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Research Article

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Posted Date: September 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10918156/v1>

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Additional Declarations: No competing interests reported.

Nutritional Composition and Sensory Properties of Biscuits As Influenced By *Zonocerus Variegatus* Powder Substitution

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Statement and Declaration

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interest

The authors declare no conflict of interest.

ABSTRACT

Edible insects are increasingly recognized as alternative sources of nutrients and sustainable food ingredients with potential applications in food product development. This study evaluated the effects of incorporating *Zonocerus variegatus* (grasshopper) powder into wheat-based biscuits on their nutritional composition and sensory properties. Four biscuit formulations containing 0% (WGF1), 5% (WGF2), 10% (WGF3), and 15% (WGF4) grasshopper powder were produced and evaluated for proximate composition, amino acid profile, mineral composition, vitamin content, antinutritional factors, and sensory properties using standard analytical procedures. Significant differences ($p < 0.05$) were observed among the biscuit formulations for several nutritional parameters. Protein content increased progressively from 9.01% in WGF1 to 15.10% in WGF4, while crude fibre increased from 2.24% to 5.22%. Moisture content ranged from 6.30% to 9.60%. Iron and zinc contents increased from 6.60 to 19.90 mg/kg and 4.20 to 5.60 mg/kg, respectively, whereas sodium decreased from 56.63 to 36.35 mg/kg. The amino acid profile showed improvements in several essential amino acids, with lysine increasing from 5.80 to 8.23 g/100 g, leucine from 7.20 to 8.60 g/100 g, methionine from 1.60 to 2.13 g/100 g, and phenylalanine plus tyrosine from 6.60 to 10.60 g/100 g. Vitamin A content increased from 340.00 to 520.00 μg β -carotene equivalent/100 g, while vitamins B9, D, and E were detected across the formulations. Antinutritional factors were present at relatively low concentrations. Sensory evaluation showed that biscuits containing up to 15% grasshopper powder remained acceptable, with mean hedonic scores above 5.0 for colour, aroma, taste, texture, crispness, and overall acceptability. Overall, incorporation of *Z. variegatus* powder, particularly at 15% substitution, improved several nutritional attributes of wheat-based biscuits while maintaining acceptable sensory characteristics, indicating its potential as an alternative nutrient-rich ingredient for biscuit production.

Key words: Edible insects, Biscuits, Grasshopper, vitamin composition

Introduction

Food security and long-term sustainability remain major global challenges, particularly in low- and middle-income regions where population growth, malnutrition, climate change, and pressure on natural resources continue to threaten the availability of nutritious foods [1]. Conventional livestock production contributes substantially to the global food supply but requires considerable land, water, and feed resources and is associated with greenhouse gas emissions and other environmental pressures. Consequently, increasing attention has been directed toward alternative and sustainable sources of nutrients, particularly protein, that can complement conventional food production systems [2,3]. Among the alternatives being investigated, edible insects have gained considerable scientific interest because of their nutritional value, resource-use efficiency, and potential contribution to food and nutrition security [4,5]. Recent evidence indicates that insects can provide substantial amounts of high-quality protein, essential amino acids, lipids, vitamins, and minerals while potentially requiring fewer environmental resources than conventional livestock systems [4,5].

In Africa, edible insects have a particular relevance because their consumption is already embedded in the food cultures of several communities and a diversity of edible insect species is available locally. In Nigeria, insect consumption has traditionally contributed to dietary diversity and provides an alternative source of animal-derived nutrients [4]. Among the edible insects consumed in the country, the variegated grasshopper, *Zonocerus variegatus*, is of particular interest. Unlike several commonly investigated edible insects such as crickets, mealworms, and black soldier fly larvae, *Z. variegatus* is a naturally occurring grasshopper widely distributed in tropical Africa and is traditionally consumed in parts of Nigeria. Its consumption has been specifically documented in southwestern Nigeria, including communities in Ondo and Kwara States, where it forms part of local food practices [6]. This established cultural familiarity provides an important advantage for investigating *Z. variegatus* as a food ingredient because product development based on a locally recognized edible species may face fewer cultural barriers than introducing an unfamiliar insect species.

The nutritional characteristics of *Z. variegatus* further support its consideration as a food ingredient. Recent studies have reported appreciable levels of protein, fat, minerals, vitamins, essential amino acids, and unsaturated fatty acids in this species, although its nutrient composition may vary according to developmental stage, sex, season, geographical location, and processing

method [7]. Comparative studies of edible insects in Nigeria have also demonstrated that *Z. variegatus* contains substantial amounts of protein and lipid together with nutritionally important minerals and amino acids [7]. Thus, converting the insect into a powdered ingredient may provide an opportunity to incorporate its nutrients into familiar cereal-based foods while reducing the sensory and psychological barriers that may be associated with consuming whole insects [8].

Biscuits are suitable vehicles for the incorporation of alternative nutrient-rich ingredients because they are widely consumed, relatively inexpensive, convenient, and have a comparatively long shelf life [9]. Wheat-based biscuits, however, are predominantly carbohydrate-based products, and their nutritional quality can potentially be improved through partial replacement of wheat powder with protein- and micronutrient-rich ingredients. The incorporation of insect powders into bakery products has therefore received increasing attention. Studies involving grasshopper powders have shown that partial substitution of cereal ingredients can increase protein, fat, fibre, ash, and selected minerals while maintaining acceptable sensory characteristics at appropriate inclusion levels [10,11]. Nevertheless, the incorporation of non-conventional ingredients may also influence important product characteristics, including colour, texture, dimensions, spread, hardness, and consumer acceptability, making formulation level and processing conditions critical considerations in product development.

Although edible insect-based bakery products have been investigated, important differences exist among the studies reported in the literature. For example, Ayo et al. evaluated biscuits produced from acha (*Digitaria exilis*) and orange-fleshed sweet potato formulations enriched with *Zonocerus variegatus*, focusing on their nutritional and quality characteristics [12]. More recently, Ayo et al. investigated the techno-functional and sensory properties of biscuits produced using toasted African bush pear (*Dacryodes edulis*) seed, acha, and *Z. variegatus* [13]. These studies demonstrate the potential of *Z. variegatus* as a biscuit ingredient but used composite cereal/tuber or seed-based formulations rather than a conventional wheat biscuit system. Furthermore, the incorporation of additional plant materials in these formulations makes it difficult to isolate the contribution of *Z. variegatus* powder to the nutritional and sensory characteristics of the final product. In contrast, the present study focuses specifically on the progressive substitution of wheat powder with *Z. variegatus* powder in a wheat-based biscuit system, thereby providing a more direct assessment of the effect of increasing grasshopper powder inclusion on the nutritional and sensory properties of the product.

Another relevant study by Ronoh et al. investigated wheat and wheat-sorghum biscuits fortified with longhorn grasshopper (*Ruspolia differens*) powder at several inclusion levels and reported improvements in protein, fat, fibre, ash, minerals, amino acids, and protein quality, together with favourable consumer responses [11]. However, *R. differens* and *Z. variegatus* are different grasshopper species with potentially different nutritional characteristics, processing behaviour, and sensory attributes. In addition, the use of sorghum-wheat composite formulations and a broader range of inclusion levels in that study differs from the formulation strategy adopted in the present investigation. Similarly, recent work on the descriptive sensory characteristics of *R. differens*-fortified biscuits demonstrated that grasshopper powder can modify sensory attributes such as colour, aroma, and texture [10]. These findings reinforce the need to evaluate the technological and sensory consequences of incorporating a specific insect species into a defined food matrix rather than assuming that results obtained with one grasshopper species can be directly generalized to another.

The selection of *Z. variegatus* in the present study was therefore based on three complementary considerations: its established consumption in Nigeria, its documented nutritional potential, and the need to generate evidence on its incorporation into a familiar wheat-based food product. Importantly, the nutritional composition of insects can be affected by processing practices, including heating, roasting, frying, drying, and seasoning [7]. Therefore, characterization of a processed *Z. variegatus* powder and its subsequent incorporation into biscuits is relevant for understanding the nutritional and sensory characteristics of the actual food product that consumers would encounter.

Despite the increasing interest in edible insects as alternative food ingredients, there remains limited information on the systematic incorporation of *Z. variegatus* powder into conventional wheat biscuits across graded substitution levels, particularly with simultaneous consideration of proximate composition, mineral and amino acid profiles, and sensory acceptability. Existing studies have largely focused on other insect species, composite cereal formulations, or broader techno-functional characteristics, leaving a need for further species-specific evidence [10–12]. Moreover, establishing an inclusion level that improves nutritional characteristics while maintaining acceptable sensory properties is important for the practical development of insect-based bakery products. Such evidence may contribute to the valorisation of locally consumed edible insects and the development of nutrient-dense foods using locally available resources.

Therefore, this study investigated the effects of substituting wheat powder with different levels of *Zonocerus variegatus* powder on the nutritional composition and sensory properties of biscuits. Specifically, the study evaluated the proximate composition, amino acid profile, mineral composition, and sensory acceptability of biscuits containing graded levels of *Z. variegatus* powder. The findings are intended to provide species-specific evidence for the development of acceptable insect-based bakery products and to contribute to ongoing efforts to diversify sustainable and nutritionally valuable food sources [4,5].

Methodology

Materials

Fresh whole *Zonocerus variegatus* grasshoppers and wheat grains were used as the principal raw materials for biscuit production. Other ingredients included margarine, sugar, milk, baking powder, salt, garlic powder, vegetable oil, and spices. All ingredients were obtained from Oja Oba Market, Ado-Ekiti, Ekiti State, Nigeria, except where otherwise stated. The brand, manufacturer, and country of origin of commercially purchased ingredients were recorded as follows: wheat grains (Golden Penny Wheat Flour, Flour Mills of Nigeria Plc, Nigeria); margarine (Blue Band Margarine, Upfield Nigeria Limited, Nigeria); sugar (Dangote Sugar, Dangote Sugar Refinery Plc, Nigeria); milk (Peak Milk, FrieslandCampina WAMCO Nigeria Plc, Nigeria); baking powder (Dona Baking Powder, Nigeria); salt (Dangote Iodised Salt, Dangote Industries Limited, Nigeria); garlic powder (processed and packaged garlic powder purchased from the local market); spices (mixed spices and seasoning, including Maggi seasoning, Nestlé Nigeria Plc, Nigeria); and vegetable oil (Mamador Pure Vegetable Oil, PZ Wilmar Limited, Nigeria). Analytical-grade reagents were used for chemical analyses.

Sample Collection and Preparation

Fresh whole grasshoppers (*Zonocerus variegatus*) were obtained during the harmattan season from the central market in Katsina State, Nigeria. The grasshoppers were transported to the laboratory in clean, covered containers and processed on the same day of collection. The processing procedure was adapted from the method described by [13].

Preparation of Grasshopper Powder

The collected grasshoppers were manually sorted to remove extraneous materials and damaged specimens. The wings were removed, and the insects were washed thoroughly with clean water. They were subsequently blanched in boiling water at 100 °C for approximately 10 min as a decontamination step.

After blanching, the insects were seasoned using salt, spices, and garlic powder. For each 1 kg batch of blanched grasshoppers, 10 g of salt, 5 g of garlic powder, and 5 g each of ground ginger and black pepper were added. The seasoning ingredients were thoroughly mixed with the blanched grasshoppers and allowed to stand for 15 min to facilitate uniform distribution.

The seasoned grasshoppers were subsequently air-dried at ambient temperature (approximately 25–30 °C) for 2 h before frying. The insects were deep-fried in vegetable oil maintained at approximately 170 °C for 5 min, using an insect-to-oil ratio of 1:5 (w/v), until a crispy texture was obtained. The frying conditions, including oil temperature and duration, were kept constant for all batches. After frying, the grasshoppers were allowed to cool to room temperature, drained to remove excess oil, and milled into a fine powder using a laboratory blender (Binatone, BLG-452, Binatone Nigeria Limited, Nigeria). The resulting powder was passed through a 500-µm mesh sieve to obtain a relatively uniform particle size and stored in airtight, food-grade containers at approximately 4 °C until use.

Because the grasshoppers were seasoned and fried before milling, the resulting ingredient is referred to throughout this manuscript as processed grasshopper powder. The possible contribution of the seasoning and frying processes to the measured nutritional and sensory characteristics was considered when interpreting the results.

Processing of Wheat Powder

Wheat powder was prepared from wheat grains using a conventional milling procedure adapted from [14,15]. The grains were manually sorted to remove damaged grains, stones, chaff, and other foreign materials. The cleaned grains were further sieved and subjected to air separation to remove adhering dust and lightweight impurities.

The cleaned grains were conditioned by the controlled addition of water to achieve a moisture content of approximately 14%, followed by a resting period of 12 h at ambient temperature to allow uniform moisture equilibration. Conditioning strengthens the bran layer and softens the endosperm, thereby facilitating efficient milling and separation of the bran from the endosperm [14].

The conditioned grains were milled using a laboratory roller mill (Brabender Quadrumat Junior, Brabender GmbH & Co. KG, Germany). The milled material was passed through a 500-µm sieve to obtain a relatively uniform wheat powder, while coarse bran and larger particles were removed. The resulting wheat powder was packaged in airtight, food-grade containers and stored under dry conditions at approximately 25 °C until biscuit production [14,15].

Biscuit Formulation

Four biscuit formulations were prepared by replacing wheat powder with processed grasshopper powder at 0, 5, 10, and 15% substitution levels. The substitution levels were calculated based on the total quantity of the wheat–processed grasshopper powder blend. The formulations were designated as follows: WGF1 (100% wheat powder + 0% processed grasshopper powder), WGF2 (95% wheat powder + 5% processed grasshopper powder), WGF3 (90% wheat powder + 10% processed grasshopper powder), and WGF4 (85% wheat powder + 15% processed grasshopper powder).

For each formulation, the quantities of the remaining ingredients were kept constant. A total flour blend of 1,000 g was used for each formulation, comprising 1,000 g wheat powder for WGF1, 950 g wheat powder and 50 g processed grasshopper powder for WGF2, 900 g wheat powder and 100 g processed grasshopper powder for WGF3, and 850 g wheat powder and 150 g processed grasshopper powder for WGF4. The other ingredients consisted of sugar (200 g), margarine (250 g), milk powder (100 g), baking powder (10 g), salt (5 g), garlic powder (5 g), and mixed spices (5 g). The complete formulation is presented in Table 1.

For each formulation, the required quantities of wheat powder and processed grasshopper powder were weighed separately and thoroughly mixed to obtain a homogeneous powder blend. The dry ingredients were subsequently incorporated into the blend and mixed uniformly. Margarine was added and thoroughly rubbed into the flour mixture until a uniform crumb-like consistency was obtained. Milk powder and the remaining ingredients were then incorporated, followed by the gradual addition of water to obtain a moderately firm and homogeneous dough. The dough was thoroughly kneaded to ensure uniform distribution of the ingredients.

The dough was rolled to a uniform thickness of approximately 5 mm and cut into circular shapes using a biscuit cutter of uniform diameter. The cut dough pieces were arranged on greased baking trays and baked in a preheated oven at approximately 180 °C for 15–20 min or until a light golden-brown colour and characteristic biscuit texture were achieved. The baked biscuits were removed from the oven, allowed to cool to room temperature, and packaged in airtight food-grade containers until further analyses.

Table 1: Biscuits Formulation

Ingredient (g)	WGF1	WGF2	WGF3	WGF4
	(0%)	(5%)	(10%)	(15%)
Wheat powder	1000	950	900	850
Processed grasshopper powder	0	50	100	150
Sugar	200	200	200	200
Margarine	250	250	250	250
Milk powder	100	100	100	100
Baking powder	10	10	10	10
Salt	5	5	5	5
Garlic powder	5	5	5	5
Mixed spices	5	5	5	5

Preparation of Biscuits

Biscuits were prepared using a modified creaming and dough-development method described by [15,16]. The same formulation and processing conditions were maintained for all treatments, except for the proportion of wheat powder replaced by processed grasshopper powder.

The required quantities of the wheat–processed grasshopper powder blend, sugar, baking powder, salt, and other dry ingredients were accurately weighed using a digital laboratory balance (insert actual brand, model, and country of origin) and thoroughly mixed. Margarine was subsequently incorporated and rubbed into the dry mixture until a homogeneous, fine breadcrumb-like consistency was obtained.

Milk was gradually added with continuous mixing until a soft, cohesive dough was formed. The dough was transferred onto a clean, lightly floured work surface and gently kneaded until uniform. It was then rolled to a uniform thickness of approximately 5–10 mm and cut into circular pieces using a biscuit cutter. The shaped dough pieces were placed on lightly greased baking trays with uniform spacing between the pieces.

The biscuits were baked in a preheated oven at 130 °C for approximately 20 min or until a characteristic golden-brown colour and desirable texture were achieved. The baking temperature and duration were kept constant for all formulations. After baking, the biscuits were removed from the oven and allowed to cool to room temperature for approximately 30 min.

For each formulation, the dough yield, number of biscuits produced, and mean weight and dimensions of the baked biscuits were recorded. The mean weight, diameter, and thickness of the biscuits were determined from representative samples from each formulation. The cooled biscuits were packaged in airtight, food-grade polyethylene bags and stored under dry conditions at room temperature (approximately 25–30 °C) until further analysis.

FIGURES AND TABLES



(a)



(b)



(c)



(d)

Figure 1: Biscuit sample obtained from 100% wheat powder, 95% wheat powder and 5% Grasshopper powder blend, 90% wheat powder and 10% grasshopper powder blend, 85% of wheat powder and 15% of grasshopper powder blend respectively.

Experimental Design and Replication

The study consisted of four biscuit formulations containing 0, 5, 10, and 15% processed *Zonocerus variegatus* powder, designated WGF1, WGF2, WGF3, and WGF4, respectively. Each formulation was produced in three independent production batches. Analytical determinations were performed in triplicate for each independent production batch, and the results were expressed as mean $\pm$ standard deviation. The control formulation (WGF1), containing 100% wheat powder and 0% processed grasshopper powder, served as the reference formulation against which the grasshopper-powder formulations were compared.

Nutritional Composition Analysis

The proximate composition of the biscuit samples was determined using standard Association of Official Analytical Chemists (AOAC) procedures [17]. Samples were ground to obtain a homogeneous material before analysis.

Determination of Moisture Content

Moisture content was determined using the oven-drying method according to AOAC method 925.10 [17]. Approximately 5 g of the homogenized sample was weighed into a previously dried and weighed moisture dish and dried in a hot-air oven at 105 °C for 4 h. The dish was subsequently transferred to a desiccator, cooled to room temperature, and weighed. Drying, cooling, and weighing were repeated until constant weight was obtained.

Moisture content was expressed as:

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{Final drying weight}}{\text{Initial weight}} \times 100$$

Determination of Crude Protein

Crude protein was determined using the Kjeldahl digestion and distillation method [17]. Approximately 1 g of sample was digested in concentrated sulfuric acid (H₂SO₄) using a catalyst mixture until the solution became clear, indicating complete digestion. After cooling, the digest was diluted with distilled water and neutralized with 40% sodium hydroxide. The released ammonia was distilled into boric acid solution containing an indicator and later titrated with standardized hydrochloric acid (0.01 N HCl).

Nitrogen content was calculated and converted to protein using:

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

Where nitrogen was derived from titration values.

Determination of Crude Fat

Crude fat content was obtained using Soxhlet extraction [17]. Approximately 2 g of dried sample was wrapped in filter paper and extracted continuously with petroleum ether (40–60°C boiling range) for about 6 hours. After extraction, the solvent was evaporated, and the flask containing the extracted fat was dried at 105°C, cooled in a desiccator, and weighed. Fat content was expressed as:

$$\% \text{ Fat} = \frac{\text{Weight of extracted lipid}}{\text{Weight of Sample}} \times 100$$

Determination of Crude Fibre

Crude fibre was analyzed using the AOAC 958.06 procedure [17]. Defatted samples (2 g) were sequentially boiled in dilute sulfuric acid and sodium hydroxide solutions for 30 minutes each. The residue obtained was filtered, washed, and oven-dried at 105°C to constant weight. The dried residue was then incinerated in a muffle furnace at 550°C for 3 hours, and fibre content was determined from weight loss after ashing.

Determination of Ash Content

Ash content was measured by incinerating about 5 g of sample in a pre-weighed crucible using a muffle furnace maintained at 550°C for 4 hours (17). After complete combustion, the crucible was cooled in a desiccator and weighed. Ash content was calculated as:

$$\% \text{ Ash} = \frac{\text{Weight of mineral residue}}{\text{Original Sample weight}} \times 100$$

Determination of Carbohydrate Content

Total carbohydrate content was estimated indirectly by difference using proximate values obtained for other components:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Crude Fibre} + \% \text{ Ash})$$

Mineral Analysis

Mineral composition of the biscuit samples was determined following wet digestion according to the method described by [18]. Approximately 1 g of each homogenized biscuit sample was accurately weighed into a digestion vessel and digested with concentrated nitric acid (HNO_3) and perchloric acid (HClO_4) under controlled heating until a clear digest was obtained. The digested samples were allowed to cool, filtered where necessary, and quantitatively transferred into volumetric flasks and diluted to a final volume of 50 mL with deionized water.

Calcium (Ca), iron (Fe), zinc (Zn), magnesium (Mg), potassium (K), and sodium (Na) were quantified using an inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent 5800, Agilent Technologies, Australia). Calibration standards were prepared from certified elemental standards, and individual calibration curves were generated for each mineral before sample analysis. Reagent blanks and appropriate quality-control samples were included throughout the analytical procedure to monitor contamination and analytical accuracy.

The concentrations obtained from the ICP-OES analysis were converted and expressed as **mg/100 g sample** on a consistent basis throughout the manuscript. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation. Where applicable, the limits of detection (LOD) and limits of quantification (LOQ) for the respective elements were recorded. Mineral concentrations below the applicable analytical detection or quantification limit were reported as <LOD or <LOQ, respectively, rather than as zero [18].

Amino Acid Determination

Amino acid composition was determined following protein hydrolysis and chromatographic analysis according to the method described by [18]. Approximately 0.5 g of each defatted and homogenized biscuit sample was weighed into a hydrolysis tube and hydrolyzed with 6 N hydrochloric acid (HCl) at 110 °C for 24 h under an inert nitrogen atmosphere. Following hydrolysis, the samples were cooled to room temperature, filtered, and evaporated to remove excess acid. The hydrolysates were reconstituted in an appropriate buffer and derivatized prior to chromatographic analysis.

Individual amino acids were separated and quantified using high-performance liquid chromatography (HPLC) with UV detection (insert actual brand, model, and manufacturer). Identification of individual amino acids was based on comparison of their retention times with those of analytical amino acid standards. Quantification was performed using calibration curves prepared from known concentrations of the corresponding amino acid standards.

Because conventional acid hydrolysis with 6 N HCl may result in the degradation or loss of certain amino acids, particularly tryptophan, tryptophan was not quantified from the conventional acid hydrolysate. Where tryptophan was determined separately, an appropriate validated method involving alkaline hydrolysis followed by chromatographic analysis was used and reported separately.

The concentrations of individual amino acids were expressed as g/100 g protein. Amino acid reference values used for nutritional comparison were converted to the same unit and reporting basis before comparison. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Vitamin Analysis

Vitamin composition of the biscuit samples was determined using high-performance liquid chromatography (HPLC) following appropriate extraction procedures as described by [17]. Sample preparation was performed according to the class of vitamins being analyzed. Fat-soluble vitamins were extracted using an appropriate organic solvent system, while water-soluble vitamins were extracted using suitable aqueous buffer solutions. The extracts were centrifuged where necessary and filtered through 0.45- μ m membrane filters prior to chromatographic analysis.

The extracted vitamin compounds were separated using a high-performance liquid chromatography system (HPLC; insert actual brand, model, and manufacturer) equipped with an appropriate detector. Identification of individual vitamins was based on comparison of their retention times with those of authentic reference standards. Quantification was performed using calibration curves prepared from known concentrations of the respective vitamin standards.

The concentrations of the identified vitamins were expressed as mg/100 g sample. Where concentrations were below the analytical detection or quantification limits, the results were reported accordingly rather than as zero. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Anti-Nutritional Factors

Phytate Determination

Phytate content of the biscuit samples was determined using the colorimetric Wade reagent method described by [19]. Approximately 0.5 g of each homogenized sample was weighed and extracted with dilute hydrochloric acid with continuous agitation for 2 h. The extract was centrifuged, and an aliquot of the resulting supernatant was transferred into a clean test tube and reacted with freshly prepared ferric chloride-sulfosalicylic acid (Wade) reagent. The intensity of the resulting colour was measured at 500 nm using a UV-visible spectrophotometer (insert actual brand and model).

A calibration curve was prepared using known concentrations of a phytate standard, and the phytate concentration of each sample was calculated from the corresponding calibration curve. The results were expressed as mg/100 g sample. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Oxalate Determination

Oxalate content was determined by acid extraction followed by titration with potassium permanganate according to the method described by [20]. Approximately 2 g of each homogenized biscuit sample was weighed and extracted with dilute sulfuric acid under controlled heating. The resulting extract was filtered, and the filtrate was titrated against a standardized potassium permanganate (KMnO_4) solution until a persistent faint pink colour was observed, indicating the endpoint. A reagent blank was determined under identical conditions and used for correction of the titre values where applicable. Oxalate concentration was calculated from the corrected titre

value and expressed as mg/100 g sample. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Tannin Determination

Tannin content was determined using the Folin–Denis colorimetric method described by [21]. Approximately 0.5 g of each homogenized biscuit sample was extracted with methanol, and the extract was filtered prior to analysis. An aliquot of the extract was reacted with Folin–Denis reagent and sodium carbonate solution to develop a blue-coloured complex. The absorbance of the resulting solution was measured at 760 nm using a UV-visible spectrophotometer (insert actual brand and model). Tannic acid was used as the reference standard, and a calibration curve was prepared from known concentrations of tannic acid. Tannin concentration was calculated from the calibration curve and expressed as mg tannic acid equivalent (TAE)/100 g sample. Each sample was analyzed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Sensory Evaluation

Sensory evaluation was conducted among 50 untrained panelists recruited from Afe Babalola University, Ado-Ekiti, Nigeria, following the procedure described by [22]. Panelists were adults who were willing to participate and who provided written informed consent before evaluation. Individuals with known allergies or previous adverse reactions to insects or any of the ingredients used in the products were excluded from participation.

The four biscuit formulations were coded using randomly generated three-digit numerical codes and presented to panelists in a randomized serving order to minimize positional and order effects. Samples were served at room temperature under standardized conditions. Panelists evaluated colour, aroma, taste, texture, crispness, and overall acceptability using a 9-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely. Drinking water was provided between samples to cleanse the palate and minimize carry-over effects. Panelists were instructed to evaluate each sample independently and not to discuss their responses during the assessment.

Ethical Approval

The sensory evaluation involving human participants was conducted in accordance with the principles of the Declaration of Helsinki and relevant institutional ethical guidelines. The study protocol was reviewed and approved by the Health Research Ethics Committee of Afe Babalola University, Ado-Ekiti, Nigeria (Approval No.: ABUAD/HREC/2025/097). Written informed

consent was obtained from all participants before participation. Participation was voluntary, and participants were informed about the purpose and procedures of the study, their right to withdraw at any time without penalty, and the confidentiality of information obtained during the study.

Statistical Analysis

All quantitative data were expressed as mean $\pm$ standard deviation (SD). The data were subjected to one-way analysis of variance (ANOVA) to determine whether significant differences existed among the four biscuit formulations. Where significant differences were detected, means were separated using Tukey's post-hoc multiple comparison test. Statistical significance was accepted at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA).

Results

Proximate Composition of Biscuit Samples

The proximate composition of the biscuits produced from wheat–grasshopper powder blends is presented in Table 2. The incorporation of processed *Zonocerus variegatus* powder resulted in variations in the proximate composition of the biscuits across the substitution levels. Moisture content ranged from 6.30 ± 0.07 to 9.60 ± 0.01 g/100 g. WGF2, containing 5% grasshopper powder, recorded the lowest moisture content (6.30 ± 0.07 g/100 g), whereas WGF4, containing 15% grasshopper powder, had the highest value (9.60 ± 0.01 g/100 g). WGF1 and WGF3 recorded moisture contents of 7.20 ± 0.02 and 7.50 ± 0.01 g/100 g, respectively.

Protein content increased consistently with increasing grasshopper powder substitution. The control biscuit, WGF1, had the lowest protein content (9.01 ± 0.02 g/100 g), whereas WGF4 had the highest value (15.10 ± 0.01 g/100 g). WGF2 and WGF3 recorded intermediate protein contents of 12.90 ± 0.01 and 14.20 ± 0.03 g/100 g, respectively. Crude fibre content also varied among the formulations. WGF1 had the lowest fibre content (2.24 ± 0.01 g/100 g), while WGF4 recorded the highest value (5.22 ± 0.15 g/100 g). WGF2 and WGF3 contained 4.97 ± 0.01 and 4.21 ± 0.01 g/100 g fibre, respectively. Thus, fibre content was generally higher in the grasshopper-powder-containing biscuits than in the control.

Fat content showed a non-linear pattern across the formulations. WGF1 recorded 19.98 ± 0.02 g/100 g fat, while WGF2, WGF3, and WGF4 recorded 25.63 ± 0.03 , 35.05 ± 0.29 , and 22.40 ± 0.05 g/100 g, respectively. The highest fat content was observed in WGF3, whereas WGF1 had the lowest value. Ash content ranged from 2.76 ± 0.01 to 3.60 ± 0.02 g/100 g. WGF3 recorded the highest ash content (3.60 ± 0.02 g/100 g), followed by WGF2 (3.49 ± 0.04 g/100 g) and WGF1 (3.48 ± 0.01 g/100 g). WGF4 had the lowest ash content (2.76 ± 0.01 g/100 g).

Carbohydrate content, calculated by difference, did not decrease progressively with increasing grasshopper powder substitution. Based on the proximate values, WGF1 had a corrected carbohydrate content of 58.09 g/100 g, while WGF2, WGF3, and WGF4 had values of 46.71 ± 0.08 , 35.44 ± 0.30 , and 44.92 ± 0.13 g/100 g, respectively. The lowest carbohydrate value occurred in WGF3, whereas WGF1 had the highest value after correction. The values therefore indicate a non-linear pattern rather than a continuous decrease with increasing grasshopper powder inclusion.

Table 2: Proximate composition of biscuit developed from wheat-grasshopper powder blend

Sample	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Fibre (g/100 g)	Ash (g/100 g)	Carbohydrate (g/100 g)
WGF1	7.20 ± 0.02	9.01 ± 0.02	19.98 ± 0.02	2.24 ± 0.01	3.48 ± 0.01	58.09 ± 0.04
WGF2	6.30 ± 0.07	12.90 ± 0.01	25.63 ± 0.03	4.97 ± 0.01	3.49 ± 0.04	46.71 ± 0.09
WGF3	7.50 ± 0.01	14.20 ± 0.03	35.05 ± 0.29	4.21 ± 0.01	3.60 ± 0.02	35.44 ± 0.29
WGF4	9.60 ± 0.01	15.10 ± 0.01	22.40 ± 0.05	5.22 ± 0.15	2.76 ± 0.01	44.92 ± 0.16

Values are reported as mean ± standard deviation, and mean values bearing different superscript letters within the same column differ significantly ($p < 0.05$).

Parameters:

Sample WGF1 is biscuit obtained from 100% wheat powder

Sample WGF2 is obtained from 95% wheat powder and 5% grasshopper powder

Sample WGF3 is obtained from 90% wheat powder and 10% grasshopper powder

Sample WGF4 is obtained from 85% wheat powder and 15% grasshopper powder.

Amino Acid Profile

The amino acid composition of the biscuit samples is presented in Table 3. Differences in amino acid concentrations were observed among the formulations, with generally higher values recorded for several amino acids as the proportion of processed grasshopper powder increased.

Among the non-essential amino acids, glycine increased from 2.29 g/100 g in WGF1 to 3.52 g/100 g in WGF4. Alanine increased from 6.27 to 7.24 g/100 g, while serine increased from 5.50 to 7.10 g/100 g. Proline increased from 7.45 g/100 g in WGF1 to 9.23 g/100 g in WGF4. Aspartic acid showed the largest increase among the reported non-essential amino acids, rising from 6.10 g/100 g in WGF1 to 13.50 g/100 g in WGF4.

Among the essential amino acids, valine increased from 4.32 g/100 g in WGF1 to 4.92 g/100 g in WGF4. Lysine increased from 5.80 to 8.23 g/100 g, while isoleucine increased from 3.70 to 4.50 g/100 g. Leucine increased from 7.20 to 8.60 g/100 g and methionine increased from 1.60 to 2.13 g/100 g across WGF1 to WGF4. Histidine increased from 4.52 to 5.28 g/100 g, while tryptophan increased from 0.79 to 1.12 g/100 g. Threonine increased from 4.71 g/100 g in WGF1 to 5.89 g/100 g in WGF3 but decreased slightly to 5.76 g/100 g in WGF4. Phenylalanine plus tyrosine increased from 6.60 g/100 g in WGF1 to 10.60 g/100 g in WGF4.

Table 3: Amino acid profile of biscuit developed from wheat-grasshopper powder blend (g/100g)

Amino acid	WGF1	WGF2	WGF3	WGF4	FAO/WHO (2007) mg/100g
Non-essential					
Glycine	2.29	2.34	2.34	3.52	0
Alanine	6.27	6.86	6.84	7.24	0
Serine	5.50	6.80	6.80	7.10	0
Proline	7.45	8.29	8.27	9.23	0
Aspartic Acid	6.10	9.80	9.70	13.50	0
Essential					
Valine	4.32	4.76	4.84	4.92	3900
Lysine	5.80	7.30	7.58	8.23	4500
Cysteine	1.29	1.79	1.79	3.27	2200
Isoleucine	3.70	3.90	4.10	4.50	3000
Leucine	7.20	7.52	8.00	8.60	5900
Methionine	1.60	1.70	1.91	2.13	3800
Phenylalanine + Tyrosine	6.60	9.10	9.40	10.60	1500
Histidine	4.52	4.67	4.83	5.28	2300
Threonine	4.71	4.88	5.89	5.76	600
Tryptophan	0.79	0.94	0.98	1.12	3900

Values are reported as mean $\pm$ standard deviation, and mean values bearing different superscript letters within the same column differ significantly ($p < 0.05$).

Mineral Composition

The mineral composition of the biscuit samples is presented in Table 4. The concentrations of the analyzed minerals varied among the formulations, with different patterns observed for individual elements.

Sodium concentration ranged from 36.35 to 56.63 mg/kg, with the highest value observed in WGF1 and the lowest in WGF4. Potassium concentration similarly decreased across the formulations, from 49.05 mg/kg in WGF1 to 24.54 mg/kg in WGF4. Phosphorus concentration ranged from 4.34 to 11.98 mg/kg, with WGF1 recording the highest value and WGF3 the lowest.

Calcium concentration varied from 12.28 to 26.59 mg/kg. WGF4 recorded the highest calcium concentration (26.59 mg/kg), followed by WGF1 (26.06 mg/kg), whereas WGF3 had the lowest value (12.28 mg/kg). Magnesium concentrations ranged from 1.69 to 2.90 mg/kg, with WGF1 recording the highest value.

Iron concentration showed an overall increase across the formulations, from 6.60 mg/kg in WGF1 to 19.90 mg/kg in WGF4. WGF2 recorded the lowest iron concentration (2.40 mg/kg), while WGF3 contained 11.40 mg/kg. Zinc concentration ranged from 1.90 mg/kg in WGF2 to 5.60 mg/kg in WGF4. Manganese concentrations were relatively low, ranging from 0.04 to 0.08 mg/kg.

The calculated Na/K ratios were 1.15, 1.06, 1.34, and 1.48 for WGF1, WGF2, WGF3, and WGF4, respectively. The corresponding Ca/P ratios were 2.18, 2.65, 2.83, and 3.86, respectively.

Table 4: Mineral composition of biscuit developed from wheat-grasshopper powder blend

Element	Wavelength (nm)	WGF1 (mg/kg)	WGF2 (mg/kg)	WGF3 (mg/kg)	WGF4 (mg/kg)
Sodium	589.29	56.63	51.39	53.15	36.35
Calcium	395.11	26.06	24.97	12.28	26.59
Magnesium	279.55	2.90	1.69	2.85	2.70
Phosphorus	213.61	11.98	9.41	4.34	6.89
Potassium	766.49	49.05	48.34	39.73	24.54
Iron	249.07	6.60	2.40	11.40	19.90
Manganese	258.49	0.08	0.04	0.06	0.06
Zinc	202.55	4.20	1.90	5.00	5.60
Cadmium	214.44	ND	ND	ND	ND
Lead	182.14	ND	ND	ND	ND
Mercury	184.89	ND	ND	ND	ND
Arsenic	188.98	ND	ND	ND	ND
Nickel	216.56	ND	ND	ND	ND

Values are reported as mean $\pm$ standard deviation, and mean values bearing different superscript letters within the same column differ significantly ($p < 0.05$).

Note: ND = not detected at the analytical detection limit.

Vitamin Composition

The vitamin composition of biscuits produced from wheat–grasshopper powder blends is presented in Figure 1. The incorporation of grasshopper powder influenced the vitamin content of the biscuits, with variations observed across the different substitution levels. Among the vitamins determined, vitamin A exhibited the highest concentration in all formulations and showed a progressive increase with increasing levels of grasshopper powder substitution.

The control sample (WGF1) recorded the lowest vitamin A content, while WGF2 and WGF3 showed moderate increases. The highest vitamin A concentration was observed in WGF4, indicating that increasing the proportion of grasshopper powder enhanced the provitamin A content of the composite biscuits. This trend suggests that grasshopper powder contributed positively to vitamin A enrichment and improved the micronutrient quality of the developed biscuits.

The concentrations of vitamins D, E, and B9 (folate) were comparatively low across all formulations, with only slight variations among the samples. Although present in smaller quantities than vitamin A, these vitamins contribute to the overall nutritional value of the biscuits by supporting immune function, antioxidant protection, and normal cellular metabolism.

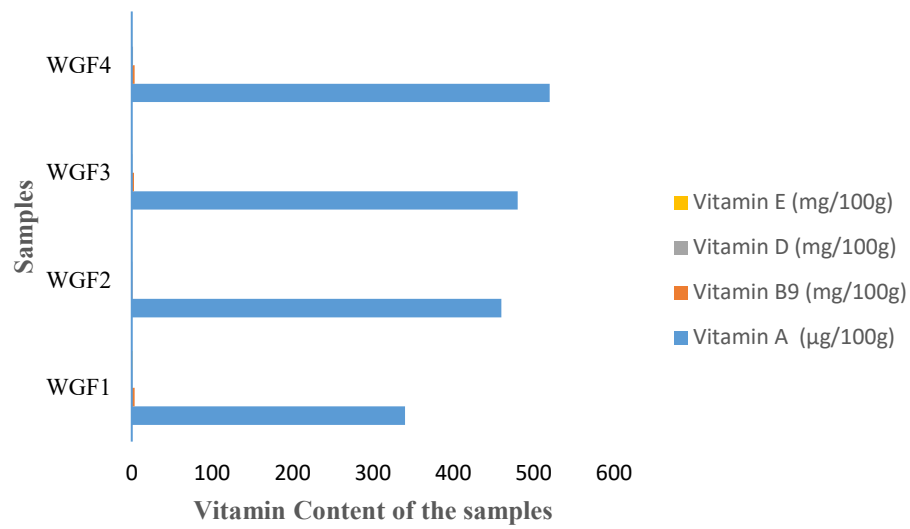


Figure 3: Vitamin content of the Biscuits

Anti-Nutritional Factors

The anti-nutritional composition (Figure 6) showed generally low levels across all biscuit samples. Saponin content remained relatively similar across formulations, ranging from 0.00 to 0.80. Tannin levels were also low, with values between 0.00 and 0.16, while oxalate content ranged from 0.18 to 0.34. These low concentrations suggest that the biscuit products are unlikely to pose significant anti-nutritional risks when consumed.

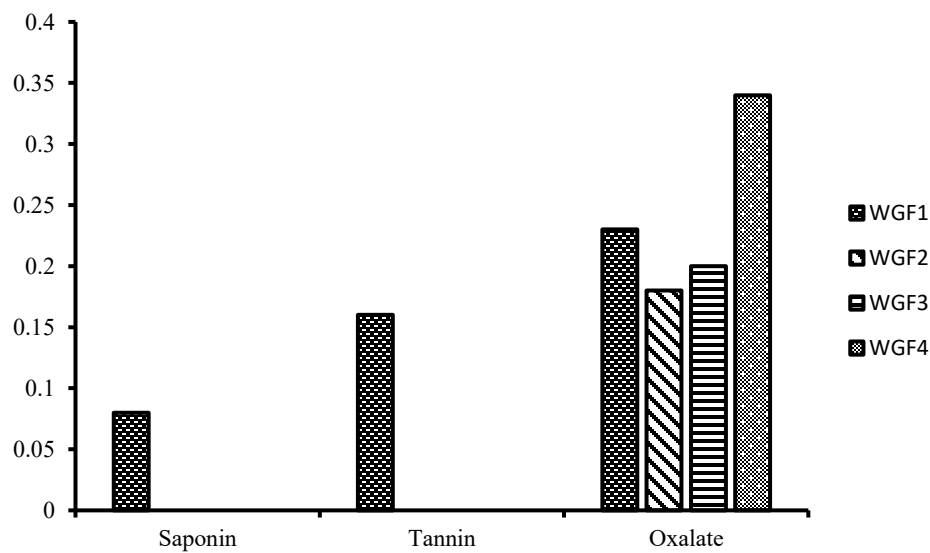


Figure 4: Anti-nutrient composition of the Biscuits

Sensory Attributes

Sensory evaluation results indicated differences in consumer preference across formulations. In terms of colour, WGF1 achieved the highest score (7.12 ± 0.75), while WGF4 recorded the lowest (5.88 ± 1.06), suggesting that higher grasshopper inclusion may have affected visual appeal. Aroma scores were highest for WGF1 (6.80 ± 0.91), while other samples showed comparable ratings. For taste, WGF1 again performed best (6.88 ± 1.13), followed closely by WGF2 and WGF3, whereas WGF4 had the lowest acceptance (5.94 ± 0.59).

Texture evaluation revealed that WGF1 had the highest rating (6.72 ± 1.14), with WGF4 again scoring the lowest (5.42 ± 1.01). This suggests that increasing substitution levels may slightly affect textural quality. Overall acceptability was highest for WGF1 (7.24 ± 0.69), while WGF2, WGF3, and WGF4 showed moderately lower but similar acceptance levels (6.14–6.40). This indicates that although nutritional quality improved with substitution, consumer preference was highest in the control formulation.

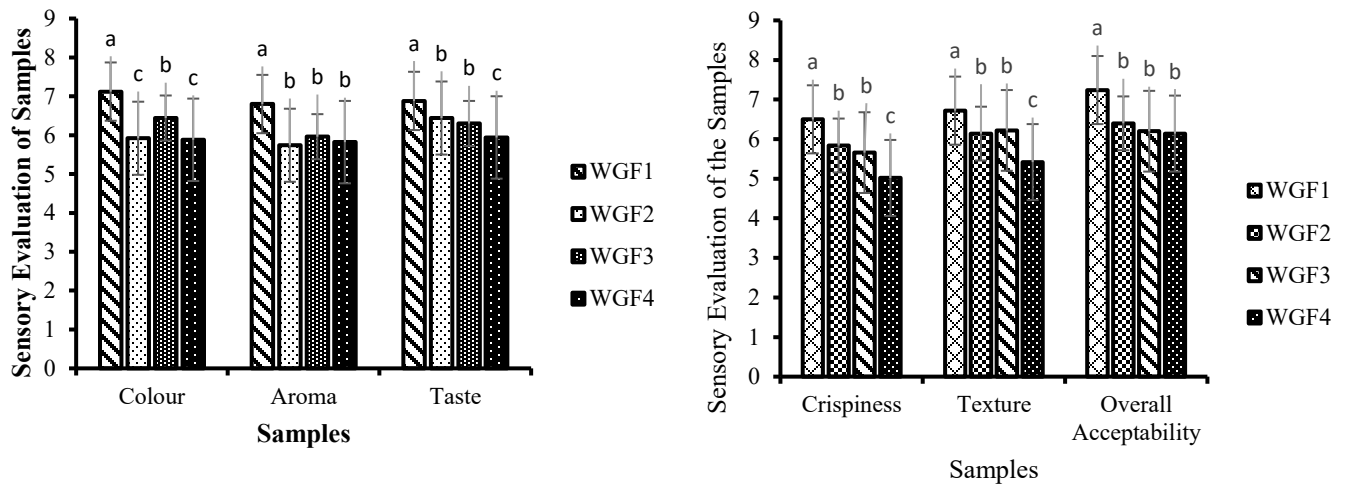


Figure 5: Sensory Evaluation of the Biscuits

Parameters:

Sample WGF1 is biscuit obtained from 100% wheat powder

Sample WGF2 is obtained from 95% wheat powder and 5% grasshopper powder

Sample WGF3 is obtained from 90% wheat powder and 10% grasshopper powder

Sample WGF4 is obtained from 85% wheat powder and 15% grasshopper powder.

Discussion

The incorporation of processed *Zonocerus variegatus* powder into wheat-based biscuit formulations resulted in changes in the proximate composition of the products. Moisture content ranged from 6.30 to 9.60 g/100 g, with WGF2 recording the lowest value and WGF4 the highest. All formulations had relatively low moisture contents, which are generally desirable in baked products because reduced water availability can limit microbial growth and contribute to improved storage stability. The variation observed among formulations may be related to differences in the composition of the blends and the interactions between the grasshopper powder and other ingredients during mixing and baking. Processing conditions, particularly baking intensity and moisture loss during heating, can also influence the final moisture content of biscuits [23]. However, because the grasshoppers in the present study were seasoned and deep-fried before milling, the contribution of these processing steps to the final proximate composition should also be considered when interpreting the differences among formulations.

Protein content increased progressively from 9.01 g/100 g in the control (WGF1) to 15.10 g/100 g in WGF4 as the level of grasshopper powder increased. This finding demonstrates the potential of *Z. variegatus* powder as a protein-rich ingredient for nutritional enhancement of cereal-based biscuits. The observed increase is consistent with previous reports on insect-enriched bakery products, including biscuits formulated with grasshopper species such as *Melanoplus cinereus* [11]. Similar improvements in protein content have also been reported in products formulated with other unconventional protein sources, including palm weevil larvae and orange-fleshed sweet potato-based blends [24]. The increase in protein is nutritionally important because protein is required for tissue growth and repair, synthesis of enzymes and hormones, and maintenance of physiological functions [10]. Nevertheless, the magnitude of the increase observed in the present study should not be attributed exclusively to the intrinsic protein content of *Z. variegatus*, since the insects were subjected to seasoning and frying before incorporation into the biscuit formulations.

Fat content varied considerably among the formulations rather than increasing linearly with increasing grasshopper powder substitution. WGF3 recorded the highest fat content (35.05 g/100 g), whereas WGF1 had the lowest value (19.98 g/100 g). The relatively high fat content may reflect contributions from both the grasshopper material and the margarine used in biscuit preparation, while the oil absorbed during deep-frying of the grasshoppers may also have contributed to the

final lipid content. Thus, the observed variation should be interpreted as a combined effect of formulation and processing rather than as a direct linear response to grasshopper powder inclusion. Although dietary fat contributes to energy density, flavour and desirable mouthfeel, higher fat levels may also increase susceptibility to lipid oxidation and influence storage stability.

Crude fibre increased from 2.24 g/100 g in WGF1 to 5.22 g/100 g in WGF4, although the increase was not completely linear because WGF3 recorded a lower value than WGF2. The higher fibre values in the substituted products may reflect the contribution of the grasshopper powder and other non-starch components of the formulation. Ash content also varied among samples, ranging from 2.76 to 3.60 g/100 g, with WGF3 recording the highest value and WGF4 the lowest. Such variation may be associated with differences in the mineral-containing components of the formulations and processing-related changes, as previously suggested [25]. Carbohydrate content did not show a progressive decrease with increasing grasshopper substitution. Based on the corrected calculation by difference, WGF1 had the highest carbohydrate content (58.09 g/100 g), followed by WGF2 (46.71 g/100 g), WGF4 (44.92 g/100 g), and WGF3 (35.44 g/100 g). The non-linear pattern indicates that changes in carbohydrate content were influenced by the relative proportions of all ingredients and not simply by the replacement of wheat with grasshopper powder. This correction is important because the original interpretation of a progressive decline in carbohydrate was not supported by the numerical results.

The amino acid profile demonstrated the presence of both essential and non-essential amino acids in all biscuit formulations. Several amino acids, particularly lysine, leucine, phenylalanine plus tyrosine, and cysteine, showed higher concentrations in the grasshopper-containing formulations than in the control. This indicates that incorporation of insect-derived protein can modify the amino acid profile of wheat-based products. However, the values should be interpreted according to a consistent reporting basis before direct comparison with FAO/WHO reference requirements or results from other studies. The relatively lower lysine concentration compared with some alternative insect-fortified formulations, such as sorghum–termite blends, is noteworthy because lysine is commonly regarded as a limiting amino acid in cereal-based diets [26]. The incorporation of insect protein may nevertheless improve the overall amino acid balance of wheat-based products.

Methionine remained comparatively low across the formulations, whereas cysteine was present at higher concentrations. Differences in the concentrations of sulphur-containing amino acids among composite products may arise from differences in the protein sources and processing conditions [27]. Histidine was detected in all formulations and increased from 4.52 g/100 g in WGF1 to 5.28 g/100 g in WGF4. Previous studies have similarly reported the presence of histidine in insect-based composite foods [26]. However, the nutritional adequacy of individual amino acids cannot be established solely from their concentration in the biscuits; interpretation should consider the quantity consumed, protein digestibility, amino acid bioavailability, and the relevant age-specific dietary requirements.

Mineral analysis showed that sodium and potassium were among the most abundant minerals detected, although their concentrations varied across the formulations. Iron and zinc were also detected in all samples, with WGF4 recording the highest concentrations of both minerals. The iron and zinc values were broadly comparable with those reported for some insect-enriched bakery products [11,24]. The relatively higher zinc concentration observed in WGF4 suggests that the incorporation of the processed grasshopper material may contribute to the mineral profile of the biscuits. Nevertheless, the mineral results should be interpreted cautiously because the grasshoppers were seasoned before incorporation. In particular, the sodium content may have been influenced by the added salt used during grasshopper preparation rather than representing the intrinsic mineral composition of *Z. variegatus*. Similarly, deep-frying and other processing operations may alter the concentration or distribution of certain minerals. Therefore, the observed mineral enhancement cannot be attributed solely to the biological contribution of the grasshopper powder.

The nutritional relevance of the detected minerals is associated with their established physiological roles in enzymatic reactions, immune function, oxygen transport, bone development and metabolic regulation [28,29]. However, the present results indicate concentrations in the finished biscuits and should not be interpreted as demonstrating that the products alone can meet recommended dietary requirements. Further assessment based on serving size and dietary reference values would be required to determine their contribution to daily nutrient intake.

The vitamin analysis indicated that vitamin A had the highest concentration among the vitamins assessed, with the values generally increasing from WGF1 to WGF4, while vitamins D, E and B9

occurred at comparatively lower levels. The higher vitamin A values in the grasshopper-containing formulations may indicate a contribution from the processed insect ingredient or differences in the overall formulation. Previous studies have reported appreciable vitamin-related nutritional contributions from edible insects [30]. However, the interpretation should correspond specifically to the analytical form measured. If the analytical procedure quantified vitamin A directly, the result should be described as vitamin A rather than as a vitamin A precursor or provitamin A. In addition, thermal processing during biscuit production can cause degradation of heat-sensitive vitamins, and the extent of such losses depends on temperature, duration and the chemical stability of individual vitamins [31]. Consequently, the values obtained represent the vitamin content retained in the final baked products rather than necessarily the amount originally present in the raw ingredients.

The detected anti-nutritional factors occurred at relatively low concentrations. Oxalate ranged from 0.18 to 0.34, while tannin ranged from 0.00 to 0.16, and saponin was detected at concentrations ranging from 0.00 to 0.80. These relatively low measured concentrations suggest that the tested compounds were present in small amounts in the finished biscuits. Similar observations have been reported for processed food products containing alternative plant or insect-derived ingredients [32]. However, the statement that the concentrations were within “safe consumption limits” should be avoided unless an established regulatory or toxicological reference value exists for each compound and the units and analytical basis are directly comparable. Likewise, low concentrations alone do not establish that nutrient bioavailability was unaffected. The effects of these compounds on mineral or nutrient absorption depend on their concentration, dietary matrix, processing, and interactions with other food components.

Sensory evaluation showed that the biscuits were generally acceptable, although the control formulation received the highest scores for several attributes. WGF1 recorded the highest colour score (7.12) and overall acceptability score (7.24), while WGF4 had comparatively lower scores for colour (5.88), taste (5.94), texture (5.42), and overall acceptability within the tested formulations. The reduction in colour scores with higher substitution may be associated with the darker colour of the grasshopper-containing material and changes occurring during baking. Differences in taste and texture may likewise result from the incorporation of the processed insect powder and its interaction with the biscuit matrix. Nevertheless, the mean scores for the formulations remained around or above the midpoint of the nine-point hedonic scale, indicating that the products retained a generally acceptable sensory profile.

The relatively favourable aroma and taste responses observed in some substituted formulations may partly be related to flavour compounds generated during baking, including Maillard reaction products. The seasoning and deep-frying of the grasshoppers may also have contributed directly to the flavour and aroma characteristics of the final biscuits. This is an important consideration when interpreting the sensory results because the observed consumer response reflects the complete processing history of the ingredient rather than the effect of unprocessed *Z. variegatus* powder alone. The general acceptability of the insect-containing biscuits is consistent with previous findings that insect incorporation can produce acceptable bakery products when inclusion levels and processing conditions are appropriately controlled [26,33].

Conclusion

The study demonstrated that incorporating processed *Zonocerus variegatus* powder into wheat-based biscuits improved selected nutritional attributes, particularly protein and fibre contents, with the 15% formulation (WGF4) recording the highest values. The incorporation also modified the amino acid, mineral and vitamin profiles, while the measured anti-nutritional factors remained relatively low. Although fat, ash and carbohydrate contents varied among formulations without a consistent dose-dependent pattern, all grasshopper-containing biscuits remained generally acceptable to the sensory panel, despite the control receiving the highest overall acceptability.

The findings indicate that processed *Z. variegatus* powder has potential as a nutritional ingredient in wheat-based biscuits. However, because the grasshoppers were salted, seasoned and deep-fried before incorporation, the observed effects reflect the combined influence of the insect ingredient and its processing rather than the intrinsic properties of *Z. variegatus* alone. Further studies should therefore assess shelf life, microbial safety, instrumental biscuit quality, consumer acceptance, processing controls, and the economic feasibility of large-scale production.

Ethics approval and consent to participate

The sensory evaluation involving human participants was conducted in accordance with the principles of the Declaration of Helsinki and relevant institutional ethical guidelines. The study protocol was reviewed and approved by the Health Research Ethics Committee of Afe Babalola University, Ado-Ekiti, Nigeria (Approval No.: ABUAD/HREC/2025/097). Written informed consent was obtained from all participants before participation. Participation was voluntary, and

participants were informed about the purpose and procedures of the study, their right to withdraw at any time without penalty, and the confidentiality of information obtained during the study.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interest

The authors declare no conflict of interest.

Funding Declaration

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors Contributions

T.J and T.D: Conceptualization, methodology, data curation, formal analysis, software, writing original draft, writing - review & editing. S.S, F.P, A.K and T. D: Data curation, formal analysis, software, writing - original draft, writing - review & editing. T.D, A.K and T.J: Conceptualization, investigation, visualization, writing - original draft, review & editing.

Acknowledgment

The authors sincerely acknowledge the invaluable support of the research assistants for their contributions to sample collection, preparation, and data acquisition. The authors also appreciate the laboratory analyst for providing technical expertise and assistance with the laboratory analyses, which contributed to the quality and reliability of the study. The support of all individuals who contributed to the successful completion of this research is gratefully acknowledged.

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