ORIGINAL RESEARCH ARTICLE

Physico-chemical characterization, acceptability and shelf stability of extruded composite flour enriched with long-horned grasshopper (*Ruspolia differens*)

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ABSTRACT

In Tanzania, chronic malnutrition rates remain high, with 32 percent of children under the age of 5 being stunted. The high cost of dietary protein sources is a contributing factor to the deterioration of the nutritional status of most children. Edible insects are suggested to provide an alternative source of protein in enriching the nutritional composition of foods. However, there is limited knowledge on the potential use of long-horned grasshopper *(Ruspolia differens)* to enhance the nutritional properties of extruded composite flour. This study intended to develop an extruded composite flour enriched with the long-horned grasshopper and further evaluate its proximate composition, mineral properties, antinutritional composition, functional properties, sensory acceptability, and shelf-life stability. Four samples of composite flour were formulated and processed using a twin-screw extruder to produce extruded composite flour (ECF) with a mixing (%wt.) ratios of 100:0 (ECF 0), 95:5 (ECF 5), 85:15 (ECF 15), 75:25 (ECF 25) for composite flour (cassava, millet, sorghum) and edible long-horned grasshopper pellets, respectively. Feed moisture content and barrel temperature were adjusted from 15 to 16% and 128 to 142 ^{0}C for Zone I, and 105 to 114 ^{0}C for Zone II, respectively. The findings show that the inclusion of long-horned grasshopper meal in the extruded composite flour significantly increased the moisture, crude fibre, crude ash, crude protein, and crude fat and mineral contents of Fe and Zn while significantly decreasing the carbohydrate contents at ($p<0.001$), and Mg contents at ($p=0.0089$). The phytate contents between ECF 0 and extruded composite flour treatments enriched with long-horned grasshopper did not differ significantly ($p<0.141$). All treatments showed a significant higher tannin content ECF 5 (0.007 mg CE/g), ECF 15 (0.012 mg CE/g) and ECF 25 (0.011 mg CE/g) when compared to values in control (0.004 mg CE/g). There was a significant difference at a 5% confidence level on the sensory properties between ECF 0 and ECF 15 and ECF 25; the latter two treatments had higher scores for colour and a lower score for aroma and taste attributes, respectively ECF 0. In addition, treatments (ECF 5, ECF 15, ECF 25) showed significantly higher peroxide values (3.52 to 5.32 mEq/Kg) than those in the control sample (2.80 to 3.33 mEq/Kg). In conclusion, the inclusion of edible long-horned grasshopper in the composite flour, significantly improved the quantity of crude protein, crude fat, and energy with an adequately appropriate amount of Zn and Fe to meet the needs of fast-growing infants and young children. Moreover, the functional

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properties of the resulted product were positively affected and its consumption is expected not to cause any harm to the human body. However, the product needs modification to improve aroma and taste attributes to increase product acceptability.

1.0 Introduction

In Tanzania, chronic malnutrition rates remain high, with 31.8 percent of children under the age of five being stunted (Tanzania National Nutrition Survey, 2018). The available traditional foods have been recommended to formulate complementary foods to maintain growth and development for fast-growing infants and young children, (WHO, 2003b). However, the commonly consumed food group is cereals, but reduced portion size and feeding frequencies indicate the possibility of deteriorating food and nutrition insecurity (MUCHALI Tanzania - February Report, 2017). Hence, there is a need to diversify cereal-based products through protein nutritional enhancement (Grasso, 2020).

One way to sustainably reduce malnutrition is by using nutritious, locally available, and affordable food sources (WHO, 2008). FAO has recognized edible insects' potential in reducing protein-energy malnutrition (PEM) and hidden hunger (Van Huis et al., 2013). The remarkable nutrient composition of edible insects gives a promising solution to providing nutrition for the growing population and eradicating poverty (Van Huis et al., 2013). Edible insects require less water, feed, and land to develop their cycle. Their by-product demonstrates a positive environmental impact with significantly fewer greenhouse gases and ammonia produced than livestock fields (Van Huis et al., 2013). Therefore, having a sustainable source of protein as an alternative source of animal protein can guarantee food security at the household and national levels (Kinyuru et al., 2018). *Ruspolia differens* possessed protein with a balanced amino acid profile, high mineral content, lipid content, carbohydrate content, and chitin as crude fibre (Ssepuuya et al., 2019; Kinyuru et al.,2010b; Fombong et al., 2017).

Nevertheless, it has been highlighted that processing methods could hinder nutritional factors and the safety of edible insects if not well controlled (Kinyuru et al., 2010a). Many traditional cooking methods require exposure of food materials to high temperatures for an extended period, which can change the nutritional composition of food (Singh et al., 2007). Preferable processes such as extrusion should be employed to maintain nutritional and food quality (Guy, 2001; Singh et al., 2007).

Extrusion is the thermal processing that involves cooking at high temperature and pressure while applying shear forces to an uncooked mass of food in an extruder (Guy, 2001). A high-temperature short time is considered preferable for improved nutritional retention, sensory properties, protein and starch digestibility, and reduced antinutrients (Singh et al., 2007). Rumpold and Schlüter (2015) suggested a need to determine the effect of processing methods on the nutrition quality of edible insects. However, few have been published on the nutritional composition and shelf stability of extruded composite flour enriched with a long-horned grasshopper.

This study intended to develop an extruded composite flour enriched with the long-horned grasshopper and evaluate its nutritional and antinutritional composition, functional properties, sensory evaluation, and shelf-life stability under ambient conditions. The findings from this study are expected to facilitate the practical way of defining the safe utilization of long-horned grasshopper in the development of nutritious extruded composite flour, and the use of edible long-horned grasshopper as an alternative source of dietary protein will guarantee nutrition and food security.

2.0 Materials and Methods

2.1 Materials

Fresh and raw long-horned grasshoppers were purchased from the Kagera region (Kemondo collection point located at Latitude: South 1^0 19' 24" and Longitude: East 31^0 48' 4" with Altitude: 1164.3m) in Tanzania during the end of the second swarming season of March-May. The raw grasshoppers were plucked off wings and paws, sorted and washed with potable water and then placed in a colander/strainer to remove excess water and packed in zip lock polyethene bags, followed by frozen at – 4 ^{0}C to maintain freshness and the microbiological quality of the sample. The frozen long-horned grasshoppers were then transported from the Bukoba region to the Morogoro region in a cool box and the Food laboratory at the Sokoine University of Agriculture and frozen at −20 °C awaiting further processing. White sorghum grain, maize grain cereal, and dried cassava chunks were purchased from Morogoro central market in Tanzania. The samples were kept in clean containers and stored adequately for further processing.

2.1.1 Study design

The already washed raw edible long-horned grasshoppers were oven-dried at 65°C for 12 hours in a convection oven (FC-612 Advance air convection oven, JAPAN) to constant dry body weight. They were then cooled to room temperature, packed in an airtight polythene bag and stored at – 20^0C. The dried cassava chunks were cleaned by sorting and removing dust and debris, then milled into flour using a commercial hammer mill, sieved (in 1mm mesh size) and stored in an airtight plastic container. First, the maize grain was sorted to remove foreign materials and then de-hulled using a commercial de-huller. Afterwards, the grains were winnowed and washed with potable water to remove the husks. The water was drained off, and the grains were sun-dried to 12% moisture content. The dried grains were milled into flour using a commercial hammer mill, sieved (in 1mm mesh size) then stored in an airtight plastic container at room temperature (25-27 ^{0}C).

The white sorghum grain was sorted to remove other foreign matter such as stones and other cereals, followed by winnowing and washing the grain with potable water, then steeped and germinated before being processed into malted flour. The malting process was conducted according to the method done by Asma et al. (2006) with the following modifications; the cleaned white sorghum grain was soaked in potable water to cover the grains 3 to 4 times the volume of potable water for 18hrs at room temperature. The water was then drained, followed by rinsing of the grains. Before setting the grains for germination, the wet grain was spread on the damp double layer muslin cloth placed in a jute bag. After that, the grains were covered with a wet double layer muslin, followed by spraying more potable water on the muslin cloth to soak the grain. The top was covered with black cloth to prevent light penetration to the grain for optimum germination conditions. Muslin cloth was used to keep moisture, thus facilitating grain germination. The germination process lasted 48hrs at room temperature. The malted grain was then oven-dried at 65 ^{0}C for 12hrs. After drying, the grains were cooled to room temperature, and sprouts were removed by hand abrasion and then milled into flour using a commercial hammer mill. Finally, it was sieved and stored in an airtight plastic container.

2.1.2 Development of extruded composite flour

The composite flour samples were formulated with malted white sorghum, cassava and maize flour, as shown in table 1. The twin-screw extruder (Kneader Model JS – 60D, Qitong Mechanical Industry Equipment Co. Ltd Yantai, China), with a screw diameter of 60mm, rate of L/D 16:1, main motor power of 22kW, and output up to 200Kg/h was used to prepare extruded composite flour in this study. The proportions of raw ingredients used in blends were prepared based on the guidelines of the World Health Organization (2003a) for feeding and nutrition of infants and young children. Four blends were formulated with a mixing (%wt) ratio of 100:0 (ECF 0), 95:5 (ECF 5), 85:15 (ECF 15), 75:25 (ECF 25) for blended flour (cassava, millet, sorghum) and edible long-horned grasshopper pellets, respectively, as indicated in Table 1. They were then mixed to obtain a homogeneous mixture. Extrusion was done under modified extrusion conditions conducted by Mahenge and Mugula (2018). Feed moisture content and barrel temperature were adjusted from 15 to 16% and 128 to 142 ^{0}C for zone I and 105 to 114 ^{0}C for zone II, respectively. The main motor and feeder speeds were 29.66 to 30.25 rpm and 09.00 to 11.82 rpm.

Table 1: Formulation (% ratio) used for the development of extruded composite flour

	Formulation ratio (%)			
Ingredients	**ECF 0 (control)**	**ECF 5**	**ECF 15**	**ECF 25**
Long-horned grasshoppers	0	5	15	25
Maize flour	33.33	31.66	28.33	25
Malted Sorghum flour	33.33	31.66	28.33	25
Cassava flour	33.33	31.66	28.33	25

2.2 Proximate compositions and energy value

Proximate analyses for raw and processed flour samples were done according to A.O.A.C (2000). Moisture content was determined by the drying method (air draft method: AOAC 2000: 925.09). Crude protein was determined by the Kjeldahl method (AOAC 2000: 923.03), whereas crude fat was evaluated by the Soxhlet method (AOAC 2000: 979.09). Total ash content was analyzed by direct ignition method (AOAC 2000: 962.09) at 550˚C for 5hrs in a muffle furnace (Carbolite CWF 1100). The crude fibre was determined using by Soxhlet refluxing apparatus (AOAC 2000: 920.39), while carbohydrate was estimated by difference methods (Manzi et al., 2004). The caloric energy was obtained by the calculation method. The values obtained for crude protein, crude fat and total carbohydrate contents were multiplied by 4, 9 and 4 Kcal/g, respectively, and added to the results.

2.3 Mineral analysis

Mineral analysis was done according to the dry-ashing procedure according to AOAC method no. 968.08 (AOAC, 2000). In a crucible, 1g of sample (raw materials and extruded composite flours) were weighed, burnt on a hot plate until the smoke subsided completely and then ashed in a muffle furnace at 500 ^{o}C for 5 hrs. The crucible was transferred into a desiccator and allowed to cool. Ashed samples were weighed and dissolved in 1ml of concentrated nitric acid. The dissolved ashed sample was evaporated to dryness on a hot plate. Five ml of 5M hydrochloric acid was added and transferred to a 100 ml standard volumetric flask. It was then made up to mark with distilled water and filtered. The prepared or digested ash of the samples were analyzed for the mineral elements: Fe, Ca, Mg and Zn using UNICAM Atomic Absorption Spectrophotometer (Model 919, Cambridge, UK) and compared with the absorption of respective standards of these minerals.

2.4 Anti-nutrient content analyses

2.4.1 Tannins

Tannin content was determined by a modified vanillin-hydrochloric acid method using catechin described by Price et al. (1978) with modification. About 0.5g of the ground sample was defatted with 5ml of 1% hydrochloric acid in methanol and allowed to stand for 15 min and centrifuged at 3000rpm for 10min. The supernatant was transferred into a volumetric flask, and the residues were further extracted by 5 ml of 1% hydrochloric acid in methanol on an electric shaker for 20 minutes. It was then centrifuged for 10 minutes at 4500 rpm. A supernatant aliquot was combined with the first one. To 1 ml of the extract, 5 ml of vanillin-hydrochloric acid reagent was added. The reaction mixture was left for 20 min at room temperature (25^{o}C), after which the absorbance was read at 500 nm against a blank using a spectrophotometer (Wagtech, CE 2021, UK). The blank was prepared using 5 ml of 4% hydrochloric acid in methanol. Tannin content was expressed as mg catechin equivalents per g (mg CE/g).

2.4.2 Phytates

Phytates content was measured according to Gemede and Fekadu (2014) method, with modification. One gram of sample was weighed into a centrifuge tube, followed by the addition of 25 ml of 3% Trichloroacetic acid solution and shaken for one hr. The mixture was centrifuged for 15 min at 2000 rpm, then 10 ml of supernatant was added to 4 ml of Ferric Chloride solution and heated in boiling water for 45 min. The mixture was then centrifuged for 15 min at 2000 rpm and decanted. The precipitate was washed twice by dispersing in 25 ml of 3% Trichloroacetic acid, heated in boiling water for 10 min, and centrifuged. Then the precipitate was washed once with distilled water and dispersed precipitate in 5 ml of distilled water following the addition of 3 ml of 1.5 N Sodium hydroxide and then mixed. This mixture was then made up of 30 ml of distilled water, heated in boiling water for 30 minutes, and filtered with Whatman Filter paper into a conical flask. The precipitate was washed with 60 – 70 ml of hot distilled water, and the filtrate was discarded. The precipitate was dissolved in 40 ml hot 3.2N Nitric acid into a 100 ml conical flask, and the precipitate was washed with several portions of water, then cooled to room temperature. A 5 ml aliquot was taken into another 100 ml flask and made up to 70 ml with distilled water. About 0.5 ml of extract was pipetted into test tubes, followed by the addition of 7.5 ml of water. About two ml of Potassium thiocyanate was added, and the absorbance was read at 470 nm using a UV-Visible spectrophotometer (Wagtech, CECIL 2021, UK). The standard solution was prepared using 100 mg of Ferric Nitrate.

2.5 Determination of functional properties

2.5.1 Bulk density

Bulk density was determined using the method Muhangi et al. (2019) described. An empty calibrated centrifuge was weighed and then filled with a sample to 5 ml by constant tapping until there was no further change in volume. The weight of the tube and its contents were taken and recorded. The weight of the sample alone was determined by difference. Bulk density was calculated from the values obtained as follows:

$$\text{Bulk density } \{g/ml\} = \frac{Weight\ of\ sample}{Volume\ occupied}$$

2.5.2 Water absorption index (WAI) and water solubility index (WSI)

Water absorption capacity/index, which indicates the amount of water available for gelatinization, was determined according to the method of Rodríguez-Miranda et al. (2012). From each sample, 1 gram was added into 10 ml distilled water in a weighed 50 ml centrifuge tube, and the mixture was vortexed for 30 seconds. Afterwards, the sample was centrifuged at 1006g for 15 min. The supernatant was decanted and evaporated to dryness at 110 ^{0}C. Water absorption (WAI) was calculated as the weight of sediment/gel obtained after removing the supernatant per unit weight of the original dry solids. Water solubility index (WSI) was obtained by diving the weight of dry solids supernatant over dry weight (original weight) of extrudate multiplied by 100 and was expressed in percentage. These were calculated as follows:

$$\text{WAI } \{g/g\} = \frac{Weight\ of sediment\ or\ gel\ after\ removal\ of\ supernatant}{Dry\ weight\ of\ extrudates\ (original\ weight)}$$

$$\text{WSI } \{\%\} = \frac{Weight\ of\ dry\ solid\ supernatant}{Dry\ weight\ of\ extrudates\ (original\ weight)} \times 100$$

2.5.3 Oil absorption index

Oil absorption capacity was determined according to the modified method of Li and Lee (1996). One half (0.5) gram of the grounded extrudate was mixed with 10 ml oil (pure soybean oil) in a 25 ml conical graduated centrifuge tube and stirred for 3 min with a vortex mixer to disperse the sample into the oil. The samples were allowed to stand at room temperature for 25 min centrifuged at 3000g using a centrifuge for 25 min. The separated oil was then removed with a pipet, and the tube was inverted for 25 min to drain the oil before reweighing. Oil absorption capacity was expressed as grams of soybean oil bound per gram of the extrudate on a dry basis.

2.6 Sensory analysis

A panel of 60 untrained consumers evaluated the consumer acceptability of the extruded composite porridge. Untrained consumers were recruited through an advert on the notice boards of the Sokoine University of Agriculture to invite panellists among the staff and student population. Those who responded to the advert were asked to fill in a consent form informing them about the samples' ingredients and ascertain their commitment to participating on the consumer panel to evaluate the four formulations of extruded composite porridge. Only those who were not allergic to any foods were allowed to participate. The extruded composite porridge samples were prepared by mixing 150mls of extruded composite flour 45g of sugar with 850mls of hot water to make extruded composite porridge. Each consumer was provided with four extruded composite porridge samples served in white disposable cups, serviettes and bottled water to cleanse their palates before and during the tasting. The consumers were asked to rate their degree of liking for appearance, aroma, smell, taste, colour, texture and overall acceptability of the product on a nine-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely. The evaluation was carried out in two sessions, each containing 30 panellists and lasted for about 30 minutes. The assessment was done in the Food laboratory of the Sokoine University of Agriculture, whereby the panellists assessed the samples seated in personal space. The evaluation was conducted using a completely randomized design (CRD), as explained in Lawless and Heymann (2010).

2.7 Microbial analyses

Microbial analyses for extruded composite flours were determined according to A.O.A.C (2002). This was done by placing a 10g extruded composite flour sample into a sterile homogenizing container and then adding 90ml sterile buffered peptone water. The mixture was then homogenized for 2 min, followed by three serial dilutions made by mixing 1 ml of sample with 9ml of sterile peptone water. Enumeration of Total viable count (TVC) was done according to (AOAC 2002: 990.12), and pre-prepared sterile Plate Count Agar (PCA) was used for culturing and enumerating bacterial colonies. Inoculating the sample onto the PCA plates was done aseptically by pouring 1 ml of the sample from each dilution onto a separate Petri dish, then pouring a sterile PCA on top of it. Incubation was done at 37°C for 24-72 hours. The number of bacterial colonies was counted and expressed as colony-forming units per gram (cfu/g) of the sample. Enumeration of yeast and mould (YMC) was done according to (AOAC 2002: 997.02). For YMC, 20ml of sterile Potato Dextrose agar (PDA) was poured and left to dry in a petri dish. Then 1ml of the inoculum from each dilution was pipetted and spread gently using a spreader onto pre-prepared plates. The inoculated plates were incubated at 25°C for five days. The plates were then examined and counted for colonies growing on the medium. The number of yeasts and moulds were expressed as colony-forming units per gram (cfu/g) of the sample.

2.8 Peroxide value

Peroxide value was determined as described by Qian and Pike (2010). About 5g sample of oil extracted from extruded flour sample in Erlenmeyer flask was mixed with 30 ml of a solvent mixture of acetic acid-chloroform 90:60(v/v) and 0.5ml of saturated potassium iodide solution was added, then shaken for 1min to make a homogeneous mixture and followed with the addition of 30ml of distilled water, then mixed well. The mixture was then titrated against standardized 0.01M sodium thiosulphate using starch solution as an indicator until the blue-black colour disappeared. The peroxide value was calculated using the formula;

$$\text{Peroxide value (mEq/kg)} = \frac{(S-B) \times N}{Sample\ weight\ (g)} \times 1000$$

Where:

S = volume of titrant (ml) for sample,
B = volume of titrant (ml) for blank,
N = normality of $Na_2S_2O_3$ solution (mEq/ml),
1000 = conversion of units (g/kg)

2.9 Statistical Analysis

Each activity value was presented as the mean and the standard deviation of each of the three replicates. Data were subjected to the R Analytics Software version 4.1.1. For nutritional, antinutritional, functional properties, microbial analyses and sensory acceptability One-way ANOVA was used to compare the mean differences across the extruded composite flours. Then, multiple comparisons were made using the post hock test (HD-Tukey). The mean differences were compared within and across the extruded composite flours for peroxide value using two-way ANOVA. Finally, the multiple comparisons were done using the post hock test (HD-Tukey). All statistical analyses were considered significant at $p < 0.05$.

3.0 Results

3.1 Proximate composition for the raw materials (flour)

The results from Table 2 show a descriptive presentation of the proximate composition of the raw ingredients used to develop extruded composite flour. The proximate composition of cassava flour, maize flour, and malted sorghum flour exhibited crude protein of 1.4%, 6.8%, 9.3% and crude fat of 0.2%, 0.7% and 2.6% respectively. The proximate composition reported for maize flour was comparable to that analyzed by Licata et al. (2013). The malted sorghum flour analyzed in this study indicated higher protein and fat content in comparison to that studied by Licata et al. (2013), which reported lower content of protein (8.94%) and fat (2.6%) in sorghum flour. However, malted sorghum flour analyzed in our study had lower content of fat, ash and crude fibre except for protein content (9.3%) and carbohydrate content (84.2%) when compared to a previous study by Mohamed et al. (2019), which reported a higher content of fat (9.26%), ash (2.01%) and crude fibre (1.4%) except for protein content (4.8%) and carbohydrate content (70.55%).

The higher protein content observed in malted sorghum could be attributed to the malting process applied to sorghum grain, which resulted in the activation of hydrolytic enzymes present in the grain, as ascertained by Ayernor and Ocloo (2007). The activity of this hydrolytic enzyme results in some biochemical modification and structurally modification which leads to the breakdown of protein, fat and carbohydrate component into their simple molecules (Adekunle, 2012; Swami et al., 2013). The protease, one of the activated hydrolytic enzymes, causes protein degradation and improves bioavailability (Pyler and Thomas, 2000). Thus, the use of malted sorghum in developing composite flour was of nutritional benefit as the malting process improved the nutritional quality of malted sorghum flour compared to unmalted sorghum.

Table 2: Proximate composition (%) for the dried raw materials (flour) used for the development of extruded composite flour

Proximate component	Maize flour	Long-horned grasshopper	Cassava flour	Malted-Sorghum flour
Moisture	5.9±0.15	2.4±0.08	1.4±0.04	2.6±0.11
Crude fibre	1.8±0.39	0.1±0.13	1.1±0.01	0.8±0.01
Crude ash	0.3±0.05	1.8±0.02	2.2±0.07	1.3±0.04
Crude protein	6.8±0.09	39.0±1.24	1.4±0.17	9.3±0.19
Crude fat	0.7 ±0.01	54.9±1.65	0.2±0.04	2.6±0.02
Carbohydrate	84.5 ±0.27	1.8 ±0.54	93.7 ±0.12	85.3 ±0.13
Dry matter	94.1±0.15	97.6±0.08	98.6±0.004	97.4±0.11

Values are mean±SD (n=3); descriptive presentation of proximate composition of raw ingredients. Carbohydrate content was estimated by the difference method.

The crude protein and fat contents of long-horned grasshoppers obtained in this study were higher than those reported by Kinyuru et al. (2010), who observed a 35.3% to 37.1% protein content range in the long-horned green and brown grasshoppers, respectively while the fat content was between 46.2% to 48.2% for green and brown grasshopper respectively. However, the reported fat and protein nutrients agree with the reported findings of Ssepuuya et al. (2019). The protein and fat content of *Ruspolia differens* was 34.2% to 45.8% and 42.2 to 54.3%, respectively. Additionally, Ssepuuya (2019) highlighted that the nutritional composition of edible insects could be affected by the geographical location of sourcing and the swarming season, which means that the observed high amount of protein and fat content could be attributed to harvesting time and the geographical sourcing area of long-horned grasshopper. These two factors were suggested to affect the nutrition content of edible insects as they influence the characteristics of the insect (*R. differens)* nutritional composition, resulting from the grass they feed as soil type influences vegetation (Joy et al., 2015). In addition, insects' availability varies with weather and harvesting season (Anyuor et al., 2021). The amount of protein in this study corresponded to that of *Ruspolia nitidula*, which was reported to have a protein content range from 36% to 40% (Ssepuuya et al., 2017). Therefore, incorporating each ingredient in the development of extruded composite flour is justified because every component plays an individual role in enriching the composition of the flour. Thus, contributing to the overall nutritional quality of the developed extruded composite flour.

3.2 Proximate composition for extruded composite flour enriched with long-horned grasshopper

In Table 3, results show a significant difference at ($p<0.05$) in proximate composition between extruded composite flour enriched with the long-horned grasshopper (ECF 5, ECF 15, and ECF 25) and extruded composite flour without long-horned grasshopper (ECF 0), except that of crude ash content ($p=0.1040$). The trends observed in proximate composition results were similar for moisture, crude fibre, crude ash, crude protein and crude fat. These mentioned proximate components tended to increase with increased content of long-horned grasshopper in composite flour, except for carbohydrates and dry matter content. The higher moisture contents were observed in extruded composite flours enriched with the long-horned grasshopper, ECF 25 (2.8%), followed by ECF 15 (2.1%) and then in ECF 5 (1.8%) when compared to ECF 0, which showed lower moisture content (0.5%). The findings for crude fibre content showed to differ significantly at ($p < 0.001$) between extruded composite flour with the long-horned grasshopper (ECF 5, ECF 15, and ECF 25) and extruded composite flour without long-horned grasshopper (ECF 0). The higher crude fibre contents were observed in ECF 25 (1.2%), followed by ECF 15 (1.0%) while lower in ECF 5 (0.9%) with comparison to ECF 0 (0.8%).

Table 3: Proximate composition and energy for different formulations of extruded composite flour enriched with long-horned grasshopper

Proximate component	ECF 0	ECF 5	ECF 15	ECF 25	F-value	P-value
Moisture	0.5±0.07^{a}	1.8±0.18^{b}	2.1±0.03^{c}	2.8±0.08^{d}	263.9	<0.001***
Crude fibre	0.8±0.01^{a}	0.9±0.0^{b}	1.0±0.001^{c}	1.2±0.01^{d}	1074	<0.001***
Crude ash	1.2±0.02^{a}	1.2±0.08^{a}	1.3±0.10^{a}	1.5±0.21^{a}	2.863	0.1040^{NS}
Crude protein	8.1±0.19^{a}	9.2±0.23^{b}	11.5±0.13^{c}	12.9±0.16^{d}	433.3	<0.001***
Crude fat	1.6±0.49^{a}	1.7±0.06^{b}	6.0±0.17^{c}	6.8±0.15^{d}	309.6	<0.001***
Carbohydrate	87.8±0.61^{d}	85.2±0.10^{c}	78.1 ±0.32^{b}	74.8±0.11^{a}	825.2	<0.001***
Dry matter	99.5±0.04^{d}	98.2±0.18^{c}	97.9±0.03^{b}	97.2±0.08^{a}	263.9	<0.001***
Energy (Kcal)	397.27±2.48^{b}	392.67±0.82^{a}	411.64±0.82^{c}	413.51±0.66^{c}	162.9	<0.001

Values are mean±SD (n=3); means with different superscript in row are significantly different ($p < 0.05$).

NS means not significant at $p > 0.05$ and *** means significant at $p < 0.001$. Carbohydrate content was estimated by the difference method. The energy values obtained by adding the crude protein, crude fat, and total carbohydrate content were multiplied by 4.00, 9.00 and 4.00 Kcal, respectively.

In terms of crude protein and fat content, the ECF 0 sample had a lower amount of these two components when compared to other treatments (ECF 5, ECF 15, and ECF 25). The protein and fat content percentage composition in ECF 0 were (8.1%) and (1.6%), respectively. The protein and fat contents for extruded composite flour were increased with an additional percentage ratio of edible insect to the composite flour, from ECF 5 to ECF 25. Since long-horned grasshopper was observed to contain higher crude fat and protein content than all raw ingredients analyzed in product development, thus justified the use of edible insects in enriching protein and energy value content. The values of protein content of ECF 5, ECF 15 and ECF 25 were increasing from 9.2%, 11.5% to 12.9% respectively, while fat content increased from 1.7%, 6.0% to 6.8%. In all three treatments, these components were observed in ECF 25, followed by ECF 15 and lower in ECF 5. This observation is similar to that observed in snacks made of wheat flour enriched with larvae of yellow mealworms powder, where the increase in the percentage of mealworms increased the crude protein content from 11.8 to 20.4g/100g and lipid content from 0.9 to 5.4g/100g while decreasing in carbohydrate content from 8.11 to 66.0g/100g. However, these reported findings (values) are lower than our findings, mainly for crude fat and carbohydrate contents except for ECF 5 (Azzollini et al., 2018).

In comparison to the previous study, all extruded composite flour enriched with long-horned grasshopper (ECF 5, ECF 15, and ECF 25) contained lower content of the crude ash and fibre than that observed in the composite instant porridge formulated from millet and termites (*Macrotermes natalensis*) which observed higher crude ash (2%) and fibre (2%) content (Musundire et al., 2021). These authors reported lower fat content (4%) and protein (11.5%) compared to ECF 15, but lower than that observed in ECF 25. The crude fat content observed in this study was higher than that observed by Ramírez-rivera et al. (2021) and which reported a range of 0.57 to 4.11g/100g in the nixtramized maize extruded snack enriched with 40% grasshopper meal. The crude protein and fat content observed in this study were lower than those reported in a previous study by Mmari et al. (2017) on the use of grasshopper in enriching the nutritional content of complementary food. Mmari et al. (2017) reported a high range of protein (21.4 to 26%) and fat content (14.3 to 19.3) in their flour formulation, which contained grasshopper (15 to 25%), soybean (35 to 40%) and sweet potatoes (40 to 50%).

Additionally, the ECF 0 (without insects' content) had a higher amount of carbohydrate content (87.7%) and dry matter content (99.5%) than ECF 5, ECF 15 and ECF 25 in the current study. The carbohydrate and dry matter contents decreased in the content of extruded composite flour enriched with long-horned grasshopper meal, whereby the low amount was contained in ECF 5 (85.2% and 98.2%), while the lower composition observed in ECF 15 (78.1% and 97.9%) and the lowest value was in ECF 25 (74.8% and 97.2%) respectively. However, the carbohydrate content reported in the current study was higher than that observed in the composite instant porridge formulated from millet and termites (*Macrotermes natalensis*) conducted by Musindire et al. (2021), which reported the carbohydrate contents of 73%. Compared to the previous studies on the formulation of complementary food, our finding shows higher carbohydrate content than those reported by Forsido et al. (2019). They reported 62.4% of the carbohydrate content in composite flour formulated from soybean, oats, and linseed. Also, the carbohydrate contents observed in all treatments (ECF 5, ECF 15 and ECF 25) were higher than that observed by Mmari et al. (2017) in three formulated composite flours (soybean-sweet potato enriched with grasshopper), whereby the higher carbohydrate content reported was 52.8%.

The total energy value (EV) from protein, fat and carbohydrates obtained in treatment ECF 15 and ECF 25 were higher than that observed in ECF 0 (397.27cal/100g), while lower content was observed in ECF 5. The highest EV was seen in ECF 15 (411.64Kcal/100g), followed by 25 (413.51Kcal/100g), and the lowest EV was observed in ECF 5 (392.67Kcal/100g), as presented in Table 3. The total energy value obtained in this study was higher when compared to that observed in the composite instant porridge formulated from millet and termites (*Macrotermes natalensis*) conducted by Musindire et al. (2021), which reported an energy value of 353kcal. Moreover, the energy value in our study was higher in comparison to other food enrichment studies by Sumath et al. (2009) and Forsido et al. (2019). They indicated energy values of 388 and 392.5 Kcal/100g, respectively. The energy derived from protein in extruded composite flour formulations was within the recommended safety level. According to Codex guidelines on the formulation of complementary foods for older infants and young children (12 to 36 months), the energy from protein should be not less than 6% of the total energy from the product and typically should not exceed 15% (Codex Alimentarius Commission, 2013). Also, the formulated extruded composite flour meets the standard of KS 2515:2014 for Formulated complementary foods for older infants and young children (GAIN, 2020), and that of TBS standard No 72:2013, which specifies the requirements for processed cereal-based foods for infants and young children (Tanzania Bureau of Standards, 2013).

3.3 Minerals content of extruded composite flour enriched with long-horned grasshopper

The results in Table 4 reported no significant difference in Ca content ($p=0.177$) between extruded composite flours without long-horned grasshopper (ECF 0) and extruded composite flours enriched with long-horned grasshopper (ECF 5, ECF 15 and ECF 25). Findings indicated no significant difference at ($p>0.05$) in mineral contents (Ca, Mg, Fe and Zn) between ECF 0 and ECF 5. The Mg content between ECF 0 and treatment ECF 15 and ECF 15 differed significantly at ($p=0.0089$). The higher Mg content was observed in ECF 0 (363.4 mg/kg) when compared to ECF 15 (306.6 mg/kg) and ECF 25 (279.3 mg/kg) ($p<0.05$) which had lower Mg contents. However, there was no significant difference at ($p>0.05$) in Mg content between ECF 0 and ECF 5 (309.5 mg/kg).

Table 4: Mineral composition (mg/kg) for different formulations of extruded composite flour enriched with long-horned grasshopper

Mineral	ECF 0	ECF 5	ECF 15	ECF 25	F-value	P-value
Ca	361.9 ± 54.29^{a}	369.3 ± 19.45^{a}	414.9 ± 8.16^{a}	399.7 ± 13.09^{a}	2.113	0.177^{NS}
Mg	363.4 ± 22.99^{b}	$309.5 \pm 19.41^{a,b}$	306.6 ± 8.82^{a}	279.3 ± 29.87^{a}	7.918	0.0089^{**}
Fe	158.2 ± 11.08^{a}	191.2 ± 9.48^{a}	262.1 ± 4.13^{b}	353.7 ± 21.57^{c}	1337	$<0.001^{***}$
Zn	47.8 ± 0.46^{a}	$52.5 \pm 2.41^{a,b}$	$53.0 \pm 0.87^{a,b}$	56.9 ± 3.24^{b}	9.657	0.0049^{**}

*Values are mean±SD (n=3); means with different superscript in row are significantly different ($p < 0.05$). NS means not significant at $p > 0.05$. *** means significant at $p < 0.001$ and **means significant at $p < 0.01$.*

The content of Fe observed in treatment ECF 15, and ECF 25 showed a significant difference at ($p<0.001$) compared to ECF 0. The Fe content estimated in ECF 25 (353.7 mg/kg) and ECF 15 (262.1mg/kg) were higher than that observed in ECF 0. Nevertheless, there was no significant difference at ($p>0.05$) in Fe content between ECF 0 (158.2 mg/kg) and ECF 5 (191.2 mg/kg). All formulations of extruded composite flour enriched with long-horned grasshopper showed higher mineral contents of Ca, Fe and Zn than that reported by Musindire et al. (2021) in the composite instant porridge formulated from millet and termites (*Macrotermes natalensis*), which reported the lower contents Ca (161mg/kg), Fe (44.3mg/kg), and Zn (8.1mg/kg). Our findings on iron content correspond to that of (Irungu et al., 2018), which indicated that the increase of adult cricket meal in the composition of the extruded aquafeeds was observed to increase iron content in the composition. Likely, as observed in our study, long-horned grasshoppers possess high iron content, as previously documented in the literature (Kinyuru et al., 2010b; Mmari et al., 2017). Additionally, the Fe content estimated in this study was higher than the value reported by Tirhas et al. (2015). They optimized complementary porridge formulated by sweet potato-soybean-moringa composite porridge and estimated Fe content was between 169 to 216 mg/kg. This shows that the inclusion of long-horned grasshopper provides the product with higher Fe content in comparison to other sources of protein. Results show that, there was a significant difference at ($p<0.05$) in Zn contents between ECF 0 (47.8 mg/kg) when compared to that ECF 25 (56.9 mg/mg). The extrusion process is said not to influence Zn content and the bioavailability of the resulted extruded product (Guy, 2001; Irungu et al., 2018). Therefore, the increased Zn content of extruded composite flour is expected from the long-horned grasshopper added to the composition.

3.4 Antinutrients composition of extruded composite flour enriched with long-horned grasshopper

The results presented in Table 5 show that phytate content between the extruded composite flour without long-horned grasshopper (ECF 0) and extruded composite flour enriched with long-horned grasshopper (ECF 5, ECF 15 and ECF 25) are not significantly different at ($p = 0.141$). However, the phytate content observed in this study was lower than that observed in malted instant complementary food made from blended malted sorghum and cooked pigeon peas (Kinyua et al., 2016). But the tannin content extracted between extruded composite flours with the long-horned grasshopper and extruded composite without long-horned grasshopper was shown to differ significantly at ($p=0.00001$). All treatments (ECF 5, ECF 15 and ECF 25) showed a slightly higher amount of tannin content (mg CE/g) when compared to values in ECF 0. The existing variations could be associated with the inclusion of long-horned grasshoppers in composite flour, considering the previous literature reported that phytates and tannins were detected in long-horned beetle, grasshoppers, and meal bugs and termites (Adeduntan, 2014). The value for tannins determined in this research was within the standard safety levels, within 1.5 to 2mg/g (WHO, 2003).

Table 5. Tannin content and Phytate content for a different formulation of extruded composite flour enriched with long-horn grasshopper

Anti-nutrient	ECF 0	ECF 5	ECF 15	ECF 25	F- value	P-value
Phytate (mg/g)	83.31±4.72^{a}	70.46±7.50^{a}	76.30±1.86^{a}	78.05±7.54^{a}	2.423	0.141
Tannin (mg CE/g)	0.004±0.0003^{a}	0.007±0.0001^{b}	0.012±0.001^{c}	0.011±0.001^{c}	55.28	0.00001***

Values are mean±SD (n=3); means with different superscript in row are significantly different ($p < 0.05$) and *** means significant at $p < 0.001$.

Tannins can bind to proteins such that the formation of tannin-protein complexes may cause digestive enzymes to inactivate, thus, affecting protein digestibility (Ifie and Emeruwa, 2011). Furthermore, the presence of phytates may affect the bioavailability of minerals such as Fe, Zn, Mg and Ca by binding them and forming insoluble complexes (Akond et al., 2011). Considering the safe level from the values established in the developed product for both tannin and phytates were at an acceptable level, thus suggesting that the extruded composite porridge could be consumed without fear of harming the consumer and would not result in micronutrient deficiency in the individual consuming rather hoped to provide nutritional benefits with safe antinutritional properties which expected to not interferes with protein bioavailability and digestibility.

3.5 Functional properties

3.5.1 Bulk density (BD)

Bulk density describes the degree of puffing experienced by the material as it exits the extruder (Igual et al., 2020). The results in Table 6 show a significant difference in bulk density at (p=0.0002) between ECF 0 and ECF 15. Treatment ECF 15 had lower bulk density of (0.5561g/cm3) than that observed in ECF 0 (0.6461g/cm3). The bulk density between ECF 0 and both treatment ECF 5 (0.61g/cm3) and ECF 25 (0.6761g/cm3) showed not to differ significantly (p>0.05) with the ECF 0. In this study, the bulk density values were observed to decrease with the increasing content of edible insects for ECF 5 and ECF 15. However, an unusual observation was made on the sample with a high insect percentage, ECF 25, as it was observed to have a high bulk density value, which was higher than that of the ECF 0 (without long-horned grasshopper), treatment ECF 5 and ECF 15. Probably, this could be associated with the processing temperature during extrusion as the ECF 25 product was processed with the lowest temperature (128 ^{0}C) in zone I where cooking takes place.

The bulk densities of extrudates were significantly affected by the processing condition during extrusions, such as barrel temperature and moisture content (Yu et al., 2013). The processing temperature could have decreased the extent of gelatinization of extrudate product, leading to low swelling and reduced volume of the extrudate, and consequently increased the bulk density (Yu et al., 2013). Another explanation for this observation could be the thermal degradation of protein, which hindered the swelling of starch, resulting in low expansion and low viscosity, and weakening of structure during extrusion, leading to an increase in the bulk density of extrudates (Niu et al., 2020). The bulk density obtained in this study is higher than that of ready to eat extruded nixtamalized maize-based snacks enriched with long-horned grasshopper reported by Cuj-Laines et al. (2018), whereby the bulk density of ready-to-eat extruded nixtamalized maize-based snacks enriched with long-horned grasshopper was observed to have BD values range of 0.47 to 1.17 kg/m3 with the highest obtained at processing temperature 150 ^{0}C with a feed moisture content of 20g/100g and 20g/100g of grasshopper meal portion. The high bulk density of ECF 0 and the decrease of bulk density with additional long-horned grasshopper amount in flour composition indicate that the complex's starch fraction contributes more to BD than protein, as starch could be organized into structures with high density (Niu et al., 2020). Additionally, the low bulk density obtained in this study is potential from a nutritional point of view because low bulk density promotes easy food digestibility (Osundahunsi and Aworth, 2002).

Table 6: Functional properties for different formulations of extruded composite flour enriched with long-horned grasshopper

Properties	ECF 0	ECF 5	ECF 15	ECF 25	F-value	P-value
Bulk density (g/cm3)	0.64±0.03^{b,c}	0.61±0.01^{b}	0.55±0.01^{a}	0.67±0.02 ^{c}	25.07	0.0002***
Water absorption index (g/g)	3.51±0.16^{a}	3.60±0.02^{a}	4.08 ±0.29^{a,b}	5.12±0.90^{b}	7.193	0.0117*
Water solubility index (%)	0.50±0.03^{b}	0.43±0.06^{b}	0.21±0.002^{a}	0.14±0.04^{a}	72.67	<0.001***
Oil absorption index (ml/g)	0.86 ±0.15^{b,c}	1.12±0.14^{c}	0.59±0.10^{b}	0.16±0.05^{a}	33.89	0.0001***

Values are mean±SD (n=3); means with different superscript in row are significantly different (p < 0.05). *** means significant at $p < 0.001$ and * means significant at $p<0.05$.

3.5.2 Water absorption index (WAI) and water solubility index (WSI)

Water absorption index (WAI) and water solubility index (WSI) represent the hydration properties of extruded products, that is, their behaviour in the presence of water. WAI describes the amount of water held by extrudates, which mainly trigger the gelatinization and melting of molecules. These properties can be used as indicators of gelatinization (Rodríguez-Miranda et al., 2011). WSI determines the number of molecules released in water from the extrudates arising from starch, protein and lipids degradation into low molecular weight compounds (Patil and Kaur, 2018; Nura et al., 2011).

Results in Table 6 indicated no significant difference in water absorption index (WAI) at (p=0.0117) between ECF 0 and ECF 25. The WAI observed in ECF 25 (5.12g/g) was higher than in ECF 0 (3.51g/g). Whereas, ECF 5 and ECF 15 were not significantly different from the WAI of ECF 0 (p>0.05). All three treatments, ECF 5, ECF 15 and ECF 25, had higher value of WAI when compared to the ECF 0. This study shows that, WAI generally increased with an increase in insect content in flour composition, thus suggesting that protein denaturation occurs at high feed moisture conditions thereby influencing hydration properties of extruded composite flour as ascertained by Kumar et al. (2015). The significant differences in WAI could result from protein denaturation and starch gelatinization due to extrusion (Mali et al., 2010). Moreover, the WAI observed in this study was lower than that indicated ready to eat extruded nixtamalized maize-based snacks enriched with long-horned grasshopper conducted by Cuj-Laines et al. (2018), which was between 4.17 to 6.84 g/g obtained at 150^{0}C with a feed moisture content of 20g/100g. However, the WAI value indicated in this current study was lower than that reported by Muhangi et al. (2019), which varies from 6.49 to 8.65 at extrusion processing of 90 ^{0}C with 11 % grasshopper meal and feed moisture content of 25%. The difference in WAI values could be related to the difference in extrusion processing parameters and raw materials used in these two studies. Then, the low WAI values observed in this study implied restricted water availability for the starch granule gelatinization due to the enhanced compact structure. Also, the higher feed moisture content and extrusion processing temperature employed by Cuj-Laines et al. (2018) facilitate greater starch gelatinization, more water retention capacity, and thus result in higher WAI. The other potential reason for the difference in WAI could be explained by adding long-horned grasshopper meals in extrudates. The previous authors observed higher WAI in extruded composite flour without grasshopper meal. Thus, the low WAI reported in this study could be related to the presence of hydrophilic balance of protein in the mixture, which cause a change in the degree of protein denaturation (Singh et al., 2007). High WAI is mentioned as preferable property in food especially that will have to be reconstituted in water before consumption, such as instant porridge (Pelembe et al., 2002). Since it gives the product ability to absorb water, as ascertained by (Ijarotimi and Ashipa, 2005). The higher WAI observed in extruded composite enriched with long-horned grasshopper compared to that of the extruded composite without long-horned grasshopper suggested that, the addition of edible grasshopper provided the composite flour with the desirable property of the porridge as was tended to increase the WAI, thus upon preparation will mix easily.

Results in Table 6 indicated a significant difference at ($p>0.05$) in the water solubility capacity (WSI) between ECF 0 and ECF 5. In contrast, WSI of ECF 0 (0.50%) was shown to differ significantly with both ECF 15 (0.21%) and ECF 25 (0.14%) at ($p <0.001$). These two treatments were observed to have lower WSI values when compared to ECF 0. Again, the results obtained in this study on WSI were lower than those stated by Muhangi et al. (2019), which varied from 5.88 to 16.004% at extrusion processing of 150 ^{0}C with 11 % grasshopper meal and feed moisture content of 18%, this indicated extensive starch dextrinization. The findings suggested that WAI and WSI were significantly affected by adding a long-horned grasshopper in the formulation. There was an increase in WAI and decrease of WSI with the incorporation of insect meal in composite flour composition, which caused less solubilization of the matrix components but increased hydration properties of the flour during extrusion. Our finding agrees with that reported by Igual et al. (2020), when crickets were extruded with cornflour to enrich the protein content of corn extrudates, where it was shown that WAI increased with the supply of protein whereas WSI decreased. In addition to that, the observed trend can be attributed to the processing method applied to develop composite flour, as the extrusion process was demonstrated to cause chemical and structural changes, thus, contributing to the increase in the hydration property of different products (Awolu et al., 2015; Hagenimana et al., 2006). Furthermore, from our findings, the values for WAI and WSI observed in extruded composite flour enriched with long-horned grasshopper were lower when compared to that observed in an insect-based extruded product formulated with cornflour with 25% of *Hermetia illucens,* which contained higher values for WAI (7.4%) and WSI (3.4%) as according to (Alam et al., 2019).

3.5.3 Oil absorption index (OAI)

Oil absorption index or capacity (OAI) is among the functional properties of extruded product, representing the amount of fat absorbed per gram of protein. OAI is an essential parameter in expressing the change of mouth feel of the extruded product due to the retention of flavour in the extrudates. From Table 6, the oil absorption index of ECF 5 and ECF 15 showed not significantly different ($p>0.05$) when compared to the ECF 0 (without insect). Nevertheless, there was a significant difference ($p<0.0001$) between ECF 0 and ECF 25, whereby the OAI of ECF 25 was higher than that of ECF 0. In this study, it was observed that the oil absorption capacity decreased with an increase in insect proportion in the extruded flour. Nevertheless, our study reported lower values for OAI observed extruded composite flour enriched with long-horned grasshopper was lower when compared to that previous for extruded product enriched with edible insects, formulated from millet and termites (*Macrotermes natalensis*) and cornflour with 25% of *Hermetia illucens* conducted by Musindire et al. (2021) and Alam et al. (2019) respectively. This two-study reported a higher OAI of 1.6% (Musindire et al., 2021) and an OAI of 1.3% (Alam et al., (2019) when compared to the current study. This could be attributed to the high percentage content of edible insects used to formulate the resulted extruded product by these studies. The main factor contributing to oil absorption capacity is protein concentration and conformational characteristics. Both hydrophilic and hydrophobic properties in protein influence their interaction with water and oil in foods. The oil absorption index depends on the availability of non-polar amino acids. Hydrophilic and polar amino acids

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reduce oil absorption capacity (Brishti et al., 2017). The observed decrease in oil absorption index in the current study corresponded to that conducted by Alonso et al. (2000), who highlighted that extrusion cooking tends to decrease the number of available sites in the extruded kidney beans, thus significantly reducing oil absorption capacity.

3.6 Sensory analysis and Consumer acceptability test

3.6.1 Colour

From Table 7, the hedonic rating score for the extruded composite porridge enriched with the long-horned grasshopper, ECF 15 and ECF 25, showed a significant colour difference compared to the extruded composite porridge without long-horned grasshopper (ECF 0). The treatment, ECF 15 and ECF 25 showed a higher hedonic score of 7.75 (like moderately) and 6.83 (like slightly), respectively, compared to ECF 0. Results for ECF 0 and ECF 5 with a hedonic score of 5.98 and 6.00, respectively, showed no significant difference at a 5% confidence level. The panellists showed their preference for brown (deep) colour than light or bright colour. The lighter colour had no addition of edible insect in their composition, ECF 0. Also, less preference in colour was observed in ECF 5 as this product was visually similar in terms of colour to ECF 0. The hedonic score for colour suggests that the addition of edible insects impacted the colour of extruded composite porridge positively and thus improved consumer acceptability. The result on colour attribute observed in this study agrees with that of Ramírez-rivera et al. (2021); these authors reported the change of colour from whiteness index (for nixtramized extruded maize snack) to browning index (for nixtramized extruded maize snack enriched with grasshopper meal). The colour change is probably contributed by the nature of the long-horned grasshopper to rich in protein content. Thus, extrusion reaction such as the Maillard reaction is likely to happen (Gulati et al., 2020). The addition of grasshopper meal *(Ruspolia differens)* extruded maize snacks increased protein content and browning extrudates due to the increased extent of Maillard reaction (Kisakye, 2019).

Table 7: Sensory scores of the sensory evaluation and acceptability for extruded composite porridge

Sensory attributes	ECF 0	ECF 5	ECF 15	ECF 25
Colour	5.98±1.14^{a}	6.00±1.21^{a}	7.75±0.86^{c}	6.83±1.21^{b}
Aroma	7.15±1.15^{b}	7.55±0.95^{b}	5.98±1.57^{a}	5.60±1.54^{a}
Texture	7.30±1.21^{b}	7.65±1.15^{b}	7.45±1.22^{b}	6.28±1.43^{a}
Taste	7.02±1.40^{b}	7.30±1.37^{b}	6.35±1.31^{a}	5.77±1.48^{a}
Overall acceptability	7.02±1.16^{b}	7.60±1.12^{b}	6.88±1.12^{b}	5.93±1.33^{a}

The hedonic scores for 60 panellists. Scores with a different superscript in a row are significantly different ($p < 0.05$). A nine-point hedonic scale: 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely.

3.6.2 Aroma

Results in Table 7, show that the aroma preferences level between ECF 0 with a score of 7.15 (like moderate) and ECF 5 with a score and 7.55 (like moderate) did not significantly differ at a 5% confidence level. Nevertheless, most panellists reported a significant difference in aroma attribute at a 5% confidence level between ECF 0 and ECF 15 with a scale of 5.98 (Neither like or dislike) and likely, between ECF 0 and ECF 25 (5.60 (Neither like or dislike). These two treatments (ECF 15 and ECF 25) were observed to have a lower hedonic score in aroma attribute when compared to the ECF 0. Additionally, the less preference in the aroma of the extruded composite porridge with long-horned grasshopper could be attributed to the fat content as they are a medium for flavour components. The addition of long-horned grasshopper in composite flour affects the aroma attribute, possibly due to the release of aroma compounds from edible insects during extrusion. The fishy smoke aroma observed by most panellists was similar to that observed in extruded brown rice-tapioca starch enriched with locusts (Tao et al., 2017). The processing technique applied to the edible insect is assumed to contribute to the product’s aroma. There is a need to study the impact of processing methods at different temperatures and the effect on the aroma component based on the temperature applied during processing.

3.6.3 Texture

The results of hedonic rating for the texture of extruded composite porridge with long-horned grasshopper were between like slightly (6.28) to like moderately (7.65). Results in Table 7 show that, there is no significant difference ($p>0.05$) in texture preferences between ECF 0 (7.30) and ECF 5 (7.65) also between ECF 0 and ECF 15 (7.45) at ($p>0.05$). However, there was a significant difference ($p<0.05$) in texture preference between porridge for ECF 0 and ECF 25. The hedonic score for texture attributes in ECF 25 was lower when compared to ECF 0. Based on panellists’ preference, ECF 5 (7.65%) was the most preferred, whereas product ECF 25 (6.28) was the least preferred. Thus, it is evident that the preference level is positively associated with the amount of long-horned grasshopper added to the composition.

3.6.4 Taste

The panellist’s preference for the taste of the extruded composite porridge samples is presented in Table 7. The hedonic score of extruded composite porridge with long-horned grasshopper was between 5.77 (Neither like nor dislike) to 7.30 (like moderate). There was a significant difference ($p<0.05$) between ECF 0 and ECF 15, and likely, between ECF 0 and ECF 25. The lower hedonic score was found in ECF 25 (5.77), followed by 15 (6.35). Based on the panellists’ preference, the hedonic score for taste preference observed between ECF 5 (7.30) and ECF 0 (7.30) showed no significant difference at ($p>0.05$). This study suggests that the addition of edible insects to the extruded composition of flour impacts the taste attributes of the product, and the preference varies with consumer evaluation. The most preferred porridge treatment had a small amount of edible insect (ECF 5) in the composition. Moreover, the panellist’s preference for taste resembled that of the aroma attribute. This indicates that panellists could differentiate the two attributes; taste from the aroma.

3.6.5 Overall acceptability

Results indicated that, there was no significant difference at ($p>0.05$) in consumer acceptability of the porridge samples between treatment (ECF 5 and ECF15) when compared to ECF 0, which had a score of 7.02 (like moderately). However, the result shows that, there was a significant difference at ($p<0.05$) in the overall acceptability of the extruded composite porridges between

ECF 0 and ECF 25, this treatment (ECF 25) had lower overall acceptability when compared to the control. Based on panellists' acceptability on the extruded composite porridge, results show that ECF 5 was rated highest for the overall acceptability with the hedonic score of 7.60 (like moderately) followed by a score of 6.88 (like slightly) for ECF15 and the least on rating score was ECF 25 with a score of 5.93 (Neither like or dislike). The higher hedonic score on overall acceptability observed in ECF 5 may be attributed to some resemblance with ECF 0, as panellists were somehow familiar with the porridge ingredients, unlike the samples that contained high insect percentage as their tastes and aroma attribute was new. Less preference for the product enriched with grasshopper could be associated with awareness of the presented product. Most panellists had never consumed insects or insect products before, thus affecting product acceptability since repeated product consumption could increase acceptability. Panellists were unfamiliar with it compared to ECF 0, which most people commonly consume.

It is worth noting that there was no significant difference at ($p>0.05$) in the overall acceptability of ECF 0 to that of ECF 5 and ECF 15. The product was accepted with the recommendation of improving the taste and aroma of the product. The recommended formulation based on panellist preference is ECF 15% (extruded composite flour with 15% of edible insect), rated with hedonic high for colour and texture. However, this product (ECF 15%) requires some modification to improve taste and aroma attributes; doing so is expected to increase product acceptance. Therefore, enrichment of the product should consider all sensory parameters to meet consumer acceptability as the addition of insects' percentage on the composition has an impact on sensory attribute but mostly when a high amount of insect is incorporated composition, as was shown that 25% addition of insect in design tends to reduce the consume acceptability based of the extruded composite porridge. The study findings corresponded to the hedonic score for colour, flavour, and overall acceptability observed in the formulation of extruded maize-sorghum flour fortified with amaranth grains with a hedonic score of 5.7 to 7.4 (Sanya et al., 2020).

3.7 Microbial quality of extruded composite flour enriched with long-horned grasshopper

From figure 1, based on the results for TVC in the initial test conducted after product development (0 weeks), results show that there was no significant difference at ($p>0.05$) in total viable count between extrude composite flour enriched with long-horned grasshopper (ECF 5, ECF 15 and ECF 25) and extrude composite flour without long-horned grasshopper (ECF 0). When compared to ECF 0 (5×10^3 cfu/g), the higher TVC was observed in ECF 15 (7×10^3 cfu/g and the lower count in both ECF 5 (2×10^3 CF cfu/g) and ECF 25 (4×10^3 cfu/g). However, ECF 25 counts were comparable to ECF 5 and ECF 15. After three weeks of storage under room temperature, the ECF 0 showed no significant difference ($p>0.05$) to TVC observed in ECF 25 but significantly differed at ($p<0.05$) to both ECF 5 and ECF 15. In comparison to ECF 0, the highest TVC (cfu/g) was observed in ECF 15, while the lowest was found in ECF 5. Nevertheless, there were no significant differences ($p>0.05$) between ECF 25 (17×10^3 cfu/g) and ECF 15 (19×10^3 cfu/g), while ECF 5 (7×10^3 cfu/g). In 6 weeks, the results showed a significant difference at ($p<0.05$) between ECF 0 and treatments (ECF 5, ECF 15 and ECF 25). After nine weeks of storage, observations showed a significant difference at ($p<0.05$) between extruded composite flour with the long-horned grasshopper and extruded blended flour without the long-horned grasshopper. Compared to the ECF 0, a higher total viable count was seen in samples ECF 15 and ECF 25 than the counts in ECF 5.

From Figure 2, yeast and mould count (YMC) results showed no significant difference ($p>0.05$) between extruded composite flour enriched with the long-horned grasshopper and extruded composite flour without long-horned grasshopper as per initial time (0 weeks). A similar trend was observed after three weeks of storage, with no significant difference ($p>0.05$) in YMC between extruded composite flour enriched with the long-horned grasshopper and extruded composite flour without the long-horned grasshopper. The higher count was in ECF 25 (6×10^3 cfu/g) and the lower count in ECF 5 (4×10^3 cfu/g) compared to the ECF 0. In week 6 of storage, the YMC in ECF 0 showed not to differ significantly ($p>0.05$) from that observed in ECF 5, while it differed significantly at ($p<0.05$) from that shown in ECF 15 and ECF 25, these two treatments were observed to compare each other. In comparison to ECF 0 (11×10^3 cfu/g), the higher total YMC was counted in ECF 15 (17×10^3 cfu/g) and ECF 25 (16×10^3 cfu/g), and lower count was in ECF 5 (11×10^3 cfu/g). After nine weeks of storage, only ECF 5, which had a lower total YMC (18×10^3 cfu/g), showed no significant difference ($p>0.05$) to ECF 0 (15×10^3 cfu/g). While a significant difference at ($p<0.05$) in YMC was observed between extruded composite flour and long-horned grasshopper and without long-horned grasshopper mainly, ECF 15 (23×10^3 cfu/g) and ECF 25 (29×10^3 cfu/g) whereby, these treatments had higher total yeast and mould count than that observed in ECF 0.

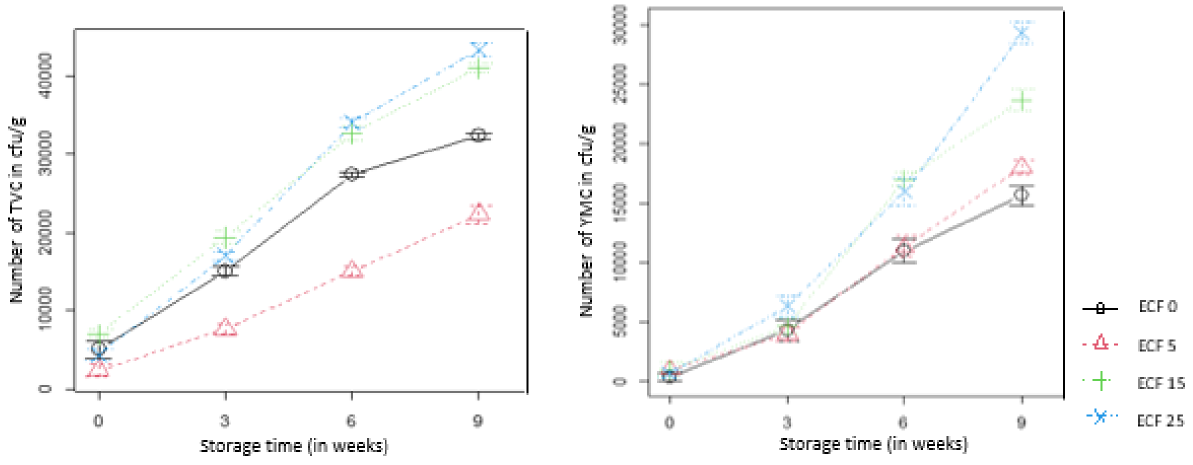

Figure 1a

Figure 1b

Figure 1a and 1b: Shows the trendlines of the TVC and YMC (cfu/g) in extruded composite flour samples during storage respectively

Microbial analyses were carried out to assess both the safety and quality of the extruded composite flour samples. The cfu/g of the total viable count (TVC) observed throughout the storage in extruded composite flours was within the acceptable limit of 10^4 (Kenya Bureau of Standards, 2020). The cfu/g of yeast and mould count (YMC) in extruded composite flours were within the acceptable limit from 0 weeks up to 3 weeks of storage, then increased above the limit from 6 weeks to 9 weeks of storage. These maximum limits are 10^2 and 10^4 for moulds and yeasts, respectively, as recommended microbiological limits for dried and instant products requiring reconstitution (Andrews, 1992). Throughout storage time, the products indicated good microbiological quality as cfu/g from TVC were within a satisfactory level, meaning were within the acceptable limit and thus defined as extruded flours enriched with long-horned grasshopper were fit for human consumption. The moisture content observed in extruded composite flour enriched with the long-horned grasshopper, which was between 1.8 to 2.8% was within the safe moisture content of 4.0% as recommended by East African Standards (RS EAC 72:2013), which specify the requirements for processed cereal-based ready to eat food for infants and young children (ISO 711) and as per international standard (Codex Alimentarius Commission, 2006).

3.8 Peroxide value of extruded composite flour enriched with long-horned grasshopper

From Table 8, the result shows a significant difference at ($p<0.05$) of all peroxide values of extruded composite flours enriched with long-horned grasshopper stored under room temperature compared to the ECF 0. Treatments ECF 5, ECF 15 and ECF 25 were observed to have higher peroxide values than ECF 0. The finding shows that the peroxide values of ECF 15 and ECF 25 extruded samples were not significantly different ($p>0.05$).

Table 8: Peroxide value for extruded composite flour stored under room temperature

Peroxide Value (mEq/kg)	ECF 0	ECF 5	ECF 15	ECF 25	F-value	P-value
PV - 0 Weeks	$2.80{\pm}0.01^{aA}$	$3.52{\pm}0.03^{bA}$	$3.97{\pm}0.13^{cA}$	$4.13{\pm}0.003^{cA}$	250.5	<0.001***
PV - 3 Weeks	$3.23{\pm}0.02^{aC}$	$3.91\ {\pm}0.04^{bB}$	$4.26\ {\pm}0.58^{b,c,AB}$	$4.68{\pm}0.06^{c,AB}$	13.44	0.0017**
PV - 6 Weeks	$3.33{\pm}0.05^{aC}$	$4.16{\pm}0.01^{bB}$	$5.03{\pm}0.08^{cB}$	$5.32{\pm}0.53^{c,B}$	33.27	0.0001***
PV - 9 Weeks	$3.18{\pm}0.03^{aB}$	$4.15{\pm}0.19^{bB}$	$4.96{\pm}0.34^{cB}$	$5.20{\pm}0.003^{c,B}$	63.92	<0.001***
F-value	157.6	27.72	6.829	12.44		
P-value	<0.001***	0.0001***	0.0135*	0.0022**		

Values are mean±SD (n=3); means with different superscripts, in lower case letters across columns, indicate significant differences and means with different superscripts, in upper case letters across rows indicate significant differences ($p < 0.05$). *** means significant at $p < 0.001$ and ** means significant at $p < 0.01$.

The peroxide value of extruded composite flours was between 2.8 to 5.32 (mEq/Kg) during all storage weeks. This value generally increased with edible insect content, as it was observed that sample ECF 0 had a low peroxide value (2.80 to 3.33 mEq/Kg). In contrast, the initial oxidation indicated in extruded flour enriched with long-horned grasshopper (ECF 5, ECF 15 and ECF 25) had higher PV than ECF 0. The PV of the flour enriched with long-horned grasshopper was between 3.52 to 5.32 mEq/Kg. The reported PV in this study was lower than observed in the developed extruded food product, which ranged between 0.59 to 7.4 mEq/1000g during 90 days of storage of homemade extruded products prepared by using malted composite flour (Gautam and Gupta, 2017). However, the PV reported in this study was higher than that reported by Mmari et al. (2017). They found that six months of storage of soybean-sweet potato-based complimentary food enriched with long-horned grasshopper (*Ruspolia differens*) showed a linear trend increasing from 3.06 to 4.2 mEq/Kg. The high PV value reported in this study could be associated with the extrusion process. The high temperature of flour during extrusion might accelerate the formation of peroxides and hydroperoxides.

Additionally, results indicated a significant difference within individual extruded composite flours at ($p<0.05$) during storage. The trend of PV observed during storage showed that initially, the peroxide value was low and tended to increase with storage time due to free radical propagation. Later by week 9, the value decreased, thus suggesting that there was peroxide decomposition. In treatment ECF 5, the initial PV observed at 0 week differed significantly at ($p=0.0001$) compared to the PV followed at 3 weeks, 6 weeks, and 9 weeks of storage time. The PV observed at 3 weeks, 6 weeks, and 9 weeks of storage time showed no significant difference ($p>0.05$). Treatment ECF 15 and ECF 25 showed a similar trend of PV observed at 0 week (initial PV), 3 weeks, 6 weeks and 9 weeks of storage time. Results showed no significant difference at ($p>0.05$) on their (ECF 15 and ECF 25) between PV reported at 0 week and that obtained at 3 weeks of storage time. In contrast, the PV at 0 week differed significantly from that obtained at 6 weeks and 9 weeks of storage time, at ($p=0.0135$) for ECF 15 and at ($p=0.0135$) for ECF 25 ($p=0.0022$). ECF 0 showed a significant difference at ($0=0.001$) between PV obtained at 0 week and observed at 3 weeks, 6 weeks, and 9 weeks of storage time. The PV reported 3 weeks showed no significant difference at 9 weeks of storage ($p>0.05$). Moreover, the PV observed at 9 weeks showed a significant difference from the PV observed at 0 week, 3 weeks and 6 weeks of storage time ($p<0.05$).

4.0 Conclusion and future research

The addition of long-horned grasshopper meal in extruded composite flour significantly increases the proximate composition (crude protein, crude fat, crude fibre, moisture content) and mineral content (Zn, Fe) of extruded composite flour, except for Ca content and Mg. Functional properties indicated that the increase in the amount of long-horned grasshopper in the composition of extruded composite flour decreased the BD significantly, and OAI and WSI were significantly increasing WAI. Moreover, the addition of a long-horned grasshopper in the composition of ECF flour was observed to affect the sensory taste and aroma attributes while significantly improving texture and colour. The developed extruded composite flours enriched with long-horned grasshopper meet the microbiological quality and peroxide value limits. The results for peroxide value were within the acceptable limit and showed stability for nine weeks of storage, thus, displaying shelf-life stability during storage. The inclusion of long-horned grasshopper in extruded composite flour is expected to substantially improve young children's nutrition and health status due to their high nutritive value. Future studies will likely provide an in-depth understanding of using fried long-horned grasshopper instead of oven-dried long-horned grasshopper because the frying method may impact the aroma and taste of extruded composite flour, will increase the acceptability of the composite flour enriched with long-horned grasshoppers. This study recommends the adoption of long-horned grasshoppers by food processors to use long-horned grasshopper to enhance the nutrition composition of extruded composite flour.

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5.2 Presentation of the study, findings, and a portion of the work

These preliminary findings were presented in abstract communication and poster presentation at the 24th AAIS meeting and 1st Hybrid Scientific Conference, held at Addis Ababa University, Ethiopia, from March 21 to March 25, 2022. This paper's abstract may be found at https://app.oxfordabstracts.com/events/2547/submissions/292815/abstract-book-view from the 24th AAIS Meeting and 1st Hybrid Scientific Conference. On March 23, 2022, this study was also presented as a poster abstract at the AAIS Meeting and 1st Hybrid Scientific Conference.

5.3 General statement

Within the *"extruded composite flour enriched with long-horned grasshopper* (*Ruspolia differens*)", research management was necessary to complete this project successfully. Mr. Ronoh Amos (IFBT, DeKUT), Eng. Isaka Gerald Barongereje (Food scientist, SUA), Stewart John Mwanyika (Laboratory scientist, SUA), Kinuthia Chuaga (Chief Technologist - IFBT, DeKUT) and Jonah Mbae (Senior -Technologist - IFBT, DeKUT), their technical support is highly appreciated.

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5.5 Declaration of interest

This research did not receive any commercial or financial relationships construed as a potential conflict of interest. All authors declare that they have no conflict of interest. The authors, *Esther Pius Shabo, Eddy Owaga*, and *John Kinyuru,* retain the copyright of this work. Ethics approval for the analysis was on 27th November 2020, reference number being DeKUT/SGSR/MSC/FST/11/13, and issued by Dedan Kimathi University of Technology ERC.

The present work fulfils the requirements for obtaining the degree "MSc Food Science and Technology." The funders were not involved in the study design, data collection, or analysis, or in the decision to write or publish the report. The manuscript studies were organized in a chosen manner based on their relevance to the subject matter and predicted work quality, rather than being exhaustive. The writers' opinions, assessments, knowledge, and conclusions in this paper are exclusively their own. The writers indicated above are responsible for the content, editing decision, manuscript composition, viewpoints expressed, acceptance of the final content, and consent to publish, if any errors remain.

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