LWT - Food Science and Technology 184 (2023) 115012

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Nutritional characteristics, microbial loads and consumer acceptability of cookies enriched with insect (*Ruspolia differens*) meal

Brian O. Ochieng [a,b,**], Joseph O. Anyango [b], Fathiya M. Khamis [a], Sunday Ekesi [a], James Peter Egonyu [a], Sevgan Subramanian [a], John M. Nduko [b], Dorothy Nakimbugwe [c], Xavier Cheseto [a], Chrysantus M. Tanga [a,*]

[a] International Centre of Insect Physiology and Ecology (icipe), P.O. BOX 30772, 00100, Nairobi, Kenya
[b] Department of Dairy and Food Science and Technology, Egerton University, P.O. Box 536, 20115, Njoro, Kenya
[c] Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, Makerere University, P.O. Box 7062, Kampala, Uganda

ARTICLE INFO

Keywords:
Edible grasshoppers
Ruspolia differens processing
Microbial quality
Nutritional composition
Safe cookie products

ABSTRACT

Utilization of *Ruspolia differens* Serville as functional food ingredient is rapidly gaining popularity. This study evaluated the nutrient quality, microbial safety and consumers' acceptability of cereal-based cookies fortified with various processed products of *R. differens* meals. Cookies fortified with blanched, boiled, and toasted *R. differens* meals had higher protein, fat and energy levels, respectively, than the control cookies. Enrichment of cookies with differentially processed *R. differens* meals had elevated levels of isoleucine and leucine. Omega-3 fatty acid, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate, was detected only in cookies prepared from wheat-insect meals blends. Blanched and boiled *R. differens* meal significantly ($p < 0.05$) boosted iron (1.70-folds) and zinc (1.12–1.16-folds) contents of the cookies. The cookie products had reduced *Enterobacteriaceae*, *S. aureus*, yeast and mould with permissible exposure limits for human consumption. The overall acceptability of insect-enriched cookie product by male and female respondents ranged between 57 and 80%. The survey revealed that the flavour, colour, mouthfeels and texture of the cookie products were important motivation for consumers to accept grasshoppers as a food source. Further research on the flavour of cookie products enriched with grasshopper meal would be required to increase acceptability to market-driven consumer appealing food products.

1. Introduction

The exponential rise in global population has consistently escalated the demand for quality proteins, prompting researchers to evaluate other sustainable sources like edible insects, which offer comparable or superior nutritional profiles to conventional animal sources (Acosta-estrada, Reyes, Rosell, Rodrigo, & Ibarra-herrera, 2021; Rumpold & Schlüter, 2013; van Huis et al., 2013). Edible insects are hence ideal for enriching widely recognized cereal-based products like wheat products with demonstrated nutritional protein inferiority (Pencharz, Elango, & Wolfe, 2016; Sozer, Nordlund, & Poutanen, 2017). The development of insect-based snacks presents a worthy vehicle for delivering essential insect nutrients to target consumers (Awobusuyi, Pillay, & Siwela, 2020) especially those with historic reluctance to consume whole insect (Olamide, Samuel, Adeniyi, & Taiwo, 2020). Of great concern is the safety of these insects before integration into products since, the collection of edible insects by entomophagists in developing countries is unregulated from the wild and the value chain is characterized by ineffective implementation of hygienic practices. (Imathiu, 2020).

Ruspolia differens Serville (Orthoptera:Tettigonidae) is a seasonally occurring edible insect in 21 African countries, constituting an important customary delicacy among various communities (Kinyuru, Kenji, Njoroge, & Ayieko, 2010; Ng'ang'a et al., 2019). Several researchers have investigated and reported its rich nutritional value, laying emphasis on their high protein content and unsaturated fats (Kinyuru, Kenji, Muhoho, & Ayieko, 2010; Rutaro et al., 2018; Ssepuuya, Smets, Nakimbugwe, Van Der Borght, & Claes, 2019). It is therefore recommended for supplementation and fortification of foods that are nutrient deficient like cereal porridges (Mmari, Kinyuru, Laswai, & Okoth, 2017) and bakery goods. However, because they are wild-sourced, they may be

* Corresponding author.
** Corresponding author. International Centre of Insect Physiology and Ecology (*icipe*), P.O. BOX 30772, 00100, Nairobi, Kenya.
E-mail addresses: bonyango@icipe.org (B.O. Ochieng), ctanga@icipe.org (C.M. Tanga).

https://doi.org/10.1016/j.lwt.2023.115012
Received 1 January 2023; Received in revised form 16 June 2023; Accepted 23 June 2023
Available online 23 June 2023

associated with microbial hazards (Belluco et al., 2013; Garofalo et al., 2019; Imathiu, 2020). Such hazards reportedly exacerbate edible insect-aversion amongst individuals unaccustomed to their consumption (Lange & Nakamura, 2021).

Ng'ang'a, Fombong, Kiiru, Kipkoech, and Kinyuru (2021) evaluated and reported microbial hazards and heavy metals as the major health risks in edible grasshopper. High total viable counts, lactic acid bacteria, *Enterobacteriaceae*, *Salmonella*, *Staphylococcus aureus*, bacterial endospores, yeasts and moulds are the main pathogenic and spoilage microorganisms that have been reported in raw harvested *R. differens*, posing health risk to potential consumers (Labu et al., 2021; Ng'ang'a et al., 2019, 2021, Nyangena et al., 2020). Interestingly, most thermal processes applicable to *R. differens* have been discovered to markedly subdue a majority of these microbes to acceptable limits (Labu et al., 2021; Ng'ang'a et al., 2019, 2021). These processing methods also destroy degradative enzymes, increase or decrease allergenicity and improve the sensory quality by introducing new aroma compounds (FAO, 2021; Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019).

Cookies have become one of the most popular snacks among both children and adults across nations due to its inexpensive manufacturing costs, increased convenience, long shelf life and ready-to-eat nature (Ayensu, Lutterodt, Annan, Edusei, & Loh, 2019; Noor Aziah, Mohamad Noor, & Ho, 2012). Cookies are the best choice as excellent carriers of a blend of different and varied functional ingredients, due to their formulation simplicity and ease of production, without compromising the sensory quality or shelf stability of the resulting products. As a result, their formulations are amenable to inclusion of other ingredients intended for nutritional enhancement (Ndife, Kida, & Fagbemi, 2017).

In this respect, several researchers have capitalized on the high value nutrient-dense biomass of edible insects to develop nutritious insect-based food products. For instance, edible insect meals such as that of mealworm (*Tenebrio molitor*), giant mealworms (*Zophobas atratus* Fabricius), silkworm (*Bombyx mori* Linnaeus), house cricket (*Acheta domesticus* Linnaeus), desert locust (Schistocerca gregaria Forskål) and termites (*Macrotermes* spp.), have been used to develop healthier nutrient-dense biscuit and cookie products (Awobusuyi, Siwela, & Pillay, 2020; Bawa, Songsermpong, Kaewtapee, & Chanput, 2020; Olamide et al., 2020; Sriprablom, Kitthawee, & Suphantharika, 2022; Tedjakusuma, Linggadiputra, Cahya, & Surya, 2022; Torres et al., 2022). The developed insect-based products were characterized by higher protein levels, essential amino acid and mineral profiles than their conventional counterparts, hence, offering valuable nutritional supply to consumers. Therefore, incorporation of insect meals into popular value-added baked products, such as cookies, could promote their fortification and utilization as functional food source. However, the use of *R. differens* in cookie products have received limited research attention, globally. The current study aimed at evaluating the nutritional content, microbial quality and consumer responses to sensory characteristics of wheat cookies enriched with differentially processed *R. differens* flours as an alternative to chicken eggs, which is conventionally used as a protein source in baking.

2. Methodology

2.1. Materials

Wild harvested and plucked (wings, appendages and ovipositors removed) *R. differens* weighing 20 kg were purchased from Masaka (0 20′ 28.0″ S 31 44′10.0″ E) and Kampala (0.3476 N, 32.5825 E) in Uganda. The samples were put into sterile sample collecting plastic containers, stored in cold boxes with flaked ice (4–7 °C) and transported to the International Centre of Insect Physiology and Ecology *(icipe)* laboratory. *Ruspolia differens* samples of 700 g each were blanched (100 °C/5 min), boiled (100 °C/15 min), toasted (for 10 min) and deep-fried in palm cooking oil (175 °C until crunchy/dark brownish) and subsequently oven-dried at 60 °C for 24 h following procedures outlined by Ochieng et al. (2022). The samples were milled in a three-speed Waring laboratory blender (Camlab, Over, UK) before being screened through a 0.1 mm stainless steel laboratory sieve. They were then vacuum packaged in sterile Ziploc bags, labeled and stored at 4 °C for further analysis. A representation of the basic nutritional characteristics of the processed *R. differens* and the wheat flour is shown in Table 1.

2.2. Blending of flours and cookies preparation

Processed *R. differens*-based cookies and the control cookies were prepared according to a method described by Noor Aziah et al. (2012), with a few modifications. Based on a continuously proven marginal acceptance of sensory features of bakery products previously manufactured with insect flours at a 10% (w/w) inclusion level, self-raising wheat was substituted with processed *R. differens* at 10% (w/w) (Adeboye, Bolaji, & Fatola, 2016; Awobusuyi, Siwela, & Pillay, 2020; González, Garzón, & Rosell, 2019; Ogunlakin, Oni, & Olaniyan, 2018; Ojinnaka, Ofoelo, & Ezenwa, 2015; Osimani et al., 2018). Also, this was based on Kenya Bureau of Standards' (KEBS) proposed minimum allowable level of edible insects in products of 10% (w/w) (KEBS, 2020a). The control cookies, contained chicken eggs as main protein source, specifically resembling conventional cookie formulations while the insect-based cookies contained only processed insect ingredients as protein sources. Approximately 172.2 g sugar and 3.4 g salt were sieved and mixed with 408.2 g of self-raising wheat flour (pre-mixed with food-grade improvers) for 5 min. Approximately 172.2 g of shortening (hydrogenated palm oil) was added and mixed in a bakery mixer (BJY-BM10, Berjaya, Malaysia) for 15 min to produce a creamy mixture. About 84 g of whole eggs (for control) or processed *R. differens* flours was added, and the mixture mixed for another 10 min. This was followed by hand-kneading of the dough for 5 min to yield a firm and uniform dough weighing about 180 g a piece. The dough was rolled out to a thickness of 5 mm using a rolling pin and cut into 5 cm diameter circles on a wooden board. The cut-out cookie doughs were placed 50 mm apart on greased baking trays and baked in a preheated oven for 15 min at 180 °C, 30% relative humidity of air (BISTROT 665; BestFor®, Ferrara, Italy). The formulation and baking process was repeated thrice for the doughs with eggs (control), blanched, boiled, toasted and deep-fried *R. differens* ingredient culminating into 15 experimental materials. Cookies from each replicate dough were placed in sterile plastic Ziploc bags and marked correspondingly for microbial analysis. The remaining cookie samples were stored in a cold room at −10 °C awaiting nutritional analyses.The schematic representation of *R. differens* processing, cookies preparation and experiments conducted is shown in Fig. 1.

2.3. Proximate determinations

Proximate parameters (moisture content, carbohydrates, protein content, ash content, dry matter, fat content and fibre content) of the cookies were assayed following the Association of Official Analytical Chemists methods (AOAC, 2000) methods. Briefly, dry matter and moisture were measured by sample drying in a conventional oven (WTB binder, Tuttlingen, Germany) at 130 °C for 3 h. Protein content was estimated using a Kjeldahl analyzer (Velp UDK 159, Velp Scientifica, Europe) and subsequently converted to protein using a factor of 6.25 for quantification as reported by Boulos, Tännler, and Nyström (2020). Fat content was solvent-extracted in petroleum ether in a Soxhlet extraction system (Velp SER 148, Velp Scientifica, Europe). Ash content was assessed by sample incineration in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 550 °C for 12 h. Fibre content was acid and base-digested in a fibre analyzer (FIWE, Velp Scientifica, Europe) and evaluated based on loss upon ignition. Carbohydrate content was estimated by subtracting the fat, protein, ash and moisture content from 100. The total energy [kcal/100 g] was calculated with the following Atwater formula: $Total\ Energy = 4 \times$

Table 1
Basic characteristics (proximate components) of processed *R. differens* and wheat flours used in cookies preparation.

Flour type	Dry matter (g/100 g db)	Moisture content (g/100 g db)	Protein content (g/100 g db)	Fat content (g/100 g db)	Ash content (g/100 g db)	Fibre content (g/100 g db)	Carbohydrate (g/100 g db)
[§]Blanched *R. differens* flour	98.2 ± 0.05[c]	1.8 ± 0.05[c]	40.1 ± 1.33[c]	43.8 ± 0.41[c]	2.2 ± 0.00[b]	11.2 ± 0.01[c]	N.D.
[§]Boiled *R. differens* flour	85.6 ± 0.10[a]	14.4 ± 0.10[e]	43.1 ± 1.60[d]	36.3 ± 1.06[b]	2.3 ± 0.09[bc]	10.9 ± 0.19[c]	N.D.
[§]Toasted *R. differens* flour	98.4 ± 0.06[d]	1.6 ± 0.06[b]	44.7 ± 1.03[d]	46.0 ± 0.82[c]	2.4 ± 0.17[bc]	9.0 ± 0.74[b]	N.D.
[§]Deep-fried *R. differens* flour	99.2 ± 0.03[e]	0.8 ± 0.03[a]	7.8 ± 0.59[a]	83.0 ± 1.54[d]	1.2 ± 0.16[a]	8.7 ± 0.39[b]	N.D.
Wheat flour	87.9 ± 0.00[b]	12.1 ± 0.00[d]	12.6 ± 0.10[b]	1.7 ± 0.10[a]	2.5 ± 0.04[c]	0.6 ± 0.04[a]	70.4 ± 0.18

Values are expressed as means ± standard deviations. Means in the same column followed by different small superscript letters are significantly different at $p < 0.05$
[§]Source: (Ochieng et al., 2022).

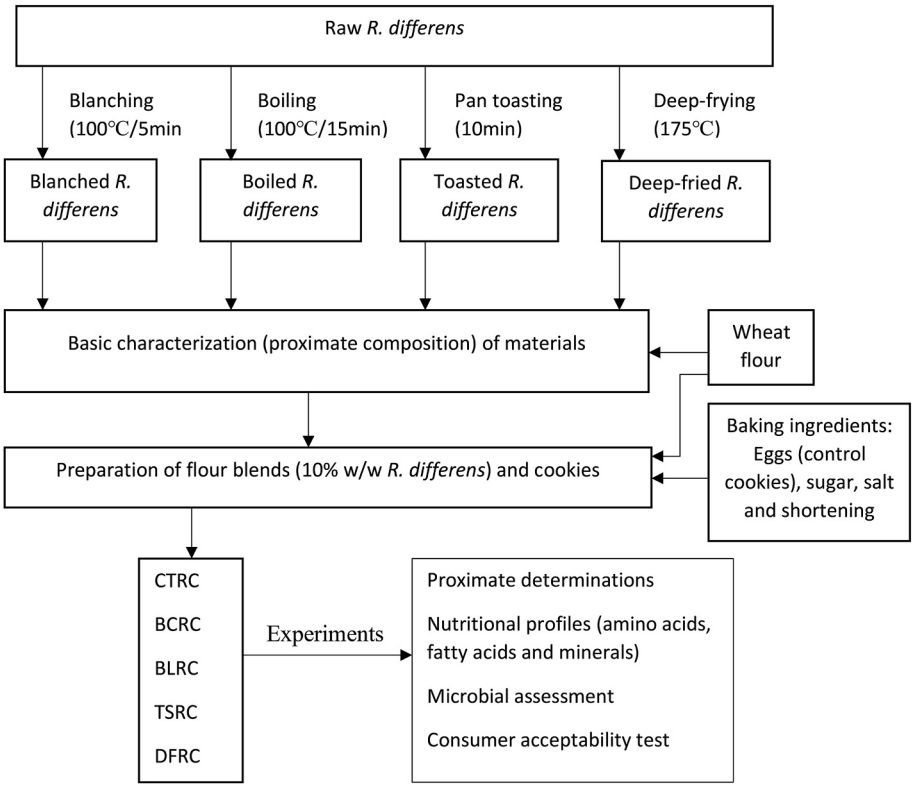

Fig. 1. A schematic summary of *R. differens* processing, cookies preparation and experiments conducted. CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

$protein\ (\%) + 4 \times carbohydrate(\%) + 9 \times fat(\%)$ (FAO, 2003). All the determinations were performed in triplicates.

2.4. Amino acid profile

The amino acid composition was determined as previously described by Musundire, Osuga, Cheseto, Irungu, and Torto (2016) with some modification. Briefly, on an analytical scale, cookie samples (10 mg) were carefully weighed and transferred into 5 mL Supelco® micro-reaction vials. Afterwards, 1.5 mL of 6 mol/L HCl was added, followed by nitrogen and finally capped. To achieve full hydrolysis, the samples were conditioned at 110 °C for 24 h. The hydrolysates were then vacuum evaporated to complete dryness, reconstituted in 1 mL of a 95:5 mixture of 0.002 mol/L formic acid and acetonitrile, vortex-shaken for 30 s, sonicated at 50 kHz for 30 min and centrifuged (Eppendorf AG, 22331 Hamburg, Germany) at 14 000 rpm. The supernatants were filtered into 2 mL clear glass vial (Supelco, Bellefonte, PA, USA) and immediately analysed (0.2 μL) by UPLC-MS/MS (Waters XEVO TQ-S, Waters Technologies, USA) in scan mode. Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford, MA) fitted with an ACQUITY UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 μm particle size; Waters Corp., Wexford, Ireland, oven temp 45 °C). The autosampler tray was cooled to a temperature of 5 °C. The mobile phase comprised of (A) water and (B) methanol (solvent B) both acidified with 0.002 mol/L formic acid. The gradient system used was 0–2 min, 5% B, 2–4 min, 40% B, 4–7 min, 40% B, 7–8.5 min 60% B, 8.5–10 min 60% B, 10–15 min, 80% B, 15–19 80% B, 19–20.5 min, 100% B, 20.5–23 min, 100% B, 23–24 min 95% B, 24–26 min, 95% B. The flow rate was maintained at 0.2 mL/min throughout the experiment. The UPLC was connected to a Waters Xevo TQ-S electrospray ionization (ESI) full scan MS in positive ionization mode. The m/z range 40–1000 with a capillary voltage of 0.5 kV, sampling cone voltage of 30 V, source temperature 150 °C desolvation temperature of 120 °C. The nitrogen desolvation flow rate was 800 L/h. Data was acquired using MassLynx version 4.1 SCN 712 (Waters). Mass spectrometric data, retention time, and co-injection of the hydrolysate with an authentic

standard amino acid mixture were used to identify the amino acids. Amino acid standard solution (AAS 18) obtained from Sigma-Aldrich (Chemie GmbH, Munich, Germany) was used for external quantification of the amounts of each amino acid present. This was repeated three times using different batches of replicated cookie samples.

2.5. Fatty acids profile

The fatty acids were assessed as fatty acid methyl esters (FAMEs) of the cookies (100 mg each) following modified protocols previously used (Cheseto, Baleba, Tanga, Kelemu, & Torto, 2020). Sodium methoxide solution (15 mg/mL) of 1 mL was introduced into each sample then the mixture vortexed for 1 min, ultrasonicated for 10 min, and incubated for 1 h in a water bath at 70 °C. To quench the reaction, 100 μL of distilled deionized water was added, followed by another 1 min of vortexing. To extract the resultant FAMEs, 1 mL of gas chromatography (GC)-grade hexane (Sigma–Aldrich, St. Louis, MO, USA) was added and centrifuged at 14,000 rpm for 5 min. The supernatant was filtered, dried over anhydrous sodium sulphate and analysed (1.0 μL) by GC-MS on a 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a 5975 C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The GC was fitted with a (5%-phenyl)--methylpolysiloxane (HP5 MS) low bleed capillary column (30 m _ 0.25 mm i.d., 0.25_μm; J&W, Folsom, CA, USA). At a flow rate of 1.25 mL/min, helium was used as the carrier gas. The oven temperature was programmed to rise from 35 to 285 °C at a rate of 10 °C min^{-1} with the initial and final temperatures set to hold for 5 min and 20.4 min, respectively. The temperatures of the ion source and quadrupole mass selective detector were kept at 230 and 180 °C, respectively. The electron impact (EI) spectrum masses were measured at a 70 eV acceleration energy. In full scan mode, fragment ions were studied throughout a mass range of 40–550 m/z. The duration of the filament delay was set at 3.3 min. Authentic standard, methyl octadecanoate (0.2–125 ng/L), prepared from octadecanoic acid (95% purity) (Sigma-Aldrich, St. Louis, MO) and serially diluted, was also analysed by GC-MS in full scan mode to generate a linear calibration curve (peak area vs. concentration) with the equation below;

$$Y = \left[5 \times 10^7 x\right] + \left[2 \times 10^7\right] \text{ with } R^2 = 0.9997 \quad \text{Equation 1}$$

Where; the gradient $= 5 \times 10^7$, y-intercept $= 2 \times 10^7$ and R^2 = coefficient of determination

External quantification of the various fatty acids from the samples was done using this regression equation. The following integration parameters were used to generate peak spectral masses using ChemStation B.02.02. acquisition software: 3 for initial threshold, 0.010 for initial peak width, 1 for initial area reject, and 'on' for shoulder detection. NIST 05, 08, and 11 mass spectra and retention durations were compared to authentic standards and reference spectra published in library–MS databases. The FAMES in all of the samples were determined in triplicates.

2.6. Mineral profile

The mineral profiles of the cookies were analysed in accordance with AOAC (2000). Ground samples weighing 0.5 g were weighed into digestion tubes containing, mixed with 8.0 mL concentrated (16.2 mol/L) nitric acid and 2 mL of 9.8 mol/L hydrogen peroxide, and permitted to stand overnight in a fume hood. The samples were then transferred to a microwave digestion machine (Multiwave Go Plus, Anton Paar, VA, US) and exposed to a temperature-time digestion schedule of 100 °C for 10 min and then adjusted to 180 °C for 10 min. Cooled clear solutions, signifying completely digested materials, were placed into 25 mL falcon tubes and adjusted to the mark with 0.4 mol/L nitric acid. Inductively coupled plasma optical emission spectrometry (ICP-OES) measurements (Optima 2100TMDV ICP-OES, PerkinElmer Massachusetts, USA) were used to examine the contents of the minerals under study in the samples and standard solution. The ICP-OES had the following operational parameters: Radio frequency power-1450 W; Plasma gas flow rate-15 L/min; Auxiliary gas flow rate-0.2 L/min; Nebulizer gas flow rate-0.8 L/min; Sample flow rate1.5 L/min; View mode-axial mode; The read-peak area; Source equilibration time-10 s; Read delay time-10 s; Replicates-1; Background correction-2-point (manual point correction); Spray chamber-Scott type; Nebulizer cross-Flow GemTip Nebulizer (HF resistant); The detector-CCD; Purge gas-nitrogen; Shear gas-air; Plasma gas-nitrogen. The distinctive elemental spectra of each mineral were measured at the following wavelengths: Magnesium – 285.213 nm, Iron – 259.939 nm, Manganese – 257.61 nm, Calcium – 317.933 nm, Phosphorous – 213.617 nm, Potassium – 766.49 nm, Aluminium – 396.153 nm, Copper – 224.7 nm and Cobalt – 228.616 nm. ICP-OES mix standard CatNo.43843 (Sigma-Aldrich, USA) prepared by serially diluting with 0.4 mol/L nitric acid to generate calibration standards of 400, 800, 2000, and 4000 g/L were also assessed by the ICP-OES, yielding linear calibration curves with elemental correlation coefficients of $R^2 = 0.999$ for all the minerals investigated. On a PerkinElmer Winlab 32 software (PerkinElmer, USA), external standard calibration and data gathering were accomplished. All of the mineral elements were quantified using the data gathered. The determinations were replicated three times with different batches of cookies.

2.7. Microbial assays

The sanitary qualities of the cookies and their respective processed *R. differens* flours were established. In a sterile filter stomacher bag (Bagmixer 400W, Interscience, St. Nom, France), 5 g of samples were blended with 45 mL of sterile peptone physiological salt solution (0.14 mol/L sodium chloride, 1 g/L peptone (OXOID LP0034), and pH of 7.0 ± 0.2 at 25 °C and homogenized at normal speed for 1 min (Seward, 400 circulator, West Sussex, UK). A 10-fold serial dilution of the homogenate with 1 mL was then performed for TVC, *Enterobacteriaceae*, *Staphylococcus aureus* and yeast and moulds except for *Salmonellae*. These microbial analyses were specifically selected because they serve as indicators of food safety and contamination. The homogenates (0.1 mL) were aliquoted and inoculated onto approximately 20 mL of freshly prepared, sterile, solidified media. TVC was determined using Plate Count Agar (Oxoid CM0463) and incubated at 30 °C for 48 h (Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). Lactose Positive *Enterobacteriaceae* was assessed on MacConkey agar (Oxoid CM0007) and incubated at 37 °C for 24 h (Nyangena et al., 2020). *Staphylococcus aureus* were evaluated on a Baird Parker agar (Oxoid CM1127) enriched with 50 g/L of Egg Yolk Tellurite Emulsion (Oxoid CM0276) and later incubated at 35 °C for 48 h. Colonies of characteristic circular (2–3 mm diameter), smooth, convex, moist appearance were enumerated (Ramashia, Tangulani, Mashau, & Nethathe, 2020). For *Salmonellae*, the samples (25 g) were first enriched in 225 mL of nutrient broth comprising 5 g peptone, 5 g NaCl, 1 g Lab-Lemco beef extract, and 2 g yeast extract per 1L of water, pH 7.4 (Oxoid CM0067), and then incubated at 35 °C for 24 h. Homogenates (25 mL) were further selectively enriched in 225 mL of Rappaport-Vassiliadis broth (Oxoid CM0669) and incubated at 37 °C for 24 h. Rappaport-Vasiliadis broth culture was collected using a sterile wire loop, streaked over Salmonella- Shigella Agar (Oxoid CM0099), and plates incubated at 37 °C for 24 h. *Salmonella* spp. were detected as colourless colonies with black centres (Nyangena et al., 2020). Yeast and moulds were cultured on Potato Dextrose Agar (PDA) (Oxoid Ltd., United Kingdom) then incubated at 25 °C for 5 days after which colonies were examined and enumerated using a magnifying lens (Ramashia et al., 2020).

2.8. Consumer acceptability

The sensory perception of the fortified cookies was assessed according to Dubost, Shewfelt, and Eitenmiller (2003). Briefly, 143

untrained panellists (72 males and 71 females) of age ranging 18-50 years, were randomly chosen for the exercise. The evaluation of the cookies was based on the following parameters: colour, flavour, texture, mouthfeel and overall acceptability. A 3-point acceptability scale (1 = dislike, 2 = neither like nor dislike and 3 = like) was adopted for the rating of the experimental cookies. The panellists were informed of the aim of the study and instructed to consent to the study on the sensory questionnaire before commencement. This sensory study was approved by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO)-Veterinary Science Research Institute (VSRI); Muguga North upon compliance with all provisions vetted under and coded: KALRO-VSRI/IACUC028/16032022. This study was reviewed and approved by Egerton University and the National Council for Science Technology and Innovation in Kenya (NACOSTI/P/21/8303). Further, we also obtained the informed consent of the participants via the statement "I am aware that my responses are confidential, and I agree to participate in this survey as well as affirming that I can withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

2.9. Statistical analysis

Statistical analyses were conducted using R Studio software version 1.3.1093–1 (R Core Team, 2020) for windows. One-way ANOVA was performed to determine significant differences in proximate components, amino acids profiles, fatty acids profiles, mineral profiles and individual microbial counts for the cookies enriched with *R. differens* flours prepared by blanching, boiling, toasting and deep-frying. Tukey HSD multiple comparison test was used to identify statistically heterogeneous subsets at $\alpha = 0.05$. All microbial counts were expressed as log cfu/g. Means and standard deviation of three replicates were reported for each analysis.

3. Results

3.1. Proximate composition of the cookies

The proximate parameters; moisture content, dry matter, protein content, fat content, ash content, carbohydrate and energy varied significantly ($p < 0.05$) across the cookie types except for fibre (Table 2). The moisture content of the control cookies was significantly ($p < 0.05$) higher than the insect-based cookies. Protein content of the insect-based cookies (BCRC, BLRC, and TSRC) were significantly ($p < 0.05$) greater than the CTRC and DFRC. The insect-based cookies (BCRC, BLRC, TSRC and DFRC) manifested significantly ($p < 0.05$) higher levels of fat and energy than the control cookies (CTRC).

3.2. Amino acid profiles of the cookies

Eleven amino acids (five essential amino acids and six non-essential amino acids) were detected from the prepared cookies (Table 3). Isoleucine and leucine of the essential amino acids and tyrosine and cystine of the non-essential amino acids were significantly ($p < 0.05$) higher in BCRC, BLRC and TSRC than in the CTRC and DFRC. DFRC were characterized with significantly ($p < 0.05$) lower amino acid levels. Valine, glutamic acid, and alanine were the most abundant amino acids in all the cookie types.

3.3. Fatty acid profile of the cookies

The fatty acid spectra and total fatty acid categories of the cookie types are shown in Table 4 and Fig. 2, respectively. A total of 32 fatty acids were detected from the four cookies types. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) constituted 52.8%, 45.8%, and 1.4%, respectively in the control cookies and 24.6–49.4%, 36.3–49.8%, and 0.8–30.0%, respectively in the cookies enriched with insect meals. The most abundant fatty acids compositionally were discovered to be methyl hexadecanoate of the SFAs, methyl 9*Z*-octadecenoate of the MUFAs, and methyl (9*Z*,12*Z*)-octadecadienoate of the PUFAs. The PUFA, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate, was only detected in insect meal enriched cookies with BCRC and BLRC exhibiting higher concentrations. However, methyl (9*Z*,12*Z*)-octadecadienoate and methyl 9*Z*-octadecenoate displayed incredible quantities in the cookies enriched with insect meals than in the control cookies.

3.4. Mineral profiles of the cookies

The mean concentration of minerals in the cookies, with the exception of P, showed significant ($p < 0.05$) variations (Table 5). Calcium level was significantly ($p < 0.05$) higher in control cookies (CTRC) than in the cookies enriched with *R. differens* meals. On the other hand, BCRC exhibited zinc (4.33 mg/100g) and iron (7.11 mg/100g) whereas BLRC exhibited zinc (4.17 mg/100g) and iron (7.12 mg/100g) which were significantly ($p < 0.05$) higher than those detected from control cookies (CTRC).

3.5. Microbial quality of the cookies and their respective insect ingredients

The total viable counts (TVC), *Enterobacteriaceae* and *Staphylococcus aureus* significantly ($p < 0.05$) differed among the four processed insect meals whereas *Salmonella* spp., and yeast and moulds colonies were both not identified (Table 6). Blanched *R. differens* meals recorded significantly higher counts of TVC, *Enterobacteriaceae* and *S. aureus* than the other processed insect meals. The cookies expressed low microbial levels characterized by non-detection of both *S. aureus* or *Salmonella* spp., low

Table 2
Proximate composition and gross energy of processed R. differens based cookies.

Enriched cookies	Moisture (g/100g wb)	DM (g/100g)	Protein content (g/100 g db)	Fat content (g/100 g db)	Ash content (g/100 g db)	Fibre content (g/100 g db)	CHO (g/100 g db)	Energy (kcal/100 g db)
CTRC	6.17 ± 1.26^{b}	93.83 ± 1.26^{a}	7.74 ± 0.40^{a}	17.76 ± 0.41^{a}	2.64 ± 0.02^{b}	0.06 ± 0.02^{a}	65.63 ± 1.12^{b}	453.33 ± 6.70^{a}
BCRC	2.83 ± 0.29^{a}	97.17 ± 0.29^{b}	11.09 ± 0.65^{b}	19.61 ± 1.08^{ab}	2.06 ± 0.01^{a}	0.17 ± 0.14^{a}	64.23 ± 1.45^{ab}	477.81 ± 4.10^{b}
BLRC	3.00 ± 0.87^{a}	97.00 ± 0.87^{b}	10.90 ± 0.08^{b}	20.68 ± 0.20^{b}	2.23 ± 0.29^{ab}	0.22 ± 0.33^{a}	62.97 ± 0.86^{a}	481.59 ± 1.28^{b}
TSRC	2.33 ± 0.29^{a}	97.67 ± 0.29^{b}	10.99 ± 0.24^{b}	19.84 ± 0.53^{b}	2.56 ± 0.01^{b}	0.07 ± 0.03^{a}	64.21 ± 0.20^{ab}	479.37 ± 3.70^{b}
DFRC	1.33 ± 0.58^{a}	98.67 ± 0.58^{b}	6.78 ± 0.14^{a}	25.82 ± 0.89^{c}	2.20 ± 0.29^{ab}	0.08 ± 0.02^{a}	63.79 ± 0.43^{ab}	514.69 ± 6.52^{c}

Values are presented as mean ± standard deviation of triplicate determinations. Means in the same column followed by different small superscript letters are significantly different at $p < 0.05$. kcal = Kilocalories; CHO= Carbohydrate; DM = Dry matter; CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

Table 3
Amino acid profiles detected in cookies enriched with different processed R. differens.

Amino acid	CTRC (mg/g)	BCRC (mg/g)	BLRC (mg/g)	TSRC (mg/g)	DFRC (mg/g)	$F_{(4,10)}$	*p*-value
Essential Amino Acids							
Phenylalanine	4.41 ± 0.28^{a}	4.91 ± 0.42^{a}	4.08 ± 0.40^{a}	4.53 ± 0.50^{a}	3.93 ± 0.54^{a}	2.4	ns
Isoleucine	4.00 ± 0.84^{a}	5.62 ± 0.82^{b}	6.07 ± 0.33^{b}	5.56 ± 0.34^{b}	3.39 ± 0.16^{a}	12.47	$p < 0.001$
Leucine	7.93 ± 0.68^{ab}	8.97 ± 0.42^{b}	8.60 ± 0.22^{b}	8.57 ± 0.22^{b}	7.32 ± 0.28^{a}	7.93	$p < 0.01$
Methionine	2.49 ± 0.16^{a}	3.08 ± 0.33^{a}	2.82 ± 0.07^{a}	2.65 ± 0.42^{a}	2.72 ± 0.18^{a}	2.11	ns
Valine	20.37 ± 0.72^{a}	22.41 ± 2.46^{a}	19.40 ± 1.82^{a}	19.77 ± 4.93^{a}	16.38 ± 1.45^{a}	1.95	ns
Non-Essential Amino acids							
Tyrosine	3.41 ± 0.06^{a}	4.18 ± 0.12^{b}	4.17 ± 0.12^{b}	4.08 ± 0.17^{b}	3.57 ± 0.11^{a}	28.08	$p < 0.001$
Arginine	11.88 ± 0.48^{b}	13.73 ± 0.25^{c}	11.27 ± 0.91^{b}	12.68 ± 0.80^{bc}	9.51 ± 0.50^{a}	18.61	$p < 0.001$
Cystine	4.54 ± 0.21^{a}	8.75 ± 1.44^{bc}	9.96 ± 0.32^{c}	6.72 ± 1.32^{ab}	5.83 ± 0.49^{a}	17.21	$p < 0.001$
Proline	4.14 ± 1.09^{a}	7.53 ± 0.55^{b}	5.25 ± 0.24^{a}	6.17 ± 0.85^{ab}	5.48 ± 0.82^{ab}	7.98	$p < 0.01$
Glutamic acid	18.00 ± '1.88^{a}	21.54 ± 0.68^{a}	19.58 ± 0.48^{a}	20.69 ± 2.66^{a}	18.67 ± 0.20^{a}	2.74	ns
Alanine	21.63 ± 0.37^{b}	24.13 ± 0.76^{b}	23.21 ± 1.37^{b}	23.14 ± 2.47^{b}	14.75 ± 0.75^{a}	23.55	$p > 0.001$

Values are presented as mean ± standard deviation of triplicate determinations. Means in the same row followed by same small superscript letters are not significantly different at $p < 0.05$. ns = not significant; CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

counts of TVC (less than 30), and no observation of *Enterobacteriaceae*, and yeast and moulds.

3.6. Consumer preferences

Fig. 3 illustrates a comparison of the consumer preference of the various cookie products based on different attributes between male and female respondents. A larger proportion of male (90.3% and 79.2%) and female (85.9% and 81.7%) respondents liked the colour of CTRC and DFRC. Only 52.8% of the male respondents and 40.8% of the female respondents liked the flavour of BCRC. However, 75% of the male respondents liked the flavour of BLRC and DFRC whereas for the females, 77.5% liked the CTRC with 71.8% liking the DFRC flavour. Both the male (73.6%) and female (73.2%) respondents preferred the texture of CTRC. Higher number of both male (83.3% and 73.6%) and female (77.5% and 78.9%) respondents generally accepted CTRC and DFRC (Fig. 4).

4. Discussion

The higher moisture and lower dry matter exhibited in control cookies (CTRC) compared to the insect-based cookies was as a consequence of direct inclusion of raw eggs in the control cookies formulation mix. Eggs reportedly contain higher moisture content of 78 g/100g wb (Ogunwole, Yinka, & Ojelade, 2015) compared with the processed *R. differens* flours (Ochieng et al., 2022) explaining the high moisture in the CTRC. The moisture content of the insect-based cookies reflected the moisture content of palm weevil flour-fortified biscuits reported by Ayensu et al. (2019). This suggest that insect enriched cookies have low moisture contents as the insect ingredients used are often subjected to dehydrative pre-processes. The low moisture content of processed insect flours and insect-based cookie products was ideal to ensure improved and extended shelf life as well as reduced the susceptibility to microbial and chemical related deteriorations.

The improved protein contents observed in the cookie products could be attributed to the source of protein used and their inclusion levels. Whole eggs used in CTRC formulation has been reported to contain protein levels of 11.54–11.56 g/100 g db (Ogunwole et al., 2015) compared to 40.1–44.7 g/100 g db in blanched, boiled and toasted *R. differens* (Ochieng et al., 2022) and may account for the differences observed in the respective cookies. Deep-fried *R. differens*, on the other hand, had a low protein content associated to a sequence of biochemical processes that markedly disintegrates proteins (Bordin, Tomihe Kunitake, Kazue Aracava, & Silvia Favaro Trindade, 2013). The low protein levels of the deep-fried *R. differens* were mirrored in the baked cookies since they were used directly in dough formulation. The protein contents in all of the insect-enriched cookies fell short of the Kenya Bureau of Standards (KEBS) requirement of 15 g/100 g (KEBS, 2020b). To enhance the protein levels in such insect-based products, utilization of defatted *R. differens* as an enrichment ingredient is imperative as earlier demonstrated in another insect (Lee et al., 2020). This strategy could be effective due to the fact that fats are compositionally the highest proximate components in *R. differens* (Ssepuuya, et al., 2019) and therefore their removal would proportionally enhance protein levels.

The fat and energy content of the insect-based cookies was higher than the control cookies. This is unsurprising because, *R. differens* has a fat content of 42.2–54.3 g/100 g (Ssepuuya, et al., 2019) which may have been reflected in the cookies. Similar results were replicated in cookies enriched with sorghum-termite blends (Awobusuyi, Siwela, & Pillay, 2020). These fats, on the other hand, are high in polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs), which may reduce the shelf life of the insect-based cookies even further if the insect ingredients are not defatted. However, this study did not consider defatting the insect ingredients as one of the aims was to compare the fatty acids profiles. These fat levels play a critical role in influencing the cookies' energy levels (Omoba & Omogbemile, 2013) since the carbohydrate levels were comparable (Table 2). Due to the carry-over effect from deep fat frying of *R. differens*, fat was conspicuously higher in the DFRC (Table 1) compared to the other cookie types. Apparently, the control cookies (CTRC) had the lowest energy compared to the insect-based cookies which corroborates the report by Awobusuyi, Pillay, and Siwela (2020).

Amino acids profiles of the CTRC compared favourably with the insect-based cookies. Alanine, aspartic acid, glutamic acid, and essential amino acids leucine, lysine, and valine have been reported in raw *R. differens* in appreciable levels (Ssepuuya, et al., 2019). This is clearly evident in Table 3 where alanine, glutamic acid and valine were the most quantitatively dominant amino acids, particularly in cookies supplemented with blanched, boiled, and toasted *R. differens*, which had higher mean protein levels (Ochieng et al., 2022). On the other hand, eggs are regarded as standard and valuable source of all essential amino acids including lysine (Attia, Al-harthi, & Korish, 2020). Despite the fact that lysine is abundant in both eggs and *R. differens*, it was not detected in the cookies, which could be due to processing-related losses (Cheng, Jin, & Zhang, 2014). Specifically, the *R. differens* underwent a two-stage cooking (initial blanching, boiling, toasting and deep-frying then baking) which may have rendered the amino acids unavailable in the baked foods. Typically, this manifested in DFCR, which had relatively low amounts of the majority of amino acids due to the fact that it was made from deep-fried *R. differens* exposed to the harshest processing in oil at 175 °C (Ochieng et al., 2022).

Methyl hexadecanoate, methyl 9*Z*-octadecenoate and methyl (9*Z*,12*Z*)-octadecadienoate were the most abundant fatty acids (FAs) in SFAs, MUFAs and PUFAs, respectively. The identified dominant FAs and

Table 4
Fatty acid composition (μg/mg) of cookies made from different processed R. differens analysed by Gas Chromatography coupled to Mass Spectrometry (GC-MS).

tR (min)	FAME (μg/mg)	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
14.81	Methyl octanoate		C8:0	N.D.	N.D.	N.D.	N.D.	N.D.
16.85	Methyl decanoate	Capric acid	C10:0	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	N.D.	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}
14.88	Methyl nonanoate		C9:0	N.D.	N.D.	N.D.	N.D.	N.D.
17.36	Methyl butanoate		C4:0	N.D.	N.D.	N.D.	N.D.	N.D.
18.95	Methyl dodecanoate	Lauric acid	C12:0	0.11 ± 0.01^{a}	0.22 ± 0.06^{a}	0.18 ± 0.05^{a}	0.17 ± 0.11^{a}	0.36 ± 0.21^{a}
20.83	Methyl 12-methyltridecanoate	Tridecyclic acid	iso-methyl-C13:0	N.D.	N.D.	N.D.	N.D.	N.D.
21.24	Methyl tetradecanoate	Myristic acid	C14:0	0.62 ± 0.10^{a}	0.69 ± 0.55^{a}	0.59 ± 0.48^{a}	0.64 ± 0.20^{a}	0.98 ± 0.22^{a}
21.8	Methyl 4,8-dimethylnonanoate		iso-dimethyl-C9:0	N.D.	0.01 ± 0.00	N.D.	N.D.	N.D.
23.52	Methyl hexadecanoate	Palmitic acid	C16:0	10.58 ± 1.33^{ab}	5.02 ± 8.32^{a}	9.99 ± 2.85^{ab}	10.37 ± 2.53^{ab}	12.73 ± 0.59^{b}
25.49	Methyl octadecanoate	Stearic acid	C18:0	2.43 ± 0.18^{a}	3.07 ± 0.20^{a}	2.84 ± 0.35^{a}	2.60 ± 0.47^{a}	2.84 ± 0.13^{a}
26.99	Methyl eicosanoate	Arachidic acid	C20:0	N.D.	N.D.	0.59 ± 0.02	N.D.	N.D.
26.99	Methyl 18-methylnonadecanoate		iso-methyl-C19:0	N.D.	0.78 ± 0.02	N.D.	N.D.	N.D.
28.61	Methyl docosanoate	Behenic acid	C22:0	0.11 ± 0.01	N.D.	N.D.	N.D.	N.D.
29.37	Methyl tricosanoate	Trycosylic acid	C23:0	0.06 ± 0.02^{a}	N.D.	0.04 ± 0.01^{a}	N.D.	0.08 ± 0.03^{a}
30.13	Methyl tetracosanoate	Lignoceric acid	C24:0	0.15 ± 0.00^{b}	0.16 ± 0.01^{b}	0.13 ± 0.02^{ab}	0.08 ± 0.01^{a}	0.14 ± 0.04^{ab}
32.08	Methyl hexacosanoate	Cerotic acid	C26:0	0.07 ± 0.01^{b}	0.06 ± 0.00^{ab}	0.05 ± 0.02^{ab}	0.01 ± 0.00^{a}	0.06 ± 0.02^{ab}
	∑ SFA			14.14	10.02	14.42	13.9	17.21
20.97	Methyl 11*Z*-tetradecenoate	Vaccenic acid	C14:1 (n-3)	0.01 ± 0.00	N.D.	N.D.	N.D.	N.D.
20.97	Methyl 4*Z*-octenoate		C8:1(n-4)	N.D.	N.D.	0.01 ± 0.00	N.D.	N.D.
22.09	Methyl 5*Z*-dodecenoate		C12:1(n-7)	N.D.	N.D.	N.D.	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}
22.15	Methyl 13-Methyl 9*Z*-tetradecenoate	Myristoleic	iso-methyl-C14:1 (n-5)	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00
23.12	Methyl 9*Z*-hexadecenoate	Palmitoleic acid	C16:1(n-7)	0.26 ± 0.03^{a}	0.77 ± 0.15^{b}	0.73 ± 0.17^{b}	0.62 ± 0.23^{b}	0.56 ± 0.03^{ab}
24.1	Methyl 10*Z*-heptadecenoate		C17:1 (n-7)	0.10 ± 0.01^{ab}	0.12 ± 0.02^{b}	0.09 ± 0.03^{ab}	0.04 ± 0.00^{a}	0.11 ± 0.03^{ab}
25.22	Methyl 9*Z*-octadecenoate	Oleic acid	C18:1(n-9)	11.55 ± 0.37^{a}	17.20 ± 2.24^{a}	13.16 ± 3.05^{a}	12.08 ± 2.17^{a}	16.19 ± 4.01^{a}
26.79	Methyl 11*Z*-eicosenoate		C20:1(n-9)	0.32 ± 0.05^{b}	0.38 ± 0.04^{b}	0.32 ± 0.09^{b}	0.14 ± 0.01^{a}	0.37 ± 0.08^{b}
28.43	Methyl 11*Z*-docosenoate		C22:1(n-11)	N.D.	0.04 ± 0.00^{a}	N.D.	0.05 ± 0.05^{a}	N.D.
28.43	Methyl 13*Z*-docosenoate	Erucic acid	C22:1(n-9)	N.D.	N.D.	0.05 ± 0.00^{a}	N.D.	0.04 ± 0.005^{a}
29.95	Methyl 15*Z*-tetracosenoate		C24:1(n-9)	0.03 ± 0.00^{b}	N.D.	0.02 ± 0.00^{a}	N.D.	0.05 ± 0.00^{c}
49.35	Methyl 8*E*-octadecenoate		C18:1(n-10)	N.D.	0.02 ± 0.00	N.D.	N.D.	N.D.
	∑ MUFA			12.27	18.53	14.38	12.94	17.34
24.69	Methyl (12,15)-octadecadienoate		C18:2(n-3)	N.D.	0.04 ± 0.00^{a}	N.D.	0.01 ± 0.00^{b}	N.D.
25.18	Methyl (9*Z*,12*Z*)-octadecadienoate	Linoleic acid	C18:2(n-6)	0.19 ± 0.05^{a}	12.10 ± 10.31^{c}	10.71 ± 9.16^{c}	5.46 ± 9.25^{b}	0.24 ± 0.05^{a}
26.3	Methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate	α Linolenic acid	C18:3(n-3)	N.D.	2.12 ± 0.97^{b}	1.55 ± 0.21^{ab}	0.49 ± 0.01^{a}	0.34 ± 0.00^{a}
26.45	Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate	Arachidonic acid	C20:4(n-6)	0.14 ± 0.09	N.D.	N.D.	N.D.	N.D.
	∑ PUFA			0.33	14.26	12.26	5.96	0.58
	∑ n-6 PUFA			0.33	12.10	10.71	5.46	0.24
	∑ n-3 PUFA			N.D.	2.16	1.44	0.50	0.34
	∑ n-6/n-3			N.D.	5.60	7.44	10.92	0.71
	∑PUFA/SFA			0.02	1.42	0.85	0.43	0.03

Values are presented as Mean ± SD (standard deviation) of triplicate determinations. N.D. = Not determined; tR= Retention time, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies; FAME=Fatty acids methyl ester.

the proportions of SFAs, MUFAs and PUFAs in the insect-based cookies reflected the pattern demonstrated in their respective processed insects (Ochieng et al., 2022). Likewise, the trend characterized by higher PUFAs in BDRC and BLRC and higher SFAs in TSRC and DFRC was witnessed by Ochieng et al. (2022) in the respective processed *R. differens* used for the cookies development. A similar trend was reported by Cheseto et al. (2020), which revealed that baking likely had no effect on the fatty acid quality and patterns.

The appearance and disappearance of particular FAs in cookies in comparison to processed *R. differens* could be due to dough formulation inconsistencies and the influence of baking circumstances. In the CTRC, the proportions of FAs followed the increasing order: SFA (14.1%) >MUFA (12.3%) >PUFA (0.3%). This implies that eggs, which were incorporated in the CTRC, contained appreciably higher levels of SFAs than MUFAs and PUFAs. The detection of appreciable levels, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate (α linolenic acid) (omega 3), methyl

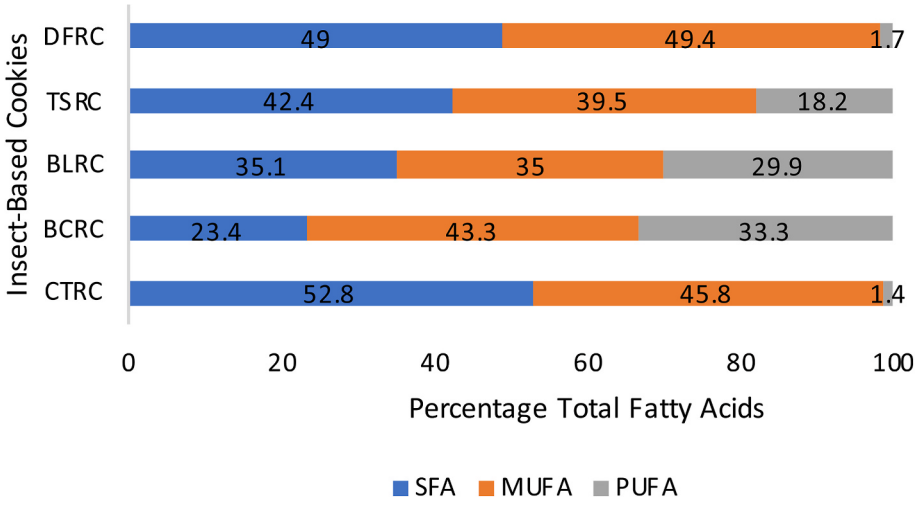

Fig. 2. Categories of the total fatty acids (%) detected in the processed *R. differens*-based cookies. CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

Table 5
Mineral profiles of processed R. differens-based cookies.

Enriched Cookies	CTRC	BCRC	BLRC	TSRC	DFRC	$F_{(4,10)}$	*p*-value
Macro minerals							
Mg (mg/100g)	24.55 ± 0.59^{b}	25.15 ± 0.01^{b}	24.12 ± 0.20^{ab}	23.53 ± 0.47^{ab}	22.34 ± 0.40^{a}	6.73	$p < 0.01$
Ca (mg/100g)	20.17 ± 0.52^{d}	17.78 ± 0.11^{c}	16.64 ± 0.85^{bc}	15.09 ± 0.76^{a}	15.30 ± 0.02^{ab}	40.97	$p < 0.001$
P (mg/100g)	290.80 ± 3.52^{a}	298.12 ± 19.80^{a}	289.33 ± 21.64^{a}	281.50 ± 4.14^{a}	275.80 ± 5.49^{a}	1.22	ns
Na (mg/100g)	905.63 ± 8.92^{b}	922.32 ± 28.50^{b}	852.89 ± 14.03^{a}	886.05 ± 3.89^{ab}	917.27 ± 8.57^{b}	10.17	$p < 0.01$
K (mg/100g)	145.22 ± 3.87^{a}	145.87 ± 6.66^{a}	151.88 ± 4.42^{ab}	160.59 ± 2.69^{b}	147.30 ± 7.30^{ab}	4.38	$p < 0.05$
Trace Elements							
Fe (mg/100g)	4.19 ± 0.01^{a}	7.11 ± 0.10^{c}	7.12 ± 0.37^{c}	5.09 ± 0.02^{b}	5.67 ± 0.43^{b}	72.69	$p < 0.001$
Cu (μg/100g)	128.83 ± 5.38^{a}	301.30 ± 7.65^{bc}	346.79 ± 21.74^{d}	331.08 ± 21.74^{cd}	273.30 ± 8.50^{b}	115.37	$p < 0.001$
Mn (mg/100g)	681.28 ± 20.80^{a}	808.71 ± 45.14^{c}	760.99 ± 16.25^{c}	748.75 ± 17.33^{bc}	692.95 ± 0.92^{ab}	13.45	$p < 0.001$
Zn (mg/100g)	3.73 ± 0.12^{a}	4.33 ± 0.08^{b}	4.17 ± 0.03^{b}	3.86 ± 0.16^{a}	3.79 ± 0.00^{a}	21.82	$p < 0.001$
Al (mg/100g)	0.75 ± 0.03^{a}	2.52 ± 0.01^{d}	1.94 ± 0.03^{c}	1.92 ± 0.11^{c}	1.45 ± 0.04^{b}	413.91	$p < 0.001$

Results are presented as mean ± standard deviation. Same small superscript letters within rows indicate no significant differences of the minerals at $p < 0.05$. CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

Table 6
Microbial levels (Log cfu/g) and detection of Salmonellae in the processed R. differens flours and respective cookie types developed.

Processing method	TVC (Log cfu/g)	Lac + *Enterobacteriaceae* (Log cfu/g)	*Staphylococcus aureus* (Log cfu/g)	*Salmonellae* (Log cfu/g)	Yeast and Moulds (Log cfu/g)
Blanching	4.54 ± 0.11^{c}	3.04 ± 0.22^{b}	4.83 ± 0.02^{b}	N.D.	N.D.
Boiling	2.57 ± 0.05^{a}	N.D.	2.61 ± 0.17^{a}	N.D.	N.D.
Toasting	3.38 ± 0.10^{b}	1.01 ± 0.03^{a}	3.70 ± 0.33^{a}	N.D.	N.D.
Deep-frying	2.42 ± 0.33^{a}	N.D.	N.D.	N.D.	N.D.
Cookie types	**TVC** (Log cfu/g)	**Lac + *Enterobacteriaceae*** (Log cfu/g)	***Staphylococcus aureus*** (Log cfu/g)	***Salmonellae*** (Log cfu/g)	**Yeast and Moulds** (Log cfu/g)
CTRC	<30	N.D.	N.D.	N.D.	N.D.
BCRC	<30	N.D.	N.D.	N.D.	N.D.
BLRC	<30	N.D.	N.D.	N.D.	N.D.
TSRC	<30	N.D.	N.D.	N.D.	N.D.
DFRC	<30	N.D.	N.D.	N.D.	N.D.

Means on the same column followed by the same small letters are not significantly different ($p < 0.05$; n = 3). N.D. = Not determined; TVC = Total viable counts; YMC=Yeast and moulds; Lac + ve = Lactose positive; CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

(9*Z*,12*Z*)-octadecadienoate (linoleic acid) (omega 6) and methyl 9*Z*-octadecenoate (oleic acid) in the insect-based cookies than in the control cookies, implies that insect-neophobic consumers can still access the health benefits of oils endowed in insects through developed products. α Linolenic acid (ALA) plays a key role as precursors for the synthesis of prostaglandin, thromboxane and leukotriene and are both neuro and cardioprotective (Blondeau et al., 2015). Oleic acid and linoleic acid are known to be anti-inflammatory, anti-dermatitis, anticancer agents, suppress coronary disorders, play a role in glycolysis, hypertension control, insulin sensitivity and resistance (Atowa et al., 2021; Glick & Fischer, 2013)

Ruspolia differens has been demonstrated to furnish considerable

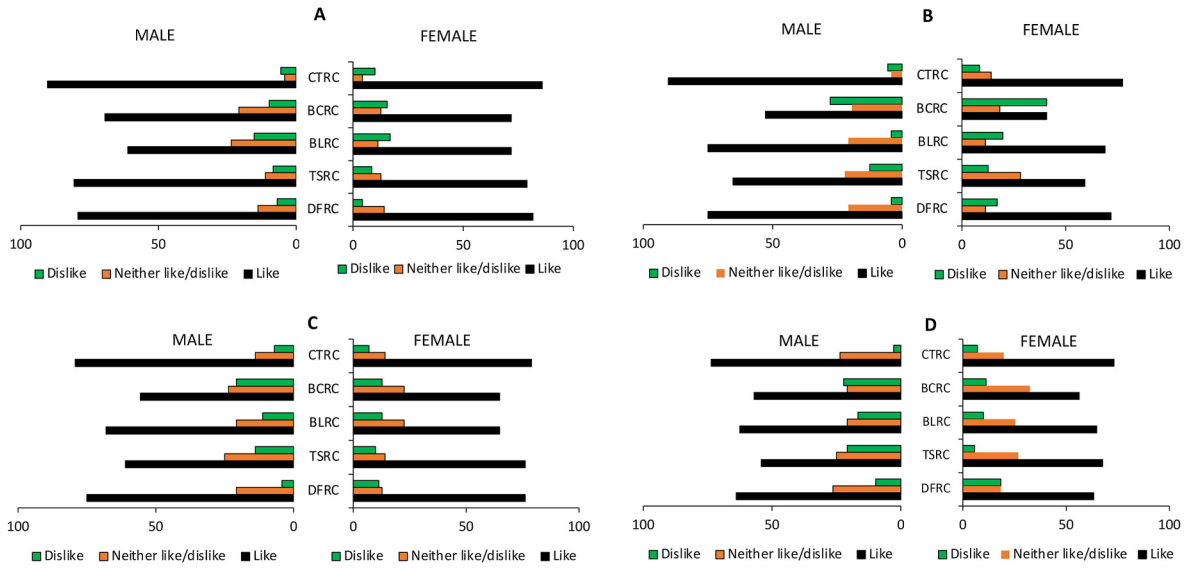

Fig. 3. Distribution of respondents (%) based on their sensory perception of the cookies. **A**: Colour; **B**: Flavour; **C**: Mouthfeel and **D**: Texture and **E**. CTRC = "Control" (i.e., cookies without processed *R. differens* meal); BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

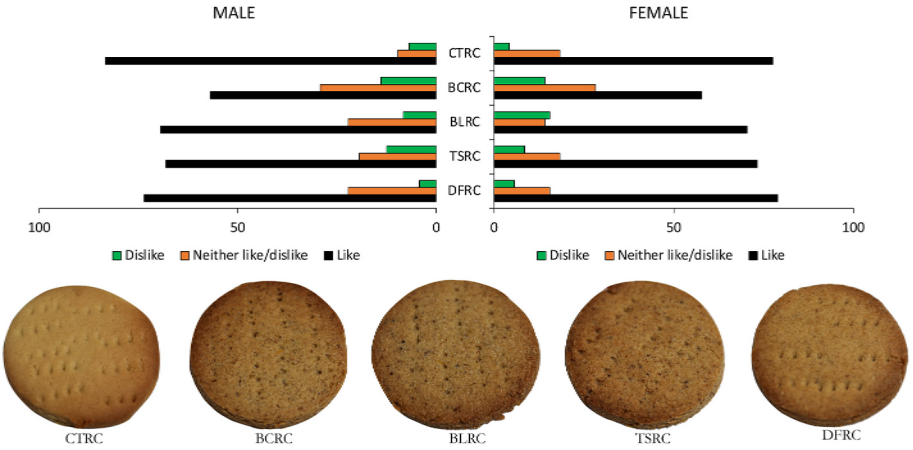

Fig. 4. Overall consumer acceptability of various cookie products. CTRC = "Control" (i.e., cookies without processed *R. differens* meal); BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC = Toasted *R. differens*-based cookies; DFRC = Deep fried *R. differens*-based cookies.

quantities of macro minerals K, P, and Mg, which, with the exception of Ca, favourably equate with or surpass animal products including beef, pork, and eggs (Ssepuuya, et al., 2019). Except for DFRC, this assumption is clearly supported by Table 5, which shows that the levels of minerals in all cookies showed negligible variation. The calcium levels in CTRC were significantly higher than those in the insect-based cookies. This is because *R. differens* has lower levels of Ca attributable to the lack of mineralized skeleton (Kinyuru, Kenji, Njoroge, & Ayieko, 2010) as opposed to chicken eggs. *R. differens* also had significant quantities of the trace elements Fe, Zn, Cu and Mn, exceeding those found in pork and chicken (Ssepuuya, et al., 2019). This study reinforces the earlier report by indicating significant differences in relation to the levels of these trace minerals between the CTRC and insect-based cookies (mainly BCRC, BLRC and TSRC) (Table 5). These findings replicate reports by Ayensu et al. (2019) where the levels of trace minerals proportionally increased with increasing levels of palm weevil incorporation. This suggests that the average mineral in the final baked products may have mirrored the patterns of minerals in the respective ingredients. Therefore, fortification of cookies with *R. differens* can be used to combat micronutrient related deficiency among populations unaccustomed to entomophagy.

Similar to other insects, *R. differens* has a lot of moisture, a good pH, and is nutrient dense, thus it's conducive for colonization by a variety of microbiological organisms (Klunder et al., 2012; Ssepuuya, et al., 2019). Furthermore, elevated microbial counts in harvested insects could be due to wild collection in an unregulated environment, unsanitary handling environments, and poorly cleansed hands, or handling equipment (Gatheru et al., 2019; Ng'ang'a et al., 2019). Therefore, eating edible insects may endanger consumers' health by offering serious health risks (Belluco et al., 2013). To reduce these health risks, proper processing, handling, and storage practices have been advocated (Rumpold & Schlüter, 2013). The processing strategies used in this investigation had a significant impact on the counts of microorganisms. Higher levels of TVC, *Staphylococcus aureus*, and *Enterobacteriaceae* observed in blanched *R. differens* demonstrate the inefficiency of blanching in suppressing a variety of microorganisms. TVC values in blanched *R. differens* are comparable to those found in blanched mealworm larvae after 10 s (Vandeweyer, Lenaerts, Callens, & Van Campenhout, 2017), 4 min blanched house cricket, 1 min blanched termites, 5 min blanched Bingula caterpillar and 1 min blanched mealworm

larvae (Caparros-Megido et al., 2017). Despite their greater levels in blanched *R. differens*, the TVC were within acceptable limits of less than Log_{10} 7 cfu/g, a measure of hygienic condition of food (Nyangena et al., 2020; Ramashia et al., 2020). This hygiene criterion was adapted from the Superior Health Council (SHC) and the Federal Agency for the Safety of the Food Chain (FASFC) guideline, which suggested using process hygiene standards for minced meat described in EU Regulation (EC) No. 1441/2007 for edible insects (Ssepuuya, et al., 2019). In comparison to toasted samples, lower TVC levels were discerned in the boiled and deep-fried samples. This is because boiling and deep-frying cause higher heat dispersion into the food matrices (Nyangena et al., 2020) than in toasting. In this investigation, deep-frying resulted in low levels of *Staphylococcus aureus*, *Enterobacteriaceae*, yeast and moulds, and completely subdued *Salmonellae*. The effectiveness of deep-frying in decontaminating edible insects has been reported by other authors (Gatheru et al., 2019; Labu et al., 2021). Because of their great heat sensitivity, *Enterobacteriaceae* numbers were entirely eliminated after boiling and deep-frying (Ng'ang'a et al., 2019). Their existence in toasted samples, on the other hand, indicates that heat transfer to the core (guts) was ineffective throughout this process (Klunder et al., 2012). The non-detection of *Salmonellae* spp. in all the samples justifies their sensitivities to heat, culminating into products compliant with microbiological guidelines on food safety (KEBS, 2020b). Low yeast and mould counts in all processed samples indicates that they were severely suppressed during processing, resulting in counts below the suggested maximum limits of 5 log10 CFU/g (Ramashia et al., 2020; Vandeweyer et al., 2017).

Despite Ssepuuya, et al. (2019) reporting pathogenic microorganisms of public health concern from raw *R. differens*, the insect based-cookies in the current study expressed permissible microbial levels and can be regarded safe for consumption. Similar results were reported on biscuits fortified with palm weevil larvae (Ayensu et al., 2019) and energy dense-biscuits enriched with silkworm pupae and locusts (Olamide et al., 2020). It is possible that use of pre-treated insect ingredients and maintenance of a high degree of human and environmental hygiene throughout ingredient formulations contributed to the low microbial counts. Furthermore, the baking temperatures (180 °C/15 min) may have markedly subdued majority of the microorganisms while the stringent adherence to post-baking hygiene standards, with prompt packaging into sterile zip lock bags may have curbed post-baking contamination from the surroundings. Finally the low water activities and pH in cookies have also been reported to restrain bacterial growth and colonization (Khan, Hashmi, & Saleem, 2017).

Consumer acceptability of insect-based products are partly a subject to organoleptic characteristics such as flavour, aroma, colour, texture, taste and appearance (Wilkinson et al., 2018; Yazici & Ozer, 2021). The larger proportion of both male and female respondents liking CTRC and DFRC colours may be due to the familiar golden-brown colour they exhibited, as in common bakery products. Relatively few respondents liked the dark-brown colours of BCRC, BLRC and TSRC which emerged from intense Maillard reaction (Ogunlakin et al., 2018), stimulated by the high levels of available proteins in the respective processed ingredients (Ochieng et al., 2022). The increased dislike of the flavour of BCRC in both male and female respondents may be linked to high concentration of residual displeasing aroma compounds since, they were formulated with mildly processed *R. differens*, characterized by limited aroma transformation. The greater disparity in the liking of the flavour of BCRC between male (52.8%) and female (40.8%) respondents is articulative to the reports elucidated by Kröger, Dupont, Büsing, and Fiebelkorn (2022) citing insect acceptance to positively correlate with masculinity but to negatively associate with femininity. The liking of flavour of DFRC and BLRC may be accredited to processing-induced formation of pleasant aroma during *R. differens* processing. A majority of the respondents preferred the texture of the CTRC as compared to the insect-based cookies. This may be explained by the gritty effect of the insect exoskeleton (Ojinnaka et al., 2015) used in the formulation of BRCR, BLRC, TSRC and DFRC. Most respondents generally accepted the CTRC and DFRC, which could be indicative of their congruity that these novel consumer familiar products could be a good alternative market-driven products.

5. Conclusion

This study demonstrates that cookies supplemented with blanched, boiled and toasted *R. differens* flour are richer in proteins, amino acids (alanine, glutamic acid, and valine), minerals (Fe, Zn, Cu, and Mn) than the control and those with deep-fried *R. differens* meal. This uncovers the detrimental effects of deep-frying on key nutrients retention in food products development. *Ruspolia differens* meal fortified cookie products showed higher energy levels than cookies without processed *R. differens*. Microbial loads were substantially suppressed in boiled and deep-fried *R. differens* meals. Remarkably, all the baked cookie products with inclusion of *R. differens* revealed that there was complete microbial elimination. The high level of acceptability of the insect meal enriched cookie products reflects the consumer preference for novel insect-based food products, thus providing more insights for advanced production of market-driven consumer insect-based food products that entices the growing entomophagists populace. However, future studies on full scale sensory evaluation including the nine-point hedonic scale would be crucial to assess the degree of consumers' liking of various insect-based food products based on their sensory appeals.

CRediT authorship contribution statement

Brian O. Ochieng: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing, and Visualization. **Joseph O. Anyango:** Conceptualization, Methodology, Visualization, Validation, Investigation, Writing – Review & Editing and Supervision. **Fathiya M. Khamis:** Conceptualization, Methodology, Investigation, Writing – Review & Editing. **Sunday Ekesi:** Project Administration, Funding Acquisition, Writing – Review & Editing. **Sevgan Subramanian:** Project Administration, Resources, Writing – Review & Editing. **James P. Egonyu:** Project Administration, Writing – Review & Editing. **John M. Nduko:** Conceptualization, Validation, Writing – Review & Editing and Supervision. **Xavier Cheseto:** Methodology, Software, Formal Analysis, Investigation, Writing – Review & Editing and Visualization. **Dorothy Nakimbugwe:** Funding Acquisition, Conceptualization, Writing – Review & Editing, Visualization and Validation. **Chrysantus M. Tanga:** Conceptualization, Methodology, Investigation, Validation, Resources, Writing – Review & Editing, Visualization, Supervision, Project Administration and Funding Acquisition. All authors read, reviewed, and approved the final manuscript.

Funding sources

Financial support for this research was provided by the BioInnovate Africa Programme (INSBIZ—Contribution ID No. 51050076), the Curt Bergfors Foundation Food Planet Prize Award, Bill & Melinda Gates Foundation (INV-032416), Norwegian Agency for Development Cooperation, the Section for research, innovation, and higher education (RAF–3058 KEN–18/0005); Australian Centre for International Agricultural Research (ACIAR) (ProteinAfrica – Grant No: LS/2020/154), the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); Australian Centre for International Agricultural Research (ACIAR), the Federal Democratic Republic of Ethiopia and the Government of the Republic of Kenya. The first author, Brian O. Ochieng, was financially supported by the Center of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) of Egerton University, Kenya. *The funders had no role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript. Therefore, the views expressed herein do not necessarily reflect the official opinion of the donors.*

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors wish to thank Jackton Ooko Ongere, Rachami Isaiah, Levi Ombura and Maureen Ong'onge for their substantial contribution in providing technical support during data collection.

References

Acosta-estrada, B. A., Reyes, A., Rosell, C. M., Rodrigo, D., & Ibarra-herrera, C. C. (2021). Benefits and challenges in the incorporation of insects in food products. *Frontiers in Nutrition, 8*. https://doi.org/10.3389/fnut.2021.687712

Adeboye, A., Bolaji, T., & Fatola, O. (2016). Nutritional composition and sensory evaluation of cookies made from wheat and palm weevil larvae flour blends. *Annals. Food Science and Technology, 17*(2), 543–547.

Atowa, C. O., Okoro, B. C., Umego, E. C., Atowa, A. O., Emmanuel, O., Ude, V. C., et al. (2021). Nutritional values of *Zonocerus variegatus* , *Macrotermes bellicosus* and *Cirina forda* insects : Mineral composition , fatty acids and amino acid profiles bellicosus and Cirina forda insects : Mineral composition , fatty acids and amino acid profiles. *Scientific African, 12*, Article e00798. https://doi.org/10.1016/j.sciaf.2021.e00798

Attia, Y. A., Al-harthi, M. A., & Korish, M. A. (2020). Protein and amino acid content in four brands of commercial table eggs in retail markets in relation to human requirements. *Animals, 10*(3), 406–416.

Awobusuyi, T. D., Pillay, K., & Siwela, M. (2020). Consumer acceptance of biscuits supplemented with a sorghum–insect meal. *Nutrients, 12*(4), 895–907. https://doi.org/10.3390/nu12040895

Awobusuyi, T. D., Siwela, M., & Pillay, K. (2020). Sorghum–insect composites for healthier cookies: Nutritional, functional, and technological evaluation. *Foods, 9* (10), 1427–1441.

Ayensu, J., Lutterodt, H., Annan, R. A., Edusei, A., & Loh, S. P. (2019). Nutritional composition and acceptability of biscuits fortified with palm weevil larvae (*Rhynchophorus phoenicis* Fabricius) and orange-fleshed sweet potato among pregnant women. *Food Science and Nutrition, 7*(5), 1807–1815. https://doi.org/10.1002/fsn3.1024

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020). Nutritional, sensory, and texture quality of bread and cookie enriched with house cricket (*Acheta domesticus*) powder. *Journal of Food Processing and Preservation, 44*(8), 14601–14609. https://doi.org/10.1111/jfpp.14601

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C., Paoletti, M., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety, 12*(3), 296–313. https://doi.org/10.1111/1541-4337.12014

Blondeau, N., Lipsky, R. H., Bourourou, M., Duncan, M. W., Gorelick, P. B., & Marini, A. M. (2015). Alpha-linolenic acid : An omega-3 fatty acid with neuroprotective properties — ready for use in the stroke clinic. *BioMed Research International*, Article 519830.

Bordin, K., Tomihe Kunitake, M., Kazue Aracava, K., & Silvia Favaro Trindade, C. (2013). Changes in food caused by deep fat frying - a review. *Archivos Latinoamericanos de Nutricion, 63*(1), 5–13.

Boulos, S., Tännler, A., & Nyström, L. (2020). Nitrogen-to-Protein conversion factors for edible insects on the Swiss market: *T. molitor, A. domesticus*, and *L. migratoria*. *Frontiers in Nutrition, 7*, 1–12. https://doi.org/10.3389/fnut.2020.00089

Caparros-Megido, R. C., Desmedt, S., Blecker, C., Béra, F., Haubruge, É., Alabi, T., et al. (2017). Microbiological load of edible insects found in Belgium. *Insects, 8*(1), 12–19. https://doi.org/10.3390/insects8010012

Cheng, L., Jin, C., & Zhang, Y. (2014). Investigation of variations in the acrylamide and Nε-(carboxymethyl) lysine contents in cookies during baking. *Journal of Food Science, 79*, T1030–T1038.

Cheseto, X., Baleba, S., Tanga, C. M., Kelemu, S., & Torto, B. (2020). Chemistry and sensory characterization of a bakery product prepared with oils from African edible insects. *Foods, 9*, 800–826. https://doi.org/10.3390/foods9060800

Dubost, N. J., Shewfelt, R. L., & Eitenmiller, R. R. (2003). Consumer acceptability, sensory and instrumental analysis of peanut soy spreads. *Journal of Food Quality, 26*, 27–42.

FAO. (2003). Food energy-Methods of analysis and conversion factors. In Food and agriculture organization of the united nations technical workshop report (Vol. 77, pp. 8-9). Agricultural Research Service, US Department of Agriculture.

FAO. (2021). *Looking at edible insects from a food safety perspective. Challenges and opportunities for the sector*. https://doi.org/10.4060/cb4094en

Garofalo, C., Milanović, V., Cardinali, F., Aquilanti, L., Clementi, F., & Osimani, A. (2019). Current knowledge on the microbiota of edible insects intended for human consumption : A state-of-the-art review. *Food Research International, 125*, Article 108527. https://doi.org/10.1016/j.foodres.2019.108527

Gatheru, J. W., Khamis, F. M., Ombura, F. L. O., Nonoh, J., Tanga, C. M., Maina, J., et al. (2019). Impact of processing methods on microbial load of reared and wild-caught edible crickets (*Scapsipedus icipe* and *Gryllus bimaculatus*) in Kenya. *Journal of Insects as Food and Feed, 5*, 171–183. https://doi.org/10.3920/jiff2018.0042

Glick, N. R., & Fischer, M. H. (2013). The role of essential fatty acids in human health. *Evidence-based Complementary and Alternative Medicine, 18*, 268–289. https://doi.org/10.1177/2156587213488788

González, C. M., Garzón, R., & Rosell, C. M. (2019). Insects as ingredients for bakery goods. A comparison study of *H. illucens, A. domestica* and *T. molitor* flours. *Innovative Food Science and Emerging Technologies, 51*, 1–26.

AOAC. (2000). In W. Horwitz (Ed.), *Official methods of AOAC international* (17th ed.). AOAC International.

van Huis, A., Klunder, H., Van Itterbeeck, J., Merten, E., Halloran, A., Muir, G., et al. (2013). Edible insects. Future prospects for food and feed security. *Food and Agriculture Organization of the United Nations*. https://doi.org/10.1017/CBO9781107415324.004

Imathiu, S. (2020). Benefits and food safety concerns associated with consumption of edible insects. *NFS Journal, 18*, 1–11. https://doi.org/10.1016/j.nfs.2019.11.002

KEBS. (2020a). *Draft Kenya standard edible insects Part 2 : Products containing specification edible insects —*.

KEBS. (2020b). *Edible insects Part 1 : Edible insects'products - specification*.

Khan, S., Hashmi, S., & Saleem, Q. (2017). Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical & Biological Archives, 3*, 38–48.

Kinyuru, J. N., Kenji, G. M., Muhoho, S. N., & Ayieko, M. (2010). Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya district, Kenya. *Journal of Agriculture, Science and Technology, 12*, 32–46. http://elearning.jkuat.ac.ke/journals/ojs/index.php/jagst/article/view/7.

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2010). Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (*Macrotermes subhylanus*) and grasshopper (*Ruspolia differens*). *Food and Bioprocess Technology, 3*, 778–782. https://doi.org/10.1007/s11947-009-0264-1

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food Control, 26*, 628–631. https://doi.org/10.1016/j.foodcont.2012.02.013

Kröger, T., Dupont, J., Büsing, L., & Fiebelkorn, F. (2022). Acceptance of insect-based food products in Western societies: A systematic review. *Frontiers in Nutrition, 8*, 1–26. https://doi.org/10.3389/fnut.2021.759885

Labu, S., Subramanian, S., Khamis, F. M., Akite, P., Kasangaki, P., Chemurot, M., et al. (2021). Microbial contaminants in wild harvested and traded edible long-horned grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae) in Uganda. *Journal of Insects as Food and Feed*. https://doi.org/10.3920/jiff2020.0069, 1–12.

Lange, K. W., & Nakamura, Y. (2021). Edible insects as future food: Chances and challenges. *Journal of Future Foods, 1*, 38–46. https://doi.org/10.1016/j.jfutfo.2021.10.001

Lee, S., Choi, Y., Jo, K., Kim, T., Yong, H. I., & Jung, S. (2020). Quality characteristics and protein digestibility of Protaetia brevitarsis larvae. *Journal Of Animal Science And Technology, 62*, 741–752.

Melgar-Lalanne, G., Hernández-Álvarez, A. J., & Salinas-Castro, A. (2019). Edible insects processing: Traditional and innovative technologies. *Comprehensive Reviews in Food Science and Food Safety, 18*, 1166–1191. https://doi.org/10.1111/1541-4337.12463

Mmari, M. W., Kinyuru, J. N., Laswai, H. S., & Okoth, J. K. (2017). Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper *Ruspolia differens* (Serville 1838) in Tanzania. *Journal of Ethnobiology and Ethnomedicine, 13*, 1–11. https://doi.org/10.1186/s13002-017-0191-6

Musundire, R., Osuga, I. M., Cheseto, X., Irungu, J., & Torto, B. (2016). Aflatoxin contamination detected in nutrient and anti-oxidant rich edible stink bug stored in recycled grain containers. *PLoS One, 11*, 1–16. https://doi.org/10.1371/journal.pone.0145914

Ndife, J., Kida, F., & Fagbemi, S. (2017). Production and quality assessment of enriched cookies from whole wheat and full fat soya. *European Journal of Food Science and Technology, 2*, 19–28.

Ng'ang'a, J., Fombong, F., Kiiru, S., Kipkoech, C., & Kinyuru, J. (2021). Food safety concerns in edible grasshoppers: A review of microbiological and heavy metal hazards. *International Journal of Tropical Insect Science, 41*, 2103–2111.

Ng'ang'a, J., Imathiu, S., Fombong, F., Ayieko, M., Vanden Broeck, J., & Kinyuru, J. (2019). Microbial quality of edible grasshoppers *Ruspolia differens* (Orthoptera: Tettigoniidae): From wild harvesting to fork in the Kagera Region, Tanzania. *Journal of Food Safety, 39*, 12549–12554. https://doi.org/10.1111/jfs.12549

Noor Aziah, A. A., Mohamad Noor, A. Y., & Ho, L. H. (2012). Physicochemical and organoleptic properties of cookies incorporated with legume flour. *International Food Research Journal, 19*, 1539–1543. https://doi.org/10.1080/23311932.2016.1172389

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., et al. (2020). Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. *Foods, 9*, 574–589. https://doi.org/10.3390/foods9050574

Ochieng, B. O., Anyango, J. O., Nduko, J. M., Cheseto, X., Mudalungu, C. M., Khamis, F. M., et al. (2022). Dynamics in nutrients, sterols and total flavonoid content during processing of the edible Long-Horned grasshopper (*Ruspolia differens* Serville) for food. *Food Chemistry, 383*, Article 132397. https://doi.org/10.1016/j.foodchem.2022.132397

Ogunlakin, G. O., Oni, V. T., & Olaniyan, S. A. (2018). Quality evaluation of biscuit fortified with edible termite (*Macrotermes nigeriensis*). *Asian Journal of Biotechnology and Bioresource Technology, 4*(2), 1–7. https://doi.org/10.9734/ajb2t/2018/43659

Ogunwole, O. A., Yinka, A., & Ojelade, P. (2015). Proximate composition and physical characteristics of eggs from laying chickens fed different proprietary vitamin-mineral premixes under two rearing systems during storage. *International Journal of Food Science and Nutrition Engineering, 5*, 59–67. https://doi.org/10.5923/j.food.20150501.08

Ojinnaka, M. C., Ofoelo, M. U., & Ezenwa, L. I. (2015). Nutritional evaluation of wheat cakes enriched with edible African termites (*Macrotermes nigeriensis*). *Agro-Science, 12*, 35–42. https://doi.org/10.4314/as.v12i3.5

Olamide, A., Samuel, O., Adeniyi, V., & Taiwo, S. (2020). Silkworm pupae (*Bombyx mori*) and locusts as alternative protein sources for high-energy biscuits. *Journal of Asia-Pacific Entomology, 23*, 234–241. https://doi.org/10.1016/j.aspen.2020.01.003

Omoba, O. S., & Omogbemile, A. (2013). Physicochemical properties of sorghum biscuits enriched with defatted soy flour. *British Journal of Applied Science & Technology, 3*, 1246–1256.

Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., et al. (2018). Bread enriched with cricket powder (*Acheta domesticus*): A technological, microbiological and nutritional evaluation. *Innovative Food Science and Emerging Technologies, 48*, 150–163. https://doi.org/10.1016/j.ifset.2018.06.007

Pencharz, P. B., Elango, R., & Wolfe, R. R. (2016). Recent developments in understanding protein needs - how much and what kind should we eat. *Applied Physiology Nutrition and Metabolism, 41*, 577–580.

R Core Team. (2020). *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ramashia, S. E., Tangulani, T., Mashau, M. E., & Nethathe, B. (2020). Microbiological quality of different dried insects sold at Thohoyandou open market, South Africa. *Food Research, 4*, 2247–2255.

Rumpold, B., & Schlüter, O. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies, 17*, 1–11. https://doi.org/10.1016/j.ifset.2012.11.005

Rutaro, K., Malinga, G. M., Lehtovaara, V. J., Opoke, R., Nyeko, P., Roininen, H., et al. (2018). Fatty acid content and composition in edible *Ruspolia differens* feeding on mixtures of natural food plants 11 Medical and Health Sciences 1111 Nutrition and Dietetics. *BMC Research Notes, 11*, 1–6. https://doi.org/10.1186/s13104-018-3792-9

Sozer, N., Nordlund, E., & Poutanen, K. (2017). Cereal side-streams as alternative protein sources. *Cereal Foods World, 62*, 132–137.

Sriprablom, J., Kitthawee, S., & Suphantharika, M. (2022). Functional and physicochemical properties of cookies enriched with edible insect (*Tenebrio molitor* and *Zophobas atratus*) powders. *Journal of Food Measurement and Characterization, 16*, 2181–2190.

Ssepuuya, G., Smets, R., Nakimbugwe, D., Van Der Borght, M., & Claes, J. (2019a). Nutrient composition of the long-horned grasshopper *Ruspolia differens* Serville: Effect of swarming season and sourcing geographical area. *Food Chemistry, 301*, 125305–125313. https://doi.org/10.1016/j.foodchem.2019.125305

Ssepuuya, G., Wynants, E., Verreth, C., Crauwels, S., Lievens, B., Claes, J., et al. (2019b). Microbial characterisation of the edible grasshopper *Ruspolia differens* in raw condition after wild-harvesting in Uganda. *Food Microbiology, 77*, 106–117. https://doi.org/10.1016/j.fm.2018.09.005

Tedjakusuma, F., Linggadiputra, J., Cahya, A. D., & Surya, R. (2022). Development of cricket flour-enriched cookies. In *IOP conference series: Earth and environmental science* (Vol. 1115)IOP Publishing, Article 012092, 1.

Vandeweyer, D., Lenaerts, S., Callens, A., & Van Campenhout, L. (2017). Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control, 71*, 311–314. https://doi.org/10.1016/j.foodcont.2016.07.011

Wilkinson, K., Muhlhausler, B., Motley, C., Crump, A., Bray, H., & Ankeny, R. (2018). Australian consumers' awareness and acceptance of insects as food. *Insects, 9*, 44.

Yazici, G. N., & Ozer, M. S. (2021). Using edible insects in production of cookies, biscuits, and crackers. *Biol. Life Sci. Forum, 3*, 1–6. S, 1.