.02 ± 1.00	–	5.47± 0.15	7.87 ± 0.04

(tR Retention time, Mean±standard deviation). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, ALA = α - Linolenic acid, EPA= Eicosapentaenoic acid, DHA = Docosapentaenoic acid, α-ESA= alpha Eleostearic acid.). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

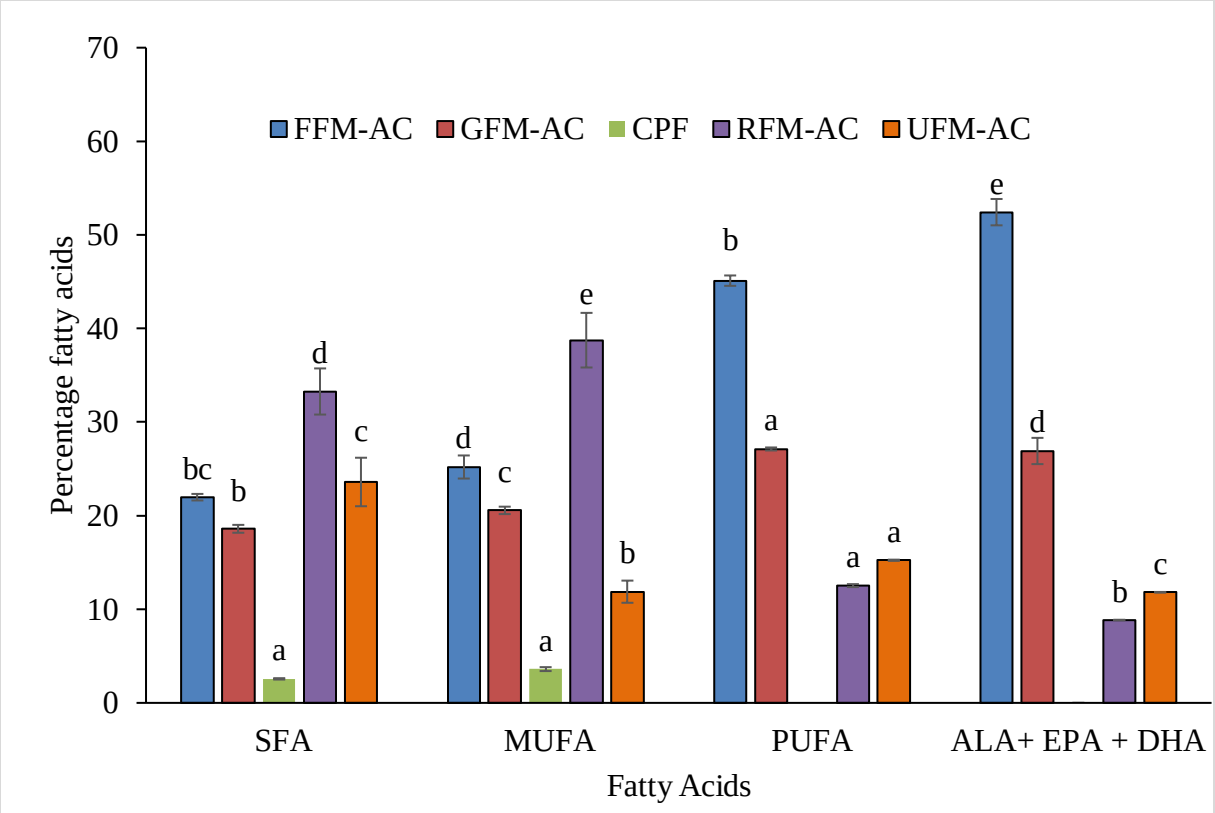

Figure 4.2: Fatty acid groups in porridge flour samples as influenced by processing. SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid. Omega-3 fatty acids (ALA+EPA+DHA). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

The proportion of each group of FAMEs (SFA, MUFA, and PUFA) in porridge flour samples is shown in Figure 4.3. Of the 24 SFAs detected, Methyl hexadecanoate (palmitic acid) contributed the highest proportion followed by Methyl octadecanoate (stearic acid) across the flour samples. In addition, Methyl 9E-octadecenoate (oleic acid) was the predominant MUFA whereas Methyl 9Z,12Z-octadecadienoate (linoleic acid, LA) accounted for the highest proportion of the PUFAS.

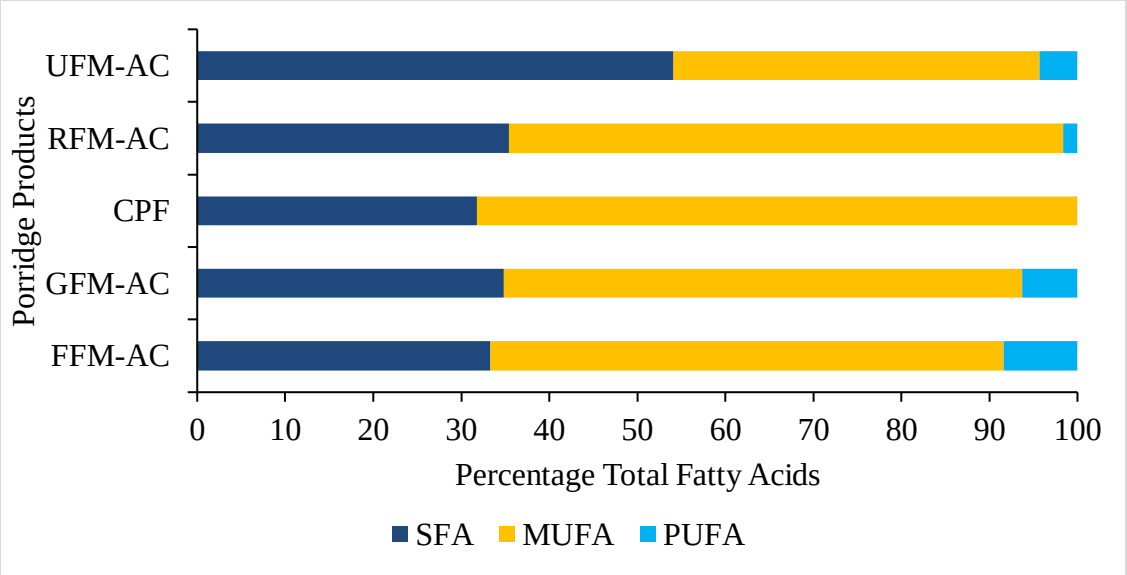

Figure 4.3: Total fatty acid composition in different porridge flour samples. SFA = Saturated fatty acid, MUFA = monounsaturated fatty acid. PUFA = polyunsaturated fatty acid (). FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour.

The proportion of omega-3 fatty acids, namely α-linolenic acid (ALA), eicosapentaenoic acid (EPA), α-eleostearic acid (α-ESA) and docosapentaenoic acid (DHA) differed considerably across the flour samples. α-linolenic acid (ALA) was detected in all the cricket-enriched samples while DHA was only present in the fermented sample. On the effect of processing, PUFAs increased significantly ($p < 0.05$) by 30% during fermentation and decreased by 3% during roasting. Besides, roasting process caused an increase ($p < 0.05$) in both MUFAs and SFAs by 27 and 10%, respectively. Germination caused a slight increase of 9 and 12% in both MUFAs and PUFAs. The ratio of omega-6 to omega-3 varied significantly among the different flour samples and ranged from 3.2 to 4.4.

A principal component analysis (PCA) biplot in which the points represent flour samples and the vectors represent a group of fatty acids is presented in Figure 4.4. PC1 and PC 2 altogether, explained 94.4% variations in the fatty acid's profiles (MUFA, SFA, PUFA, and Omega 3) of the samples. Omega 3 equaled ALA+ EPA+ DHA. Total SFA and MUFA were closely related whereas the Omega 3 and PUFAs are closely related. These FAMEs varied insignificantly in CPF and UFM-AC. Comparatively, there was little difference between FFM-AC and RFM-AC although, GFM-AC, UFM-AC and CPF can be grouped. Whereas GFM-AC and UFM-AC were highly correlated, their difference from CPF is minimal in terms of Fatty acids.

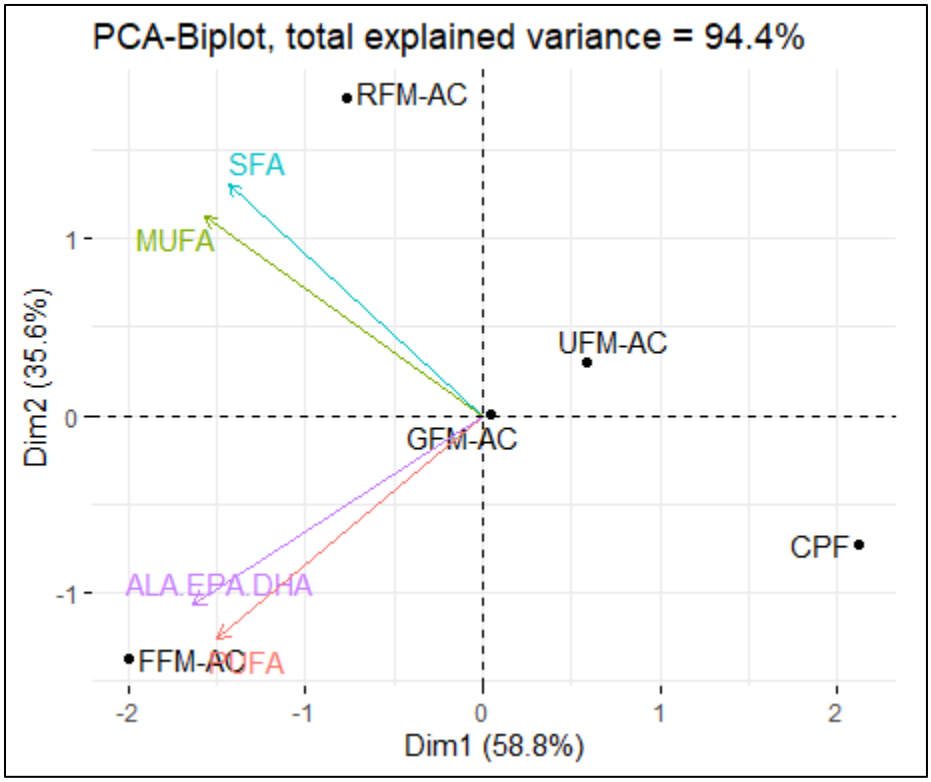

Figure 4.4: Principal component analysis (PCA) biplots showing the variation of fatty acid methyl esters (FAMEs) among the different porridge flour samples. Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and Omega 3 fatty acids (ALA+EPA+DHA) FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth+ cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth+ cricket. CPM = Commercial porridge flour.

4.3. Vitamin content

The results of vitamin content in porridge flour samples are shown in Table 4.3. The results show significant variations in the vitamin content. Cricket enriched formulations had significantly ($p < 0.05$) higher content of vitamin B_{12}, vitamin B_5, B_6, nicotinamide and thiamine. Thiamine content ranged from 4.3–39.5 mg/100 g while vitamin B_{12} was in the range of 3.2–37.7 mg/100 g. Processing methods had a significant ($p < 0.05$) effect on the vitamin concentrations. Germinated and fermented samples had enhanced levels of vitamin C, nicotinamide, vitamin B_5, folate and vitamin B_6. However, there was a significant ($p < 0.05$) decrease to undetectable levels in the contents of nicotinic acid and thiamine during fermentation and germination respectively. Vitamin B_{12} showed significant reductions ($p < 0.05$) in all the processed porridge flours while riboflavin content was not affected by processing. Roasting process had a negligible effect on vitamins except for significant ($p < 0.05$) reductions in nicotinic acid and vitamin $B_{12,}$ and a slight reduction in vitamin C. The retinol, α, and γ-tocopherol content differed significantly ($p < 0.05$) in different porridge flour samples. Retinol content increased slightly during fermentation while germination process decreased the levels of retinol and increased the α-tocopherol content. However roasting process did not affect the levels of α and γ-tocopherol.

Table 4.6: Vitamin content (mg/100 g) in porridge flour products.

Vitamins	*Porridge products				
	FFM–AC	**RFM–AC**	**GFM–AC**	**UFM–AC**	**CPF**
Vitamin C	146.5±2.8^{c}	55.2±2.9^{a}	72.0±6.5^{b}	58.0±5.1^{a}	149.6±2.1^{c}
Thiamine (B_1)	39.5±3.0^{c}	4.3±0.2^{b}	–	5.9±0.4^{b}	–
Nicotinic acid (B_3)	–	6.1±0.5^{b}	19.5±1.2^{d}	10.6±0.7^{c}	27.7±2.9^{e}
Pyridoxine (B_6)	10.8±1.1^{c}	–	6.0±0.3^{b}	–	0.5±0.1^{a}
Nicotinamide	47.9±2.1^{d}	7.1±0.2^{b}	33.9±1.1^{c}	8.8±0.4^{b}	3.0±0.3^{a}
Pantothenic acid (B_5)	423.3±3.4^{d}	314.4±18.4^{c}	453.8±44.5^{d}	209.6±3.8^{b}	26.4±2.5^{a}
Folate (B_9)	38.8±1.5^{b}	41.8±0.3^{b}	42.4±3.7^{b}	29.3±1.8^{a}	28.6±2.6^{a}
Cyanocobalamin (B_{12})	21.9±1.4^{c}	12.4±0.9^{b}	13.7±4.0^{b}	37.7±4.0^{d}	3.2±0.3^{a}
Riboflavin (B_2)	41.6±1.0^{a}	45.5±2.4^{a}	34.8±2.9^{a}	41.6±5.0^{a}	74.2±8.2^{b}
Retinol	0.54±0.08^{c}	0.29±0.01^{ab}	0.07±0.01^{a}	0.38±0.19^{bc}	0.55±0.03^{c}
γ-Tocopherols	0.17 ±0.01^{a}	0.54 ±0.00^{b}	0.19±0.01^{a}	0.52±0.02^{b}	0.88±0.04^{c}
α-Tocopherols	0.35±0.09^{a}	0.76±0.08^{b}	1.48±0.03^{c}	0.83±0.03^{b}	0.46±0.06^{a}

*__Porridge product__: FFM–AC = Fermented finger millet–amaranth + cricket, RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPF = commercial porridge flour. Values are mean (± standard deviation). Mean values with different superscript letters within rows are significantly different at $p < 0.05$ according to the Tukey test.

4.4. Mineral content

The mineral content varied significantly across the porridge flour products as shown in Table 4.4. The iron content of the formulated flours ranged between 8.59–19.48 mg/100 g with germinated sample having the highest content (19.48 mg/100 g). Zinc content was in the range of 3.08–3.70 mg/100 g while the range obtained for calcium was from 234.87 mg–278.61 mg/100 g. The

calcium content for commercial porridge flour was significantly ($p < 0.01$) higher at 312.69 mg/100 g when compared to the formulated samples, while the Zn content was significantly low (1.86 mg/100 g). There were no significant variations in copper and zinc levels across the processed formulations when compared to the unprocessed sample. The fermented sample had significantly ($p < 0.01$) lower levels of magnesium, and phosphorous, but the levels of calcium, copper, iron, and zinc did not vary when compared with the unprocessed formulation. Calcium and iron content in the germinated sample were comparatively higher than in other formulations. Roasting process did not significantly affect the mineral content.

Table 4.7: Mineral content of the porridge flour formulations on dry weight basis (dwb).

Minerals	Mg (mg/100g)	Fe (mg/100g)	Ca (mg/100g)	Zn (mg/100g)	P (mg/100g)	Mn (mg/100g)	Cu (µg/100g)
FFM–AC	169.57±8.03 [b]	8.56±1.45 [a]	234.87±17.60 [a]	3.23 ±0.28 [b]	372.71±19.14[b]	10.92±0.63[b]	728.78 ±15.37[b]
RFM–AC	210.84±1.45[c]	9.18±1.18 [a]	257.69±1.37[ab]	3.39±0.31 [b]	458.70±3.76[c]	9.45±0.15[a]	724.94±22.80[b]
GFM–AC	207.82±11.93[c]	19.48±6.69[b]	278.61±17.90 [b]	3.71±0.18 [b]	469.28±9.55[c]	9.32±0.67[a]	787.20±42.28[b]
UFM–AC	203.09±5.14[c]	9.55±2.42 [a]	244.69±4.94 [a]	3.08±0.16 [b]	476.72±17.46[c]	8.64±0.04[a]	736.28±14.4 [b]
CPM	145.08±0.25 [a]	9.86±2.08 [a]	312.69±0.57 [c]	1.86±0.04 [a]	221.63±5.57[a]	22.44±0.20[c]	477.42±1.88[a]

[a]Porridge flour product: FFM–AC = Fermented finger millet –amaranth + cricket, RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket meal, UFM–AC = Untreated finger millet–amaranth + cricket. CPM = Commercial porridge flour. Values are mean (± standard deviation). Mean values with different superscripts within rows are significantly different at $p < 0.05$ according to the Tukey test.

4.5. Phytic acid, tannins, and flavonoids

The phytic acid, tannin and flavonoid contents of flour samples are summarized in Figure 4.5. The phytic acid content of roasted sample was significantly ($p < 0.05$) higher (1029 mg/100 g) followed by the unprocessed sample (837 mg/100 g) while germinated sample had the lowest (279 mg/100 g) among the processed formulations. The highest ($p < 0.05$) tannin content (683 mg/100 g) was recorded for fermented sample and the lowest content (381 mg/100 g) was detected in the germinated sample. The flavonoid content of the porridge flours was in the range of 60 mg –166 mg/100 g, with the highest value (166 mg/100 g) recorded for commercial flour.

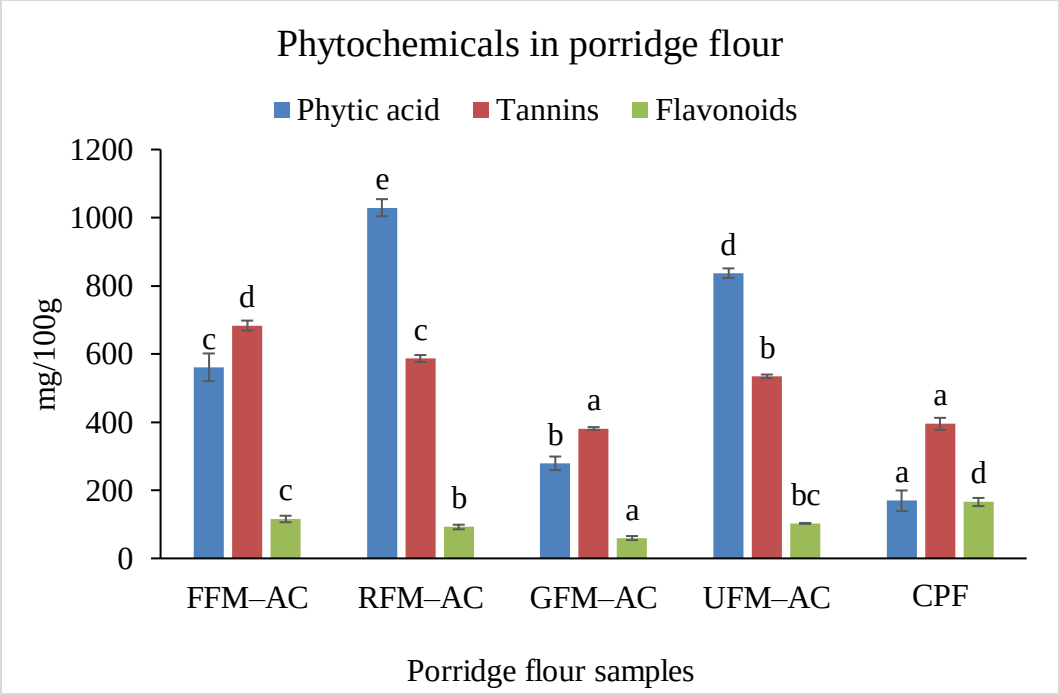

Figure 4.5: Phytochemical content in porridge flour samples. The bars represent mean and error bar standard deviation of phytochemical contents. Different letters above the error bar indicate significant differences in phytochemical content. FFM–AC= Fermented finger millet–amaranth + cricket. RFM–AC= Roasted finger millet–amaranth + cricket, GFM–AC=Germinated finger millet–amaranth + cricket, UFM–AC=Untreated finger millet–amaranth + cricket. CPF = Commercial porridge flour. ǂ mg tannic acid equivalents (TAE)/100 g of sample, ᵠ mg catechin equivalents (CEQ)/100 g of dry sample.

4.6. Phytic acid/mineral molar ratios

Processing methods had a significant ($p < 0.05$) influence on the phytate/mineral molar ratios (Table 4.5). Germinated sample had the lowest ratio for phytate/Fe (1.31) and phytate/zinc (7.43) whereas commercial flour had the lowest ratio for phytate/calcium (0.03). Phytate/Ca ratios recorded in all the flours were below the limit threshold except for roasted sample (0.24). Roasted sample had the highest values (above threshold) for all phytate/mineral molar ratios.

Table 4.8: Mineral bioavailability as influenced by processing methods.

[b]Product	[a]Molar ratios		
	Phytate/iron	**Phytate/zinc**	**Phytate/calcium**
FFM–AC	5.69±1.38[b]	17.25±1.40[b]	0.14±0.00[c]
RFM–AC	9.63±1.41[c]	30.48±5.39[c]	0.24±0.01[e]
GFM–AC	1.31±0.42[a]	7.43 ±0.64[a]	0.06±0.01[b]
UFM–AC	7.76±1.90[bc]	26.90±2.04[c]	0.21±0.00[d]
CM	1.46±0.05[a]	9.03±1.97[a]	0.03±0.01[a]
*Limit	1.0	15.0	0.24

* Reference based on Norhaizan and Nor (2009).
[a]Molar ratios: Mean (± standard deviation) of phytate/mineral molar ratios in flour samples.
[b]Product**:** FFM–AC = Fermented finger millet–amaranth + cricket. RFM–AC = Roasted finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket, UFM–AC = Untreated finger millet–amaranth + cricket. CPF = Commercial porridge flour. Different superscript letters within columns indicate significant difference at $p < 0.05$ according to the Tukey test.

4.7. Flavor optimization

Results of sensory evaluation showed that there were significant differences ($p < 0.05$) between the samples for all attributes which include color, texture, flavor and overall acceptability (Table 4.6). The sensory panelists were able to distinguish the four formulations with a biplot PCA for the hedonic scores accounting for 82% of the variance between consumer scores for the formulations, an indication that all the formulations had significant dissimilarities (Figure 4.6). Sample CF4 (20% cricket) was opposite sample CF2 (10% cricket) with minimal differences in

the scores for sample CF1 (0% cricket). CF2 had higher differences in the hedonic scores among consumers.

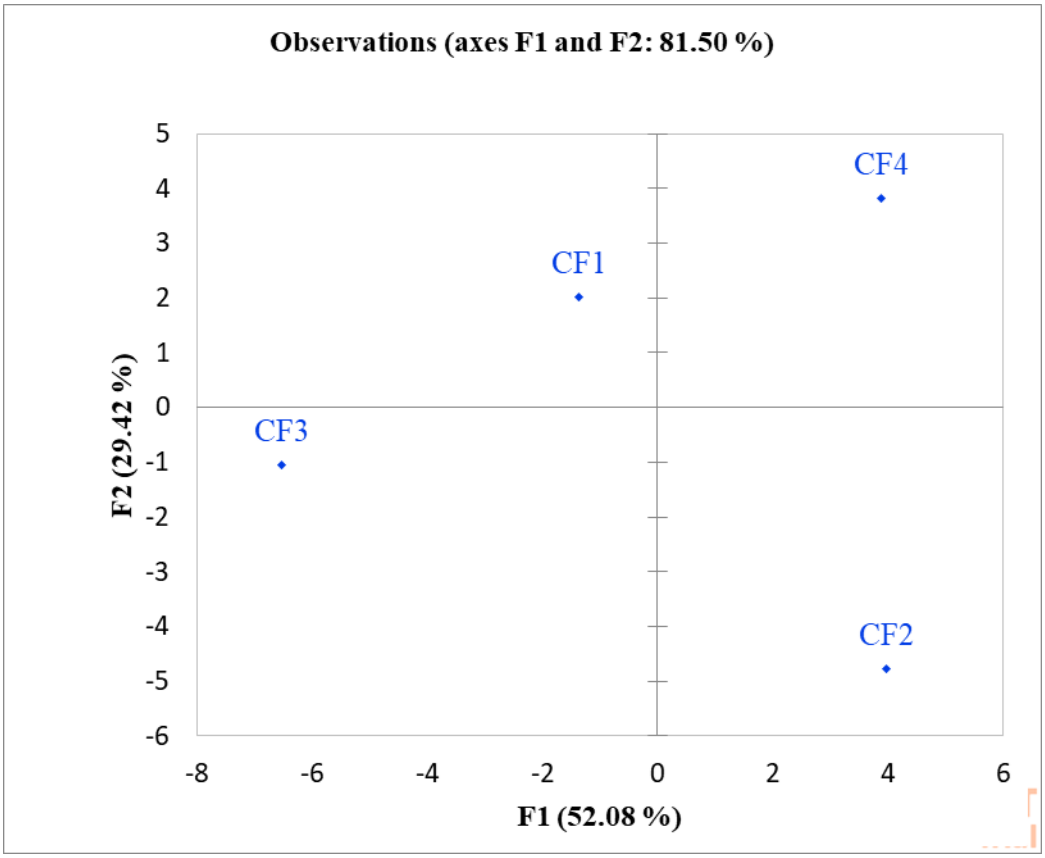

Figure 4.6: PCA plot for formulation similarities. CF1 (56% finger millet: 44% amaranth: 0% cricket), CF2 (50% finger millet: 40% amaranth: 10% cricket), CF3 (47% finger millet: 38% amaranth: 15% cricket) and CF4 (45% finger millet: 35% amaranth: 20% cricket).

The mean sensory scores for each quality attribute of the porridge prepared from the composite flours are presented in Table 4.6. The statistical analysis of the data showed there were significant differences ($p < 0.05$) in the quality attributes evaluated. With exception of color, the average scores for the hedonic scales showed that sample CF2 (10% cricket) had the highest scores for all the variables under study while sample CF3 (15% cricket) and CF4 (20% cricket) were also acceptable but had lower scores than CF1 (0% cricket) and CF2 (10% cricket). Attributes for

sample CF1 (0% cricket) were not significantly different ($p > 0.05$) from those of sample CF2 (10% cricket) but had relatively lower mean scores compared to the former.

Table 4.9: Sensory scores for different attributes of flour enriched with cricket powder

Product	Color	Aroma	Consistency	Mouthfeel	Taste	Acceptability
CF1	6.2± 0.9^{c}	5.8± 1.0^{b}	6.0±1.0^{bc}	6.0±1.1^{b}	5.8 ±1.2 ^{c}	5.9±0.9 ^{bc}
CF2	5.9± 1.1^{bc}	6.0±1.1^{b}	6.3±0.8 ^{c}	6.3±0.7 ^{b}	6.4 ±0.8 ^{c}	6.3±0.6 ^{c}
CF3	5.4±1.2 ^{ab}	5.0± 1.3^{a}	5.5±1.1 ^{b}	5.7±1.2 ^{b}	5.0±1.3 ^{b}	5.5±1.0 ^{b}
CF4	5.1±1.3 ^{a}	4.2±1.6^{a}	4.6±1.3 ^{a}	4.9±1.4 ^{a}	4.2 ±1.4^{a}	4.4±1.2 ^{a}

Mean value (± standard deviation), means with different superscripts along each column differ significantly ($p < 0.05$). CF1 (56% finger millet: 44% amaranth: 0% cricket), CF2 (50% finger millet: 40% amaranth: 10% cricket), CF3 (47% finger millet: 38% amaranth: 15% cricket) and CF4 (45% finger millet: 35% amaranth: 20% cricket).

The PCA analysis with the first two dimensions explaining 71% of the variability in scores for the hedonic scores showed that the color of the samples had no significant correlation with the preference of the porridge products (Fig 4.7). However, the aroma and taste of the products were positively correlated while there was a weak association between mouthfeel and the product consistency. The mouthfeel was also independent of the product taste.

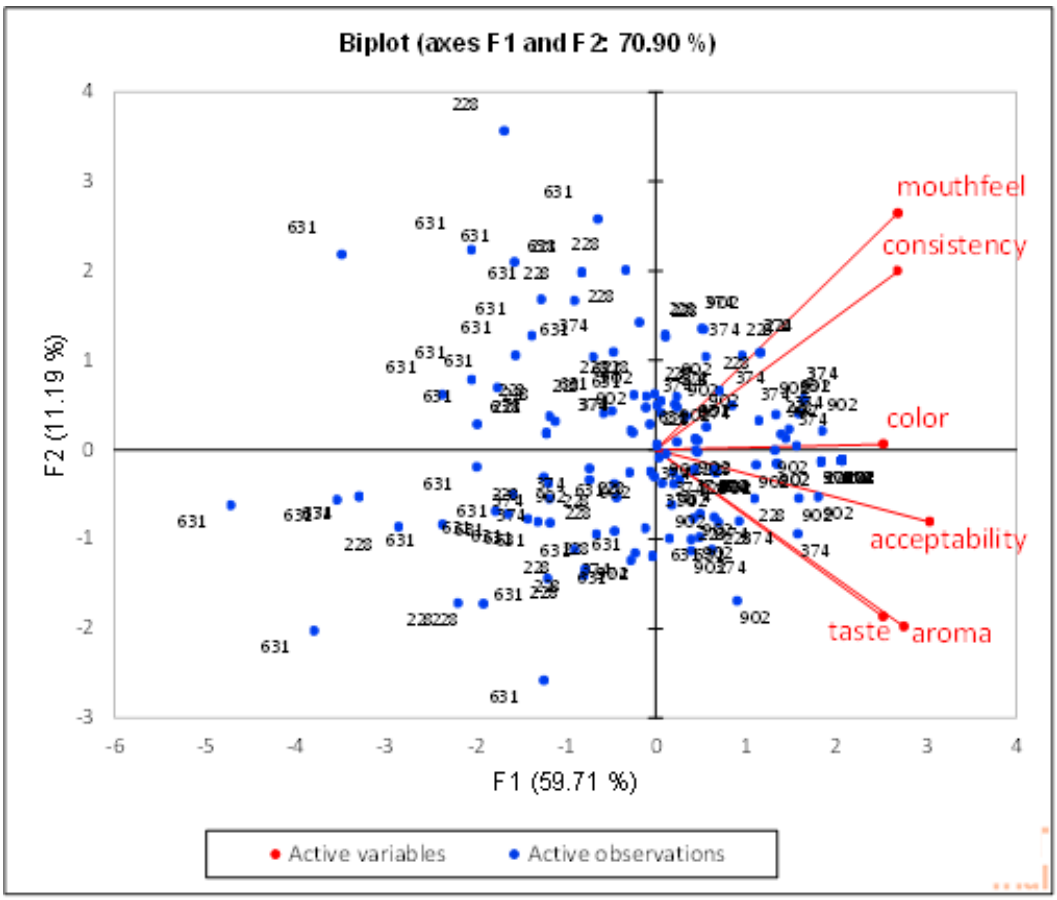

Figure 4.7: PCA plot for organoleptic scores for different product formulations

4.8. Effect of different processing methods on sensory acceptability

Four processed and formulated porridge samples used for the sensory test are illustrated in Figure 4.8. The mean sensory scores as affected by processing methods for various quality attributes of the porridge prepared from the composite flours are presented in Table 4.7. The statistical analysis of the data showed there were significant differences among the sensory attributes ($p < 0.05$). The average scores showed that porridge samples coded T1 (roasted), T2 (untreated), and T3 (fermented) had the highest scores for all the attributes (5.9, 5.7 and 5.9 respectively) and were the most preferred as compared to T4 (germinated) which scored the least (1.7) in all the attributes.

There was no significant difference ($p > 0.05$) between samples T1 (roasted), T2 (untreated) and T3 (fermented) scores for all attributes.

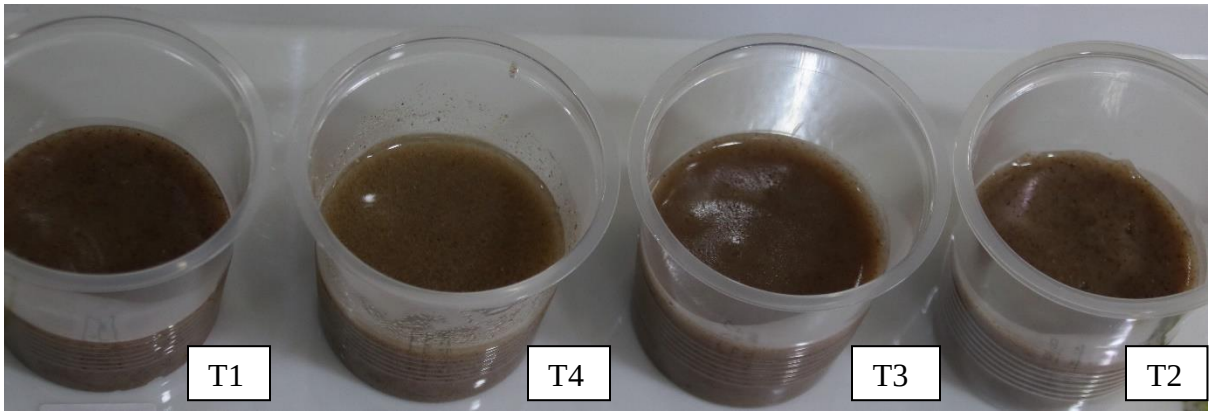

Figure 4.8: Porridges from the formulated flours. T1=Roasted, T2=Untreated, T3=Fermented T4=Germinated.

Table 4.10: Effect of processing on sensory scores for different attributes of finger millet–amaranth flour enriched with cricket powder

Porridge Product	Acceptability	Aroma	Color	Consistency	Taste	Texture
RFM–AC	5.9±0.9[a]	5.8±1.0[a]	6.1±0.7[a]	5.8±1.0[a]	6.2±0.8[a]	5.8±1.1[a]
UFM–AC	5.7±1.0[a]	5.8±1.0[a]	6.0±1.1[a]	5.9±1.0[a]	5.9±1.1[a]	5.9±1.1[a]
FFM–AC	5.9±1.1[a]	5.2±1.6[a]	6.2±1.1[a]	5.9±1.3[a]	5.7±1.4[a]	6.2±0.9[a]
GFM–AC	1.7±1.0[b]	2.5±1.5[b]	2.3±1.5[b]	1.9±1.1[b]	1.8±1.1[b]	1.8±1.1[b]

Mean±standard deviation and different superscripts along each column differ significantly ($p < 0.05$). T1=RFM–AC = Roasted finger millet–amaranth + cricket, T2=UFM–AC = Untreated finger millet– amaranth+ cricket, T3=FFM–AC = Fermented finger millet–amaranth + cricket, T4=GFM–AC = Germinated finger millet –amaranth + cricket.

Sensory panelists were able to distinguish the four formulated porridge products. The products had significant dissimilarities with about 56.4% of the total variance for attributes explained (Figure 4.9). Product T4 (germinated) was opposite with the higher differences in the hedonic scores than products T1 (roasted), T2 (untreated) and T3 (fermented) which had the smallest differences in the hedonic scores among them (Figure 4.9).

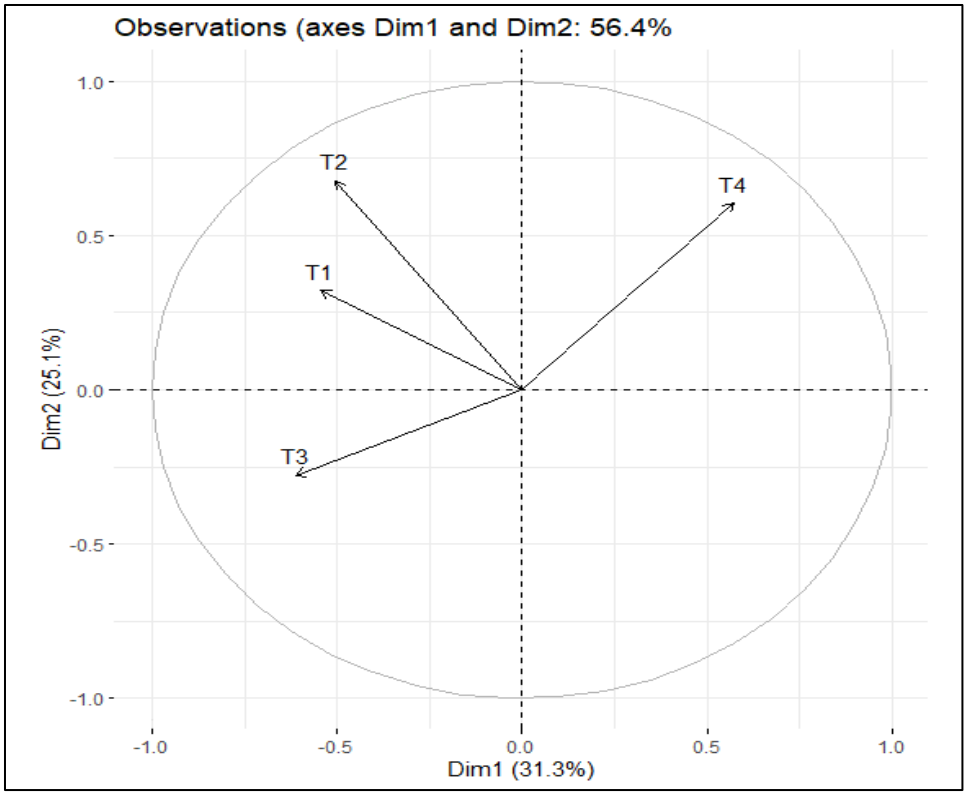

Figure 4.9: PCA plot for sensory scores for different treatment formulations. T1 = RFM–AC = Roasted finger millet–amaranth + cricket, T2=UFM–AC = Untreated finger millet–amaranth + cricket T3=FFM–AC = Fermented finger millet–amaranth + cricket, T4=GFM–AC = Germinated finger millet–amaranth + cricket powder

Two–dimensional PCA explained 90.6% of the variation of six sensory attributes and helped to group the porridge samples according to their differences (Figure 4.10). The first PC accounted for 86.0% of the total variance of the sensory data while the second PC accounted for 4.6% of the total variance of the sensory data. Germinated sample was separated from other samples due to the lower hedonic scores. Roasted, untreated and fermented flour formulations were grouped and had more pronounced taste, acceptability, consistency, texture and acceptability. There was a strong correlation between taste, acceptability, consistency, texture and acceptability for determining the sensory analysis of the four porridge samples. However, for the four porridge samples, aroma did significantly not correlate with either taste, acceptability, consistency, texture or acceptability.

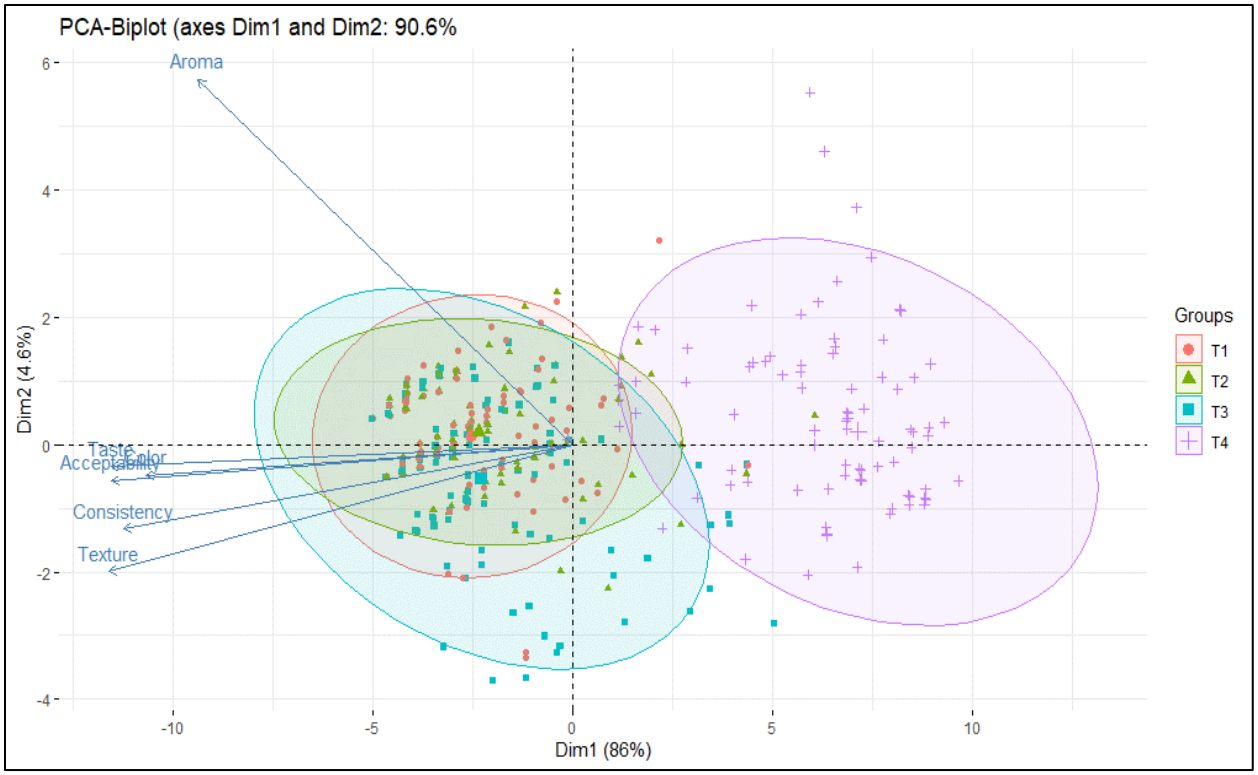

Figure 4.10: Principal component analysis (PCA) of six sensory attributes perceived significantly different among flour formulations T1 = RFM–AC = Roasted finger millet–amaranth + cricket, T2=UFM–AC = Untreated finger millet–amaranth + cricket T3=FFM–AC = Fermented finger millet–amaranth + cricket, T4=GFM–AC = Germinated finger millet–amaranth + cricket.

4.9. Microbial quality of porridge flour samples

The porridge flour was analyzed for microbial quality to guarantee safety during consumption. The microbial load (total bacterial plate, mould and yeast counts) on the porridge flours is presented in Table 4.8. There were statistical differences in microbial load among germinated, fermented, roasted and untreated samples made of finger millet, amaranth and cricket at different proportions ($p < 0.05$). The total viable plate count for untreated samples was 3.81 $\log_{10}$ CFU/g whereas mould and yeast count was 1.37 $\log_{10}$ CFU/g. The roasted samples had the least total viable counts and zero yeasts and moulds.

Table 4.11: Total viable, yeast and mold count (CFU/g) in different flours enriched with cricket

Flour	Total Viable Counts	Yeasts and Moulds Counts
UFM–AC	3.81 ± 0.04^{c}	1.37 ± 0.09^{b}
GFM–AC	4.05 ± 0.03^{c}	1.61 ± 0.00^{c}
FFM–AC	3.19 ± 0.05^{b}	1.67 ± 0.05^{c}
RFM–AC	2.43 ± 0.22^{a}	0.00 ± 0.00^{a}

Mean±standard deviation and different superscripts along each column differ significantly ($p < 0.05$). RFM–AC = Roasted finger millet–amaranth + cricket, UFM–AC = Untreated finger millet–amaranth + cricket, FFM–AC = Fermented finger millet–amaranth + cricket, GFM–AC = Germinated finger millet–amaranth + cricket.

4.10. Influence of packaging material on microbial load and stability of flours

Figure 4.11 shows that samples packaged in aluminium did not significantly change over time with regard to total microbial counts. The flour samples packaged in paper bags exhibited significant variations in terms of microbial loads ($p < 0.05$) but in both packages (paper and alluminium), the microbial limit stayed acceptable. Roasted flour formulation had a low count of bacteria (2.4-2.9 $\log_{10}$ CFU/g), yeast and mould (1.2-1.4 $\log_{10}$ CFU/g) compared to fermented, untreated and germinated formulations in both packaging materials. However, there were no significant ($p > 0.05$) differences in the bacteria, yeast and mould loads between the different times of storage. Except for moulds, bacteria and yeasts were present in all the porridge flour formulations.

Unlike with fermentation and germination processes, the moisture content declined significantly ($p < 0.05$) with roasting (3.03%) (Figure 4.11). The flour samples packaged in paper bags exhibited noticeable variations related to the moisture content within the storage environment. The flour samples stored for 6 months had higher moisture content in both packaging materials compared to the initial flour formulations. The highest moisture content (11.5%) was recorded in the germinated flour sample after the sixth month of storage.

Figure 4.11 shows the effect of storage conditions and packaging on free fatty acids (FFA). FFA is an indicator of oxidative degradation in products. Both packaging material and storage period affected the levels of FFA. In both storage periods, flour formulations stored for 6 months consistently had higher concentrations of FFA (2.0-6.7 mg/g) compared to those stored for 3 months (0.9-4.9 mg/g). The highest FFA value (6.7 mg/g) was recorded in the germinated flour sample after 6 months of storage. Samples stored for less than three months had low concentrations of FFAs. In both storage periods, germinated and fermented finger millet cricket flours had higher concentrations of FFAs compared to untreated and roasted flours.

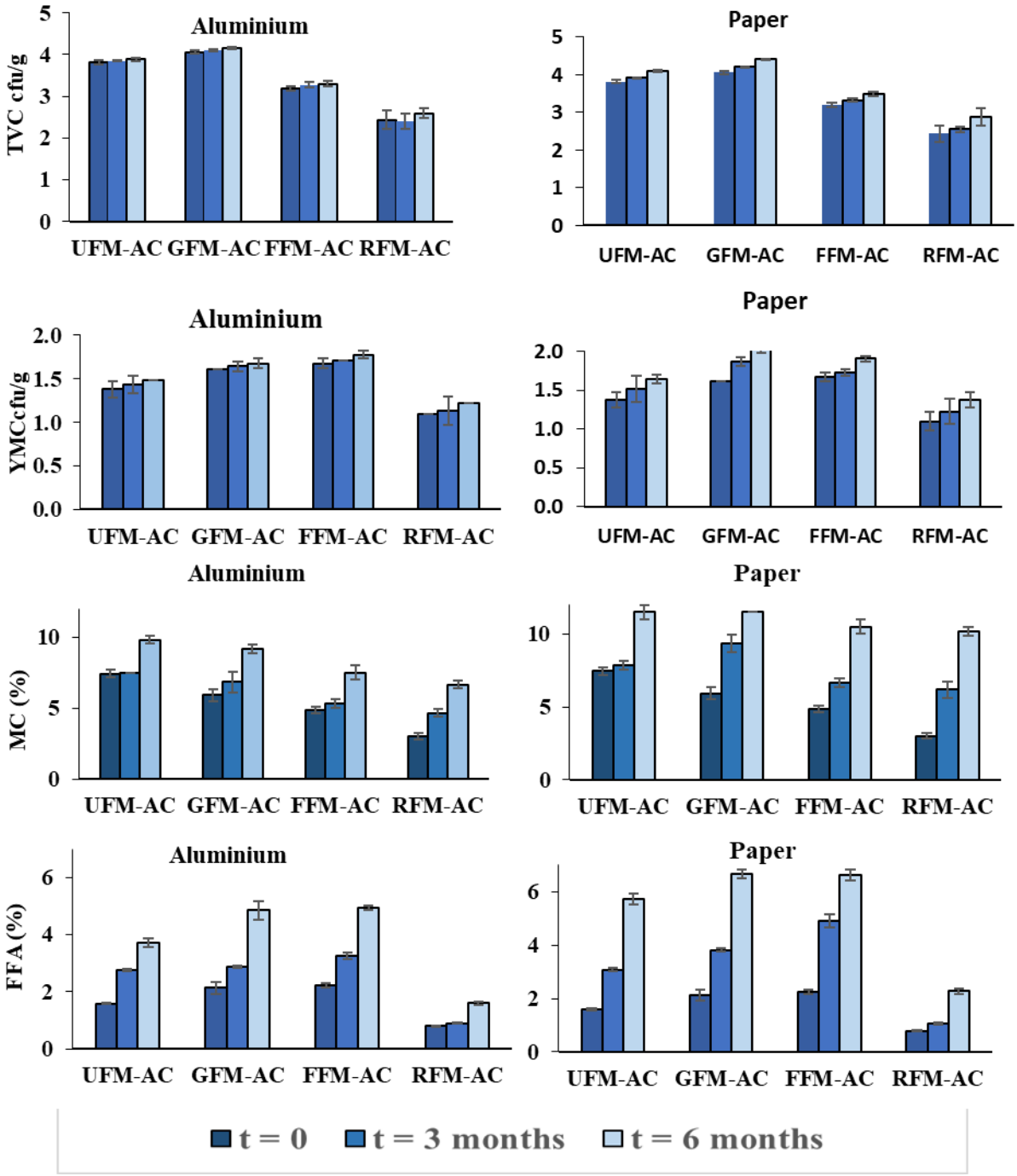

Figure 4.11: Influence of packaging and storage duration (from 0 to 6 months) on the microbial count, moisture concentration, and free fatty acids in flour formulations. The bars indicate mean±standard deviations. TVC=Total viable counts, YMC= Yeast and mould count, MC=Moisture content, FFA= Free fatty acids. FFM–AC= Fermented finger millet–amaranth + cricket. RFM–AC= Roasted finger millet–amaranth + cricket, GFM–AC=Germinated finger millet–amaranth + cricket, UFM–AC=Untreated finger millet–amaranth + cricket. CPF=Commercial porridge flour

CHAPTER FIVE: DISCUSSION

5.1. Proximate composition

Traditional cereal–based porridge has been considered one of the major causes of protein energy–malnutrition in developing countries. The traditional complementary foods are usually low in protein and energy density while containing high anti–nutrient content. This study shows that formulating porridge products with edible cricket increases protein, energy and mineral contents compared to the commercial porridge flour and, therefore, enriched porridge products could be better substitutes for traditional complementary foods. The recommended daily intake for protein in children aged 1–3 years is 13g/day (WHO/FAO/UNU, 2007), and our study showed that the protein content in the cricket enriched flour ranged from 15.34 –16.12 g/100 g (dwb). The observed protein content in cricket-enriched porridge flour is higher than those reported by Agbemafle *et al*. (2020)) on complementary food with orange–fleshed sweet potato enriched with palm weevil larvae, and this could be accredited to the addition of cricket powder which is highly rich in protein (Rumpold & Schluter, 2013). The suitable serving size of this porridge product, when prepared to porridge, would be 20 g of dry product prepared with up to 125 ml water at the time of consumption.

The fermented product had significantly lower protein content when compared with the untreated sample and this could be associated with amino acids being metabolized into ammonia and other volatile flavor compounds during the process of fermentation (Pranoto *et al*., 2013). A similar trend was reported during fermentation of pearl millet by Osman (2011). However, there was a slight increase in protein content in the germinated product and this could be attributed to the mobilization of storage nitrogen and synthesis of enzymatic proteins by the sprouting seeds during germination (Nnam, 2000).

Fat content is an important factor influencing the energy density of foods as its energy density is more than double that of carbohydrates and proteins. It also provides essential fatty acids and improves the absorption of fat-soluble vitamins and the sensory quality of food (Onabanjo *et al.*, 2008). The fat content of cricket-enriched products was higher than that of non–cricket-enriched products. However, the results are lower than amaranth-based complimentary food blended with edible termites (10%) and fish (3%) (Kinyuru *et al*., 2015). These observations were due to the incorporation of termites and fish which contain a high amount of fat content (Kinyuru *et al*., 2013). Fermentation process caused a 13% reduction in fat content and this could be attributed to fat being utilized by microorganisms as a source of energy. These findings are consistent with observations by Assohoum *et al*. (2013) who observed a reduction in fat content on fermented maize. Fiber content significantly increased during germination but reduced during fermentation and roasting. The increase in fiber content during germination could be attributed to the loss of sugars in the grains as it's usually used up by spouting seeds leaving behind fibrous seeds (Ikenebomah *et al*., 2019).

According to Codex Alumentarius (1991), the energy requirement for complementary foods is 400 kcal/100 g on a dry weight basis. The energy contents in all the processed and formulated porridge products ranged from 408.12 to 413.92 kcal/100 g and were significantly ($p < 0.05$) higher compared to commercial flour (381.28 kcal/100 g) due to the increase in protein and fat amounts as a result of blending. Roasted and fermented porridge products yielded the highest energy density than germinated or unprocessed products.

5.2. Mineral composition

The study shows that processed porridge flour products had increased amounts of essential minerals including zinc, iron, and calcium. The iron content in the formulated products (8.6–19.5 mg/100 g) met the 7 mg/day recommended daily allowance (RDA) for iron in young children aged 1–3 years. Zinc content was in the range of 3.08–3.70 mg/100 g which contributes about 75–90% RDA for zinc (4.1 mg/day) in young children. All the porridges were high in calcium content (234.9–312.8 mg/100 g) but did not meet the RDA (500 mg/day) for the target age group (WHO/FAO, 2004). Fermentation processing did not affect the levels of Ca, Cu, Fe, and Zn but caused a reduction in the concentrations of Mg and P and an increase in the Mn content. Reduction in the mineral content during fermentation has also been observed in cowpea flour (Difo *et al*., 2014). Germination processing did not affect the levels of Zn, Cu, Mg, P, and Mn but increased the levels of Ca and Fe. Increments in Ca and Fe amounts during germination processing have been previously reported (Sow *et al*., 2019; Tizazu *et al*., 2010). The increase in mineral content could be attributed to losses of water-soluble compounds during soaking. However, roasting processing did not affect the mineral content. This is in contrast with other studies (Malik *et al*., 2002; Sade, 2009) which indicate a reduction in mineral content during roasting due to loss of nutrients at high-temperature heating.

5.3. Fatty acid composition

Results show that cricket-enriched flours had higher levels of PUFAs as compared to commercial flour. The formulations showed different variations in the composition of fatty acid with the fermented sample having the highest proportion of PUFAs including the omega-3 fatty acids. Omega-3 fatty acids are considered one of the most vital functional food components in the market.

These essential PUFAs have enormous health benefits ranging from promoting brain development and immune function, to prevention of cancers, cardiovascular complications and inflammatory ailments (Islam *et al*., 2018; Martínez *et al*., 2018). Intake of omega-3 fatty acids during pregnancy and lactation has been associated with reduced mortality and allergies in infants as well as improved cognitive function that extends throughout adulthood (Gunaratne *et al*., 2012; Stark *et al*., 2016). The four omega 3 fatty acids include alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Naturally, ALA is found in plant oils while EPA and DHA are found in fish and other sea foods (Verma *et al*., 2019). Conversion of ALA to EPA and finally to DHA occurs in the body through desaturation and elongation reactions, however, in humans, this process is inefficient due to the lack of desaturase enzyme required to add a double bond at the C-15 position of a carbon chain of fatty acid to form n-3 PUFAs (Flock *et al*., 2013). The rate of conversion varies and is about 3-6% in men and up to 9% in women (Coates *et al*., 2010). Therefore, there is a need to supplement these essential FAs through diet to prevent deficiencies. The proposed RDA for omega 3 (EPA and DHA) is 250-500mg per day for general health in adults (Flock *et al*., 2013; Harris *et al*., 2009). The omega-6 to omega-3 ratios of all flour oils found in the current study are within the recommended range of the required daily intake (< 5:1) recommended by WHO. The ratios were lower than those reported by Paucean *et al*. (2018) on wheat-lentils composite flour and Cheseto *et al*. (2020) on insect oils and cookies.

Fermentation process caused an increase in the levels of PUFAs and MUFAs and a decrease in the SFAs. Four omega-3 fatty acids were detected in the flour formulations but DHA was only present in the fermented sample and this indicates biosynthesis of DHA during fermentation. The increase in PUFAs, particularly omega-3 FAs during fermentation could be attributed to microbial synthesis

(Kannan *et al*., 2021). Several researchers have investigated the production of omega-3 fatty acids through microbial synthesis including bacteria (Yazawa, 1996), fungi (Gayathri *et al*., 2010) and yeast (Xie *et al*., 2017). In this study, roasting increased the amounts of SFAs and oleic acid while the amounts of unsaturated linoleic acid and omega 3 fatty acids decreased. Studies indicate that the rate of fatty acid oxidation increases as the number of double bonds increases thus PUFAs will isomerize at a higher rate than MUFAs (oleic acid) (Yoshida *et al*., 2001) and this could explain the increase and decrease of oleic and linoleic acid respectively during roasting. Similar results have also been previously reported on roasted hazelnuts and almonds (Amaral *et al*., 2006; Lin *et al*., 2016). Germination caused a 9% decrease in MUFAs and an increase (12%) in the levels of PUFAs. The results on increase in PUFAs during germination are similar to those reported by Mariod *et al*. (2012) on germinated black cumin seeds.

5.4. Vitamin content

Vitamins are organic compounds that are required in small amounts for the maintenance of normal health and biological reactions in the cells (Walther & Schmid, 2017). In this study, fermentation process caused an increase in vitamin C, thiamine (B_1), pyridoxine (B_6), pantothenic acid (B_5), folate (B_9) and nicotinamide. The increase in water–soluble vitamins particularly of the B–group during fermentation could be a result of microbial synthesis. Several bacteria genera including lactic acid bacteria (LAB) have the potential to synthesize vitamins particularly those in the B–group (Barrios–González, 2012). The results agree with those reported by Kaprasob *et al*. (2018) which included an increase in B–group vitamins during fermentation of cashew apples using probiotic strains of LAB. However, there were reductions in nicotinic acid and vitamin B_{12} in the fermented sample, and the losses might be attributed to microbial utilization by LAB in their

metabolic biosynthesis for growth (Tabaszewska *et al*., 2018). Germination process enhanced the concentrations of vitamin C, nicotinic acid, vitamin B_6, B_5 and B_9. The increase could be ascribed to the synthesis of these vitamins by the germinating seeds (Zilic *et al*., 2015). The increase in vitamin C during germination has been reported by other researchers and is attributed to starch hydrolysis by amylases and diastases leading to an increase in the bioavailability of glucose needed for the biogenesis of vitamin C (Huang *et al*., 2014; Zilic *et al*., 2015). However, loss of thiamine to an undetectable level was recorded in the germinated sample and this might be due to leaching in the spouting medium (Nkhata *et al*., 2018). The negligible effect of roasting on riboflavin and nicotinamide in porridge flours suggests the high thermal stability of B group vitamins (Fuliaş *et al*., 2014). Germination process increased the α-tocopherol content and these results are comparable to those reported by Young *et al*. (2012) on germinated rough rice seeds. Roasting process did not, however, affect the levels of tocopherols because tocopherols are resistant to thermal degradation (Alamprese *et al*., 2009). Contrary, Stuetz *et al*. (2016) reported a loss of tocopherols in roasted compared to raw nuts, the variation might be explainable by different roasting conditions used in the study.

5.5. Phytic acid, tannins and flavonoids

Phytic acid is the principal storage form of phosphorous in plant seeds (Zhang *et al*., 2020) and is known to reduce the bioavailability of dietary Zn, Fe, and Ca in humans and monogastric animals owing to their chelating properties (Konietzny & Greiner, 2003). The reduction in phytic acid in the fermented product may be attributed to the effect of microbial phytase and endogenous phytase activity during fermentation (Castro–Alba *et al*., 2019; Osman, 2011). Phytic acid degradation during germination process could be due to increased activity of enzyme phytase in spouting grains

which hydrolyze phytic acid into lower inositol phosphates (Inyang & Zakari, 2008). Germination and fermentation significantly decreased phytic acid levels, however, both methods led to varying reductions of phytic acid. Germination decreased phytic acid by 67% as compared to fermentation at 33%. The effect of germination on phytic acid levels could be attributed to a combination of processes such as phytase activity and the leaching of phytate ions into soaking in water before germination of grains. The minor degradation of phytic acid during fermentation could also be attributed to the high phytate content polyphenols in both finger millet and amaranth grains. Fermentation is more effective when carried out in grains with low anti-nutrients levels. The presence of endogenous phytase inhibitory compounds such as tannins could also be a major contributing factor (García-Mantrana *et al*., 2014; Sandberg & Svanberg, 1991). The phytic acid content increased during roasting. The increase in phytic acid could be a result of an increase in lower phosphorylated inositol phosphates which in turn increased the total phytic acid content. Similar results were observed by Frontela *et al*. (2008) who noted an increase in inositol pentaphosphate (IP5) during roasting of raw cereal flours and infant cereal mixtures. However, our study did not differentiate between the classes of phytates but rather focused on the total phytic acid content. Besides, the roasting temperature (120 °C) restricted endogenous phytase activity which has an optimum temperature of 55°C and is deactivated at temperatures above 65°C (Konietzny & Greiner, 2003). Contrary, this observation in the current study is inconsistent with some previous studies reporting that roasting of maize (Chukwuma, 2016; Oboh *et al*., 2010) soybean (Agume *et al*., 2007) and pearl millet (Sade, 2009) had a significant decrease in phytic acid and the decrease is attributed to the heat treatment. The low levels of phytic acid in the commercial porridge flour could be due to the use of exogenous phytate degrading enzymes during the industrial processing of the flour.

Flavonoids are a group of polyphenolic compounds found in plants (Havsteen, 2002). Flavonoids exhibit powerful antioxidative properties which makes them significant in the prevention of degenerative diseases (Panche *et al*., 2016). However, flavonoids are also regarded as anti–nutrients with the ability to chelate metals such as Fe and Zn reducing their bioavailability (Spencer, 2003). The flavonoid values in our products ranged between 59.8–165.5 mg/100 g. In this study, flavonoid content increased during fermentation. This is consistent with the observations of Adetuyi & Ibrahim (2014) who reported an increase in total flavonoid content during fermentation of okra seeds. The increase in flavonoid content could be attributed to the release of simple phenolic compounds during acid and microbial hydrolysis of complex phenolic compounds during fermentation (Hur *et al*., 2014). A reduction in flavonoid content was noted during germination (42%) and roasting (10%). The results are similar to the findings of other authors (Modgil & Sood, 2017; Zhang *et al*., 2010). The reduction of flavonoids during roasting could be attributed to flavonoid breakdown due to high temperature applied during roasting since flavonoids are heat sensitive (Chaaban *et al*., 2017).

Tannins are regarded as antinutrients because of their ability to inhibit digestive enzymes, lowering the digestibility of most nutrients, particularly proteins (Ali *et al*., 2003). An increase in tannin content in formulations might be due to the blending effect of amaranth seeds and finger millet which are both high in tannins. Tannin content was reduced during germination and the reduction could be due to the complexing of tannins with seed proteins and metabolic enzymes and not due to actual degradation (Shimelis & Rakshit, 2007). Leaching into the sprouting medium due to the solubility of tannins could have also led to a reduction of tannin contents (Kunyanga *et al*., 2011). A significant increase in tannin content observed during fermentation might be due to the hydrolysis of various components including condensed tannins by catabolic enzymes. Similar

findings were reported by Osman (2011) during fermentation of pearl millet. On the contrary, reductions in tannin content during fermentation have also been reported (Abdelhaleem *et al*., 2008; Osman, 2004). However, tannin content increased during roasting process. An increase in tannin content during roasting has been reported by other investigators (Hithamani & Srinivasan, 2014; Pradeep & Guha, 2011) during pearl and finger millet processing.

5.6. Phytate/mineral molar ratio

Phytate/mineral molar ratio is an indicator of mineral bioavailability in plant–based foods (Norhaizan & Nor, 2009). Flour samples from germinated grains had good bioavailability of Zn while all porridge products except for the roasted sample had good bioavailability of Ca. The increased bioavailability of minerals corresponds with the reduction in phytic acid. The commercial flour had good bioavailability of minerals except for Fe, and this could be attributed to low levels of phytic acid as well as the addition of extra minerals during fortification (as stated in the label).

5.7. Sensory evaluation

While CF3 (15% cricket) and CF4 (20% cricket) were considered somehow "earthy", CF1 (0% cricket) and CF2 (10% cricket) were perceived to have aromas that were just right. The decrease in likeness for aroma and taste in CF3 (15% cricket) and CF4 (20% cricket) could be attributed to the earthy flavor of the cricket powder. The earthy flavor could be explained by the fact that crustacean shellfish and insects including crickets are arthropods, hence they would have comparable flavors. From the comments, formulation CF2 (10% cricket) was also considered more

"creamy" or "richer". The significant difference observed in the sensory attributes including aroma, consistency and taste of formulation CF2 (10% cricket) may be due to the enrichment with cricket flour which is known to contain substantial amounts of protein and fat. Cricket flour has an auspicious well–balanced amino acid profile which is an important element of food nutrition and also contributes to sensory properties (Magara *et al*., 2021). On the mouthfeel attribute, sensory panelists made remarks on cricket-enriched porridges being too grainy or gritty. The levels of graininess were higher in CF3 (15% cricket) and CF4 (20% cricket). The addition of cricket powder may alter the texture of the product making it grainy or sandy. Cricket flour contains greater quantities of fiber in the form of chitin and chitosan (Finke, 2007; Ibitoye *et al*., 2018). The insoluble fiber could be the reason for the grainy and rough mouthfeel which is considered as an undesirable sensory attribute in porridge (Wanjala *et al*., 2016). In contrast, a sensory evaluation study on insects prepared using different methods revealed that crispy/crunchy preparations were more desirable to the panelists compared to the boiled insects (Megido *et al*., 2014). The significant differences observed in mouthfeel, taste and acceptability of formulation CF2 (10% cricket) suggest that the content of crickets should be moderated. High content of cricket flour may result in undesirable flavor, rough mouthfeel, unpleasant taste and lower acceptability. Panelists recommended that the acceptability of cricket enriched porridges could be improved through processes like fermentation and roasting, or by addition of flavors such as vanilla or chocolate to ensure that the final product is appealing to the consumers.

The processing methods such as roasting and fermentation have a significant effect on the overall quality of grain and the final product. However, germinated porridge sample (T1) had the least liked taste and acceptability. This may be due to the bitter taste and off–flavors that are produced as a result of germination. The results are similar to those obtained by Nefale and Mashau, (2018)

who reported the same on germinated finger millet porridge. The mean sensory scores for all attributes for roasted, fermented and untreated samples were higher and more accepted by sensory panelists. The processing methods and time combinations neutralize anti–nutrients locked into the bran and seed coating. Roasting improves organoleptic qualities through aroma development (Griffith & Castell–Perez, 1998). According to Agume *et al*. (2017) and Sade (2009), roasting, and soaking significantly decrease phytate content that contributes to undesirable tastes. Sensory attributes are affected by increased lactic acid content and a drop in pH levels due to fermentation which also affected phytates degradation. During fermentation, an acidic medium in the process of souring is introduced, and volatile compounds that act as flavor compounds such as lactate and acetic are also produced (C. Onyango *et al*., 2000). According to Castro–Alba *et al*. (2019) porridge prepared with fermented flour has a better taste when compared with flour from germinated grains.

5.8. Microbial quality and storage stability

The results of microbial load in the flours were within the acceptable limits recommended by KEBS (Kenya Bureau of Standards, 2020) for insect-enriched foods of $<10^5$ for total aerobic counts and $<10^2$ for yeasts and moulds. The lower count of microbial load in four porridge flour formulations could be as a result of the preliminary processes such as roasting at high temperatures and fermentation that could have killed a large number of microorganisms. The low counts could also be a result of the high standard of personal hygiene and maintenance of good manufacturing practices observed during the flour formulation process. This also indicates that the flour products produced through traditional processing methods can have a longer shelf life when packaged well.

For flour packaged in kraft paper bags, an increase in moisture content could have resulted in an increase in the microbial load over time. The presence of these microbes in the fermented flour agreed with the study by Odumodu and Inyang (2006) where fermentation of cereal flour involved the combined action of bacteria, fungi and yeasts. Fermentation is a functional approach for reducing microbial contamination (Chibueze *et al*., 2016) while roasting of grains has a significant impact on the microbial quality in the final product. The temperature and time during roasting detect the microbial quality. Hence, roasting temperature and time combination is of great importance in grain processing for making flour which may include destroying undesirable microorganisms, toxins, allergens, and inactivation of destructive enzymes (Mridula *et al*., 2007). For instance, when almonds were roasted, bacterial load was significantly reduced at temperatures of 130, 140 and 150°C with corresponding times of 21, 11 and 5 min, respectively (Pan & Atungulu, 2010). Agume *et al*. (2017) reported that roasting reduced the moisture content of soybean from 7.2 to 5.7 g/100 g. Agume *et al*., (2016) explained this phenomenon by suggesting that since the moisture content in the flour is associated with its hygroscopic character, therefore, roasting reduces the interactions of flour with water. The low moisture content in roasted flours probably results in changes in the physical characteristics.

During storage free fatty acids accumulation results from fat breakdown/hydrolysis by enzymes such as lipases (Lin *et al*., 2019). However, lipases are denatured during thermal processing and this could explain the low concentration of FFA in the roasted flour samples. In this study, a high accumulation of fatty acids was observed in paper bags which allowed higher moisture gain when compared to aluminium.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Supplementation of finger millet–amaranth porridge meal with edible cricket results in porridge product with higher energy density and nutritional value as evidenced by improved fat, protein, and micronutrient contents. Edible cricket flour can be blended with staple flour and are arguably a sustainable, culturally suitable alternative to less nutrient staple flour. The use of traditional methods to increase nutrients such as mineral and vitamin concentrations in foods is a better-accepted alternative to current fortification systems. Germination and fermentation processes led to a reduction in phytic acid improving the bioavailability of minerals in flours.

Consumer sensory analysis revealed that the addition of 10% cricket powder was the most preferred than the others suggesting that insect powder should be added moderately to ensure appealing aroma, tastes and acceptability. Traditional processing methods such as roasting and fermentation improve organoleptic properties in porridge flour formulations resulting in porridges with better sensory qualities. Moreover, the use of fermentation and roasting help in reducing the microbial loads in flours.

Porridge flour enriched with edible cricket is shelf life stable under room conditions for six months. The study also shows the choice and quality of suitable packaging material are of critical importance in protecting insect–based flour from both moisture and oxygen. Packaging in aluminium bags is more effective but the cost implications should be evaluated.

6.2. Recommendations

Although a complete degradation of phytic acid was not achieved, this study demonstrated that germination and fermentation can help in improving mineral bioavailability. However, for complete phytic acid degradation, it is necessary to look at better approaches such as the use of exogenous phytate degrading enzymes, application of uptake enhancers and combined process approaches.

Furthermore, studies of *in vitro* bioavailability can be carried out for reproducibility of reported results on mineral and trace elements bioavailability considering other factors such as pH, the concentration of enhancers, and inhibitors such as dietary fiber and polyphenols also dictate the inhibitory effect of phytic acid on mineral bioavailability. More research is also needed on protein digestibility.

In promoting the use of insects as foods, more strategies beyond environmental sustainability and the nutritional value of insects should be considered. Efforts such as consumer interaction and awareness through mass media communication and food festivals could be of importance in promoting entomophagy. In addition, locals with indigenous knowledge in entomophagy could be consulted regarding the use of insects as food, including edible species, handling and preparation, storage, safety and strategies for promoting insects as food.

The cost-effectiveness of large-scale industrial production and processing of insect-based foods should be evaluated to ascertain the economic feasibility. Furthermore, the impact of different packaging materials and storage conditions on the quality of insect–based foods should be assessed.

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APPENDICES

Appendix 1: Type of grains and cricket used in this study

Finger millet grains

Amaranth millet grains

Cricket *Scapsipedus icipe*

Appendix 2: Sensory evaluation questionnaire

SENSORY EVALUATION QUESTIONNAIRE

Panelist Code: _____

Date: __ __ 2021

A) CONSENT FORM

Sensory evaluation of porridge enriched with edible cricket flour. You are invited to participate in a research study of the perception of insect–based products. We ask that you read this form and ask any questions that you may have before agreeing to be enrolled in the study. This is a voluntary exercise to determine the acceptability of porridge enriched with edible cricket. Kindly let us know if you are allergic or intolerant to insect–based foods, so you are excluded from the study. The results of your assessment as a panelist are strictly confidential. Kindly fill in your details in the section below.

I have read the information about the conditions of this sensory evaluation and all my concerns about the study have been addressed. I hereby give my voluntary consent for participation in this study.

Name : _____

GENDER	**TICK HERE**
Male	
Female	
AGE	**TICK HERE**
Less or equal to 20	
21–25	
26–30	
31–35	
36–40	
41 and above	

Signature : _____

B) SENSORY EVALUATION
INSTRUCTIONS
Below is a sensory assessment evaluation sheet of porridges from different kinds of flours.
You are required to assess their **taste, aroma, color, texture, consistency, and overall acceptability** using a scale of 1–7. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your reference (1–7) in the column against each attribute. Put the appropriate number against each attribute as follows:

- 7– like extremely
- 6– like moderately
- 5– like slightly
- 4– neither like nor dislike

- 3– dislike slightly
- 2– dislike moderately
- 1– dislike extremely

Attributes	**Sample Codes**			
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Color				
Aroma				
Consistency				
Texture				
Taste				
Overall Acceptability				
Would you recommend this product for complementary feeding?	**Yes**	**Yes**	**Yes**	**Yes**
	No	**No**	**No**	**No**

Additional comments:

Thank you for participating in the study.