Heliyon 10 (2024) e25331

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effect of cooking methods on nutritional value and microbial safety of edible rhinoceros beetle grubs (*Oryctes* sp.)

Marliyn W. Muthee, Fathiya M. Khamis, Xavier Cheseto, Chrysantus M. Tanga, Sevgan Subramanian, James P. Egonyu [*,1]

International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772 – 00100, Nairobi, Kenya

ARTICLE INFO

Keywords:
Campylobacter
Fatty and amino acid profiles
Proximate composition
Salmonella
Shigella
Staphylococcus aureus

ABSTRACT

Although edible rhinoceros beetle (*Oryctes* sp.) larvae are popularly consumed in many countries worldwide, they are prepared using different methods such as boiling, roasting, toasting, and deep-frying, whose effect on nutritional value and microbial safety is scarcely known. Here we investigated the effect of these methods on the nutritional value and microbial safety of *Oryctes* sp. larvae. Our hypothesis was that cooking the grubs using the four methods had no effect on their nutritional content and microbial loads and diversity. The grubs were analyzed for proximate composition, and fatty and amino acid profiles using standard chemical procedures; and microbial safety using standard culturing procedures. Deep-frying reduced protein and carbohydrate content, but elevated fat content. Boiling lowered ash content, but increased fibre and carbohydrate composition. Roasting and toasting increased protein and ash contents, respectively. Forty fatty acids were detected in the larvae, of which levels of only five were not significantly affected by cooking method, while the levels of the others were differentially affected by the different cooking methods. Amino acid profiles and levels were largely comparable across treatments, but lysine and arginine were higher in all cooked grubs than raw form. All the cooking methods eliminated Enterobacteriaceae, *Shigella* sp. and *Campylobacter* sp. from the grubs. Except boiling, all methods reduced total viable count to safe levels. *Salmonella* sp. were only eliminated by toasting and roasting; while boiling promoted growth of yeast and moulds. *Staphylococcus aureus* levels exceeded safety limits in all the cooking methods. These findings offer guidance on the type of method to use in preparing the grubs for desired nutritional and safety outcomes.

1. Introduction

The rapidly growing world population which is expected to reach approximately 10 billion by 2050, with most growth occurring in sub-Saharan Africa and Central, Southern, Eastern and South-Eastern Asia, presents a challenge to sustainable food security and achievement of the United Nations Sustainable Development Goal (SDG) 2, of zero hunger in these regions [1,2]. As such, the demand for animal source foods (ASF) like meat, fish, eggs and milk as good sources of high quality nutrients like protein, iron, iodine, vitamins A and B_{12}, zinc, energy and fatty acids is increasing drastically [3]. This challenge calls for a search for sustainable food systems which

* Corresponding author.
E-mail address: jpegonyu@gmail.com (J.P. Egonyu).
[1] Current address: Faculty of Science and Education, Busitema University, P. O. Box 236, Tororo, Uganda.

https://doi.org/10.1016/j.heliyon.2024.e25331
Received 9 March 2023; Received in revised form 10 January 2024; Accepted 24 January 2024
Available online 1 February 2024

will ensure food security for future generations in a way that is economically, socially and environmentally uncompromising [4]. Conventional livestock farming exerts a lot of pressure on arable land, water, and energy resources, and contributes significantly to increased environmental footprints [5]. It also releases 14.5 % of all the greenhouse gases attributed to human activity [5]; hence posing a threat to environmental sustainability. Edible insects, classified among regenerative future foods, generally contain all the essential macro and micronutrients found in conventional ASF in comparable or superior levels [6–8].

Although not yet validated in human trials, there's evidence of insect-derived peptides and bioactive compounds with antioxidant, anti-angiotensin-converting enzymes, anti-dipeptidyl peptidase-IV, anti-glucosidase, anti-lipase, anti-lipoxygenase, anti-cyclo-oxygenase, anti-obesity, and hepatoprotective activities [9,10]. Such reports indicate that insect consumption could solve human health problems related to inflammation, free radical damage, diabetes, hypertension, and liver damage, among others, which affect both the under-nourished and the over-nourished populations alike. Insect consumption, either directly as human food and indirectly as insect meal, could potentially help in easing the problem of protein malnutrition [11]. Additionally, some insects like crickets have chitin which during digestion by the enzyme chitinase which is present in the human gastric juice is degraded to chitosan, which stimulates the growth of beneficial gut bacteria [12,13]. Moreover, greenhouse gas emissions by insects may be mitigated by using renewable energy sources during production [14]. On this basis, insect production and consumption needs to be promoted among all populations globally, regardless of their food security situation.

Entomophagy (consumption of insects) began as early as the second and first millennia CE in the East and 384–322 BCE in Europe [15]. Several reports such as Tao and Li [16] and van Huis et al. [8] have indicated that over 2000 species of insects are consumed by two billion people in over 113 countries worldwide. However, van Huis et al. [17] opined that two billion is and over estimation of the number of people who consume insects globally, as the exact number is difficult to determine because of changes in the patterns of insect consumption over time, lack of knowledge about frequency of insect consumption and non-representative data on actual percentage of the national populations that consume insects. Common insects that are consumed include beetles, caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leafhoppers, plant-hoppers, termites, dragonflies and flies [18]. Most countries in Africa practice entomophagy, with the common species consumed varying from one country to another [7], depending on tradition, nutrition, ecology and economic factors [19].

The coprophagous beetle grubs (Coleoptera: Scarabaeidae) are among the popular edible insects in Africa, southeast Asia and the Pacific, Central and South America [20–22]. They are the fifth most consumed insects in Kenya after termites, grasshoppers, saturniids and crickets [23], especially in the western region where they are consumed in ~39 % of the households [24]. Although the grubs are typically sourced from the wild in farmyard composts of rural animal keepers, they could be reared commercially once the rearing protocols are developed [24]. It is however not yet known whether the methods used for cooking these grubs prior to consumption such as boiling, toasting, roasting, sun drying and frying guarantee their nutritional integrity and safety from microbial contaminants.

The methods of cooking edible insects for consumption may enhance their flavour and taste, eliminate food-borne microbial pathogens and toxins, increase shelf life, and improve bioavailability of nutrients, sensory characteristics and functional properties [25]. Cooking methods have different effects on the physicochemical and nutritional compositions of foods depending on the duration and temperature of heating, and magnitude of shear forces [26,27]. Cooking conditions (dry air or steam) and temperature influence cooking loss (mass of meat before and after cooking), total collagen content, moisture loss and tenderness and juiciness of the cooked meat product [28]. The effect of processing on the nutritional value of various conventional foods has been widely researched [29–31]. Similar studies have also been carried out for some species of edible insects. For instance, Manditsera et al. [32] reported that boiling at ~100 °C for either 30 min or 60 min reduces protein content and bio-accessibility of iron and zinc in edible beetles (*Eulepida mashona*) and crickets (*Henicus whellani*) as a result of leaching and formation of complexes with other constituents that are more resistant to digestion. However, studies on the same for several popular edible insects including rhinoceros beetle grubs are scarce. Wild collected edible insects may also contain different levels of inherent pathogenic and spoilage microorganisms, depending on insect species and cooking method [33]. For example, Labu et al. [34] detected potentially pathogenic bacteria like *Bacillus cereus* and fungi such as *Trichoderma asperellum* in wild harvested edible grasshoppers (*Ruspolia differens),* but frying the insects before consumption reduced the bacterial and fungal loads to recommended food safety limits of <5 log cfu/g and 2–3 log cfu/g, respectively. Gahukar [20] reported that the bacterial diversity and loads of both fresh and processed edible forest insects depended on the geographical area, swarming season, trading and collection periods. Fermentation of edible insects reportedly induces antimicrobial properties due to production of lactic acid [35]. Additionally, Belluco et al. [36] reported that although processing reduces microbial load in insect products, a reduction in coliforms and pathogenic bacteria during the entire production life cycle is still needed. However, the effects of different cooking methods, on the nutritional and microbial quality of coprophagous edible beetle grubs has scarcely been investigated.

In this study, we investigated the effect of boiling, roasting, toasting and deep-frying on the nutritional value and microbial safety of edible *Oryctes* sp. larvae, relative to their raw form.

2. Materials and methods

2.1. Insects

Live edible beetle grubs (10 kg) were collected from cattle dung compost in the backyards of various households in Bumula, Bulondo and Sangalo villages in Bungoma County, western Kenya. The amount of grubs collected in each farm varied greatly from none to unrecorded quantities, hence the insects were pooled together to amass sufficient quantities which were later subdivided equally for the different treatments and replicates. The samples were transported alive in the cattle dung compost in plastic bins to the

International Center of Insect Physiology and Ecology (*icipe*), Nairobi, for nutritional and microbial analyses. The live samples were hand-picked from the compost into a clean plastic container and degutted by incising the posterior end —using a clean sharp kitchen knife — through which undigested gut contents were squeezed out. The degutted grubs were washed using warm flowing tap water (~30 °C). The grubs were then subdivided into five equal portions which were separately cooked by boiling, roasting, toasting and deep-frying. Raw grubs were used as an untreated control. Representative images of the grubs cooked differently are presented in Fig. 1.

2.2. Sample preparation

2.2.1. Toasting

A clean dry stainless steel pan, 26 cm diameter, (RT203, Ramtons) was placed over an open flame on a kitchen gas cooker and heated to about 150 °C [37] which was verified using a digital kitchen thermometer. Raw edible beetle grubs (1200 g) were placed on a hot stainless-steel pan and turned regularly for approximately 15 min when they turned golden brown. The toasted insects were then removed and placed on an aluminum foil and allowed to cool at room temperature (~25 °C). The insects were then packed separately in airtight plastic containers that had been sterilized using 70 % ethanol and stored in a refrigerator (4 °C) and deep freezer (−20 °C) awaiting microbial and nutritional analyses, respectively.

2.2.2. Roasting

The raw grubs (1200 g) were placed on a clean oven tray and placed in an oven (Bistrot 665) set at 180 °C [38] and a desired dryness of 30 % was also set for approximately 15 min when they turned golden brown. Other procedures for handling the grubs prior to microbial and nutritional analyses were as described above for toasting.

2.2.3. Boiling

Potable water (2 l) was placed in a clean saucepan on a kitchen gas cooker and allowed to boil (100 °C). Raw beetle grubs (1200 g) were transferred to the saucepan and boiled for 20 min [39] after which they were drained in a kitchen strainer for about 1 min. Other procedures for handling the grubs prior to microbial and nutritional analyses were as described above for toasting.

2.2.4. Deep-frying

Vegetable cooking oil (2 l), (Fresh Fri, Pwani Oil Ltd, Kenya) was placed in a clean stainless-steel pan (RT203, Ramtons) (26 cm diameter) over a kitchen gas cooker and allowed to boil (180 °C). Raw beetle grubs (1200 g) were then submerged in the oil and fried for about 7 min when they turned golden brown [40]. The fried grubs were subsequently placed on an aluminum foil, lined with a paper towel to soak up excess oil for about 1 min. Thereafter, the grubs were prior to microbial and nutritional analyses handled as described above for toasting.

2.3. Sample drying

Owing to preliminary observations that oven drying resulted in leakage of oil through the incision on the larvae, we preferred to dry the insects using freeze drying which extracts frozen moisture through sublimation [41]. Three replicates (200 g each) of grub samples

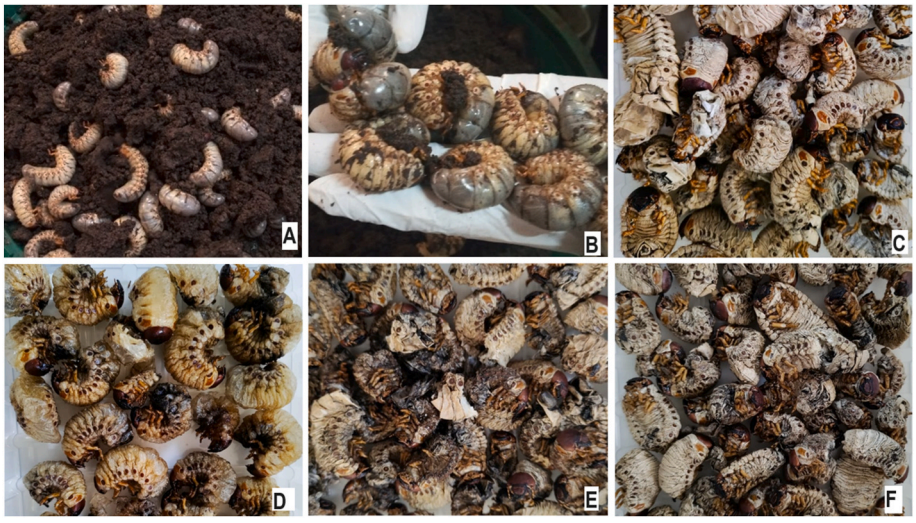

Fig. 1. *Oryctes* sp. grubs. A: grubs in the cattle dung compost; B: raw undegutted grubs removed from compost; C: boiled degutted grubs; D: deep-fried degutted grubs; E: roasted degutted grubs; and F: toasted degutted grubs.

cooked differently and preserved at −20 °C for nutritional analysis were placed in clean and dry plastic containers and dried in a freeze dryer (Wizard 2.0, Advantage EL-85, SP Scientific) at −80 °C and 116 psi for three days. After freeze drying, the samples were separately ground to fine powder using a kitchen blender for approximately 2 min. The kitchen blender was washed and sterilized using 70 % ethanol after each sample. The powders were transferred into sterile plastic containers and the lid tightly closed. The airtight containers (prior sterilized using 70 % ethanol) were sealed with para-film at the lid area to further prevent entry of air or moisture as the samples awaited nutritional analysis.

2.4. Proximate composition analysis

Proximate analysis of boiled, toasted, roasted and deep-fried beetle grubs was determined following standard methods of the Association of Official Analytical Chemists [42].

2.4.1. Dry matter

Dry matter was determined as described in AOAC [42] method 930.15. Briefly, 2 g of the sample was weighed into a pre-weighed porcelain crucible and dried in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 135 °C for 2 h after which the sample was allowed to cool in a desiccator. The sample was later weighed, and its moisture content was calculated. The moisture content of the sample was calculated as follows:

$$Dry\ matter\ content\ (\%) = \frac{W_{D2} - W_{D0}}{W_{D1} - W_{D0}} \times 100 \quad (1)$$

Where:

W_{D0} – weight of crucible (g)

W_{D1} – weight of crucible + undried sample (g)

W_{D2} – weight of crucible + dried sample (g)

Based on the results from dry matter content computation, moisture was computed as follows:

$$Moisture\ content\ (\%) = 100\% - dry\ matter \quad (2)$$

2.4.2. Ash content

Ash content was determined following AOAC [42], method 942.05. Approximately, 2 g of ground sample was weighed into empty crucibles and incinerated in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) set at 550 °C for 3 h, after which they were allowed to cool in a desiccator. The crucible was then weighed, and the weight used to calculate the ash content as illustrated below:

$$Ash\ (\%) = \frac{W_{A2} - W_{A0}}{W_{A1} - W_{A0}} \times 100 \quad (3)$$

Where:

W_{A0} – weight of crucible (g)

W_{A1} – weight of crucible + undried sample (g)

W_{A2} – weight of crucible + dried sample (g)

2.4.3. Crude protein

Crude protein was determined using the Kjeldahl method as described in AOAC [42], method 984.13. To 1 g of the sample, 2 tablets of Kjeltabs, 13 ml concentrated sulphuric acid and 2 tablets of antifoam were added. The mixture was digested at 420 °C on the digestion block heater (Velp DKL 20) for approximately 3 h until the liquid became clear. The mixture was allowed to cool, and the tubes transferred to a Kjeldahl distillation and titration unit (Velp UDK 159). The nitrogen amount was generated and the protein amount determined using 5.33 as the nitrogen-to-protein conversion factor which accounts for non-protein nitrogen in insects [43]. The formula used is follows:

$$Crude\ Protein\ (\%) = N \times F \times \frac{100}{DM} \quad (4)$$

Where:

N – Percentage of Nitrogen in the Sample.

F – Nitrogen to protein conversion factor (5.33)

DM – Dry Matter Content of Sample.

2.4.4. Crude fat

Crude fat in the sample was determined using the Randall technique in a solvent extraction unit (Velp SER 148, Velp Scientifica, Europe) as illustrated in AOAC [42], method 920.29. Briefly, 1 g of the sample was weighed into a labeled filter paper and placed into extraction thimbles. Exactly 70 ml of diethyl ether were measured into pre-weighed glass cups which were then placed under

extraction columns. The extraction thimbles were connected to the extractor and lowered into the corresponding glass cup (immersion) and allowed to boil for 30 min. The thimbles were raised out of the solvent and the fat extracted for 60 min. The solvent was distilled (recovery) after which the glass cups were removed and placed in a fume hood for 30 min to evaporate the solvent. The glass cups were then placed in an oven set at 105 °C for 30 min to remove any traces of the solvent and moisture and allowed to cool and weighed. The fat content was calculated as shown below.

$$Crude\ Fat\ (\%) = \frac{W_F - W_0}{W_S} \times \frac{100}{DM} \tag{5}$$

Where:

W_F – weight of extraction cup + fat (g)
W_0 – weight of empty extraction cup (g)
W_S – weight of sample (g)
DM – Dry Matter Content of Sample.

2.4.5. Crude fibre

Crude fibre was determined by acid digestion and loss on ignition in a fibre analyzer (FIWE, Velp Scientifica, Europe) according to Method 962.09 of AOAC [42], with few modifications. Ground sample (1 g) was weighed into a glass crucible and 150 ml of 1.25 % sulphuric acid and n-octanol (antifoam agent) added, and the mixture was boiled for 30 min. The sulphuric acid was drained, and the contents washed with 30 ml of hot deionized water. After draining, 150 ml of preheated sodium hydroxide and 3 drops of n-octanol were added, and the mixture boiled for 30 min. The mixture was filtered and washed 3 times with hot deionized water. The contents were washed with cold deionized water to cool the crucibles followed by washing thrice with 25 ml of acetone. The acetone was drained, and the crucibles removed and placed in an oven set at 105 °C for 1 h and allowed to cool. The crucibles were weighed, and the weight recorded to get the dry weight of the crude fibre plus ash content. The contents were then incinerated in a muffle furnace at 550 °C for 3 h, allowed to cool and the weights recorded and used to calculate the crude fibre content as follows:

$$Crude\ Fibre\ (\%) = \frac{F_1 - F_2}{F_0} \times \frac{100}{DM} \tag{6}$$

Where:

F_1 – weight of crucible and sample after fibre extraction and oven drying (g)
F_2 – weight of crucible and sample after incineration (g)
F_0 – weight of empty crucible (g)
DM – Dry Matter Content of Sample.

2.4.6. Neutral Detergent Fibre

Neutral Detergent Fibre (NDF) was determined in a fibre analyzer (FIWE, Velp Scientifica, Europe) following the procedure outlined in van Soest et al. [44]. Ground sample (1 g) was weighed into a pre-weighed dry crucible and the weight recorded. The crucibles were then attached to the fibre extractor and 100 ml of neutral detergent solution. The mixture was then heated to boiling and refluxed for 60 min followed by filtration and washing once with hot water and twice with cold acetone. The crucibles were then removed, and the contents dried in an oven set at 135 °C for 2 h, allowed to cool in a desiccator and the weights recorded. The contents were then incinerated in a muffle furnace at 550 °C for 2 h, cooled and the weights recorded and used to calculate the NDF content as follows.

$$NDF\ (\%) = \frac{W_{N-W_0}}{W_S - W_0} \times \frac{100}{DM} \tag{7}$$

Where:

NDF _ Neutral Detergent Fibre.
W_N _ weight of crucible + sample after fibre extraction and oven drying (g)
W_0 _ weight of empty crucible (g)
W_S _ weight of crucible + sample (g)
DM – Dry Matter Content of Sample.

2.4.7. Acid detergent fibre

Acid Detergent Fibre (ADF) was determined in a fibre analyzer (FIWE, Velp Scientifica, Europe) following the procedure outlined in van Soest et al. [44]. Ground sample (1 g) was weighed into a pre-weighed dry crucible and the weight recorded. The crucibles were attached to the fibre extractor and 100 ml of acid detergent solution. The mixture was heated to boiling and refluxed for 60 min. The mixture was then filtered and washed once with hot water and twice with cold acetone. The crucibles were then removed, and the contents dried in an oven set at 135 °C for 2 h, allowed to cool in a desiccator and weighed The ADF content was computed as follows:

$$ADF\ (\%) = \frac{W_{A-W_0}}{W_S - W_0} \times \frac{100}{DM} \tag{8}$$

Where:

ADF _ Acid Detergent Fibre.
W_A _ weight of crucible + sample after fibre extraction and oven drying (g)
W_0 _ weight of empty crucible (g)
W_S _ weight of crucible + sample (g)
DM – Dry Matter Content of Sample.

2.4.8. Available carbohydrates

Amount of available carbohydrates was determined as follows:

$$Available\ carbohydrates = 100 - (moisture + ash + crude\ fat + crude\ fiber) \quad (9)$$

2.5. Oil extraction

[45] As a prerequisite for efficient gas chromatography analysis, oil was extracted from three replicates of beetle grubs cook using different methods described above according to Igiehon et al. [46], with modifications. Briefly, 10 ml of dichloromethane and methanol (2:1 v/v) were added to 1 g of each sample in 50 ml falcon tubes. The mixtures were vortexed and sonicated at 20 kHz for 10 min in an ice bath and centrifuged at 4200 rpm for 10 min. The supernatants were transferred into clean falcon tubes and placed in a hooded chamber to evaporate any remaining solvent, leaving only the crude oil extract. The extracts (100 mg) from each replicate were separately weighed into clean narrow-necked vials.

2.6. Analysis of fatty acids

To each vial containing 100 mg of oil extract, 1 ml sodium methoxide solution was added. The solution was prepared by dissolving 2000 mg of sodium methoxide into 20 ml of dry methanol. The mixture was vortexed for 1 min, sonicated for 10 min and incubated over a water bath (70 °C) for 1 h. Hexane (1 ml) was added to each vial, vortexed for 1 min and transferred to Eppendorf tubes. The mixture was centrifuged at 14000 rpm for 20 min. The supernatant (100 μl) was filtered into clean wide necked vials and 900 μl hexane added by passing it through anhydrous sodium sulphate on inserts fitted in 1 ml pipette tips. Aliquot (1 μl) of the mixture was analyzed in a 7890A gas chromatograph linked to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The carrier gas was helium at a flow rate of 1.25 ml min^{-1}. The oven temperature was programmed from 35 °C to 285 °C at 10 °C/min. The standard, methyl octadecanoate (0.2–125 ng/μL) prepared from octadecanoic acid (≥95 % purity) (Sigma-Aldrich, St. Louis, MO) was analyzed by GC–MS to generate a linear calibration curve (peak area vs. concentration) with the equation; [y = 5E +07x +2E +07] which gave $r^2 = 0.9997$. The fatty acids in the samples were quantified using this regression equation. ChemStation B.02.02 software was used for the data acquisition.

2.7. Analysis of amino acids

Each sample (200 mg) was separately transferred into a 5 ml micro-reaction vial into which 2 ml of 6 N HCl was added and closed after careful introduction of nitrogen gas. For tryptophan analysis, 100 mg from each of the samples was separately transferred into a 5 ml micro-reaction vial into which 2 ml of 6 N NaOH was added and then capped after careful introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110 °C. After the hydrolysis, the mixtures were evaporated to dryness under a vacuum. The hydrolysates were reconstituted in 1 ml 90:10 water: acetonitrile, vortexed for 30 s, sonicated for 30 min, and centrifuged at 14,000 rpm. The supernatant was analyzed by LC-MS. The compounds were identified and quantified using authentic sample mixture (amino acid standard solution (AAS 18) purchased from Sigma-Aldrich (Chemie GmbH, Munich, Germany). Serial dilutions of the authentic standard containing 18 amino acids (1–105 μg/μl) was also similarly analyzed by LC-MS to generate linear calibration curves (peak area vs. concentration) used for the external quantification of the sample amino acids. The chromatographic separation was achieved on an Agilent system 1100 series (MA, USA) using ZORBAX SB-C18, 4.6 × 250 mm, 3.5 μm column, operated at 40 °C. Mobile phases used were made up of water (A) and acetonitrile (B) each with 0.01 % formic. The following gradient was used: 0–8 min, 10 % B; 8–14 min, 10–100 % B; 14–19 min, 100 % B; 19–21 min, 100-10 % B; 21–25 min, 10 % B. The flow rate was held constant at 0.5 ml min^{-1} and the injection volume was 10 μL. The LC was interfaced to a quadruple mass spectrometer. The mass spectrometer was operated on ESI-positive mode at a mass range of *m/z* 50–600 at 70 eV cone voltage.

2.8. Assessment of microbial quality

Three replicates of each cooked grub sample (5 g) were ground using a kitchen grade blender and suspended in 45 ml sterile peptone water in 50 ml falcon tubes to form stock solutions. For microbial analysis, the cattle dung compost from which the grubs were obtained was also analyzed in triplicates. Serial dilution was done by adding 1 ml from the stock solution into 9 ml peptone water in a 15 ml falcon tube using sterile pipette tips. This was repeated to obtain solutions between 10^{-2} and 10^{-6} for isolation and analyses of total viable count (TVC), *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., Enterobacteriaceae and fungi. Isolation and analyses were done on six different types of media namely plate count agar (PCA), mannitol-salt agar, salmonella-shigella agar, Campylobacter blood free selective agar base, violet red bile agar (VRBA) and potato dextrose agar (PDA) (Fig. 2).

2.8.1. Enumeration of total viable count, Enterobacteriaceae, Staphylococcus aureus and yeast and moulds

Plate count agar (PCA) (Oxoid, UK), mannitol-salt agar (Himedia, India) and potato dextrose agar (PDA) (Oxoid, UK) were prepared according to the manufacturers' instructions for enumeration of total viable count, *Staphylococcus aureus,* and yeast and moulds count, respectively. All the media were autoclaved at 121 °C for about 3 h, and allowed to cool to 45 °C. An aliquot of each sample (0.1 ml) was drawn from stock solutions of 10^{-4}, 10^{-5} and 10^{-6} and poured into sterile culture plates. Each medium (20 ml) was poured into each culture plate containing the sample and swirled to homogenize the mixture. The mixture was left for approximately 20 min to solidify. Culture plates containing PCA and mannitol-salt agar were incubated at 35 °C for 48 h, whereas those containing PDA were incubated at 25 °C for 72 h. Violet red bile agar (VRBA) (Oxoid, UK) used for enumeration of Enterobacteriaceae was also prepared as per manufacturer's instructions, boiled on a hot plate to dissolve the contents and allowed to cool to 50 °C and removed from the hot plate into a sterile hooded chamber. Aliquots of each sample (0.1 ml) were drawn from stock solutions of 10^{-4}, 10^{-5} and 10^{-6} and pipetted into sterile culture plates, followed by the media. The mixtures were swirled and left for 20 min to solidify. The plates were incubated at 35 °C for 48 h. Counting was done and colonies expressed as colony forming units per gram (cfu/g). For calculation of TVC, Enterobacteriaceae and yeast and moulds, colonies growing on 10^{-4} culture plate was used to determine the colony count. For *Staphylococcus aureus,* colonies growing on 10^{-5} culture plate was used to determine the colony count. For statistical accuracy, culture plates should have a countable number of colony forming units (30–300) and not be overcrowded to avoid miscalculation due to overlapping colonies which explains the culture plates selected in this study [47].

2.8.2. Detection of Salmonella, Shigella and lactose fermenting bacteria

Salmonella-shigella (SS) agar (Oxoid, UK) was prepared as per the manufacturer's instructions and boiled to dissolve the medium. The homogenized medium was allowed to cool to about 45 °C, poured into sterile culture plates and left in a sterile hooded chamber for 8 h to solidify. The sample was then streaked onto the medium using a sterile loop and incubated at 35 °C for 24 h. Presence of black colonies indicated presence of Salmonella; whereas pink colonies indicated the presence of lactose fermenters; and colourless colonies indicated the presence of Shigella sp [48].

2.8.3. Campylobacter sp.

Campylobacter blood free selective agar base (Oxoid, UK) was prepared as per the manufacturer's instructions and autoclaved at 121 °C for about 3 h. The medium was then allowed to cool to about 45 °C, poured into sterile culture plates inside a sterile chamber and left for 8 h to solidify. The specimens from 10^{-3} stock solutions prepared as described above were streaked onto the solidified media using a sterile loop and incubated at 37 °C for 72 h in a microaerobic atmosphere. Presence of white colonies indicated presence of *Campylobacter* sp. in the specimens.

2.9. Data analysis

Statistical analyses were carried out using IBM® SPSS® Statistics 20 (IBM Corporation, Armonk, NY, USA) at $\alpha = 0.05$. Analysis of variances were carried out to determine effect of cooking methods on nutritional composition and logs of microbial counts. Means

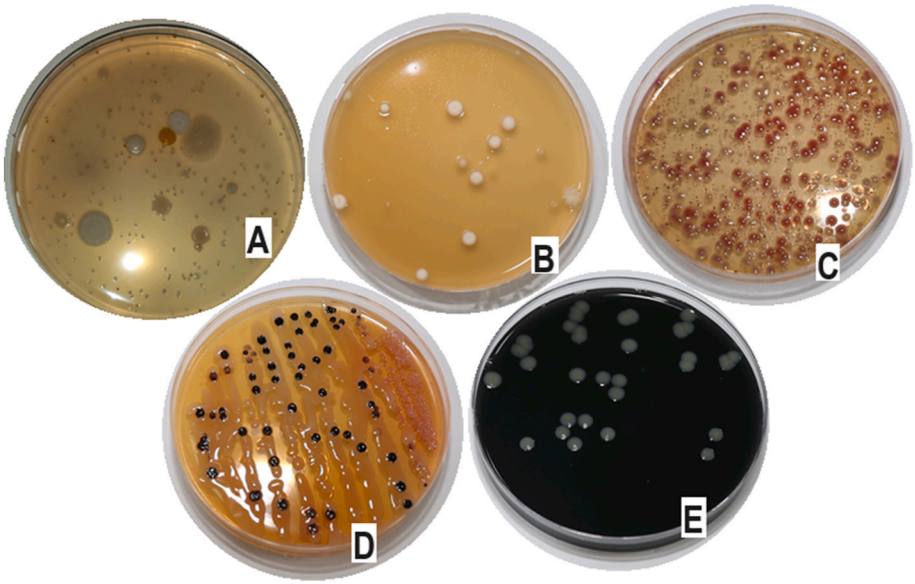

Fig. 2. Pathogens detected in cooked *Oryctes* sp. larvae products. A: microbial colonies on plate count agar (PCA); B: colonies of yeast and moulds on potato dextrose agar (PDA); C: Enterobacteriaceae colonies on violet red bile agar; D: bacterial colonies on Salmonella-Shigella agar–Black and colourless for *Salmonella* sp. and *Shigella* sp., respectively; and E: *Campylobacter* sp. colonies

were separated using Tukey's Honestly Significant Difference (HSD) test. Fatty acids which were detected in grubs from only one treatment were not statistically analyzed, but their means and standard deviations of the means were computed and presented.

3. Results

3.1. Effect of cooking method on nutritional composition

Apart from crude fibre content, other proximate compositions of the beetle grubs were significantly affected by the cooking method (Table 1). Compared to raw samples, boiling significantly reduced dry matter content of the grubs, whereas other cooking methods statistically increased the dry matter content. The highest increase in dry matter content arose from deep-frying.

Ash contents of raw, toasted and deep-fried grubs were statistically comparable. However, significant increase and decrease in ash content relative to raw grubs were recorded with roasting and boiling, respectively.

Compared to raw grubs, toasting, roasting and boiling significantly increased crude protein content of the grubs; whereas deep-frying statistically reduced the crude protein content. The highest crude protein content (48.4 %) was recorded in the roasted grubs, but this value was comparable to the crude protein contents of the toasted grubs.

Unlike protein content, crude fat significantly decreased in toasted, roasted and boiled grubs compared to raw samples, while deep-fried grubs had statistically higher crude fat content than the raw grubs. The lowest crude fat content (19.9 %) was recorded in the toasted grubs, and it was statistically comparable with that of roasted grubs, but lower than that of boiled grubs.

The contents of NDF in toasted and boiled grubs were comparable to that of raw grubs. However, roasting and deep-frying significantly reduced NDF content compared to raw grubs, although the content in roasted grubs was comparable to those of toasted and boiled grubs.

Compared to raw grubs, all the cooking methods increased ADF contents of the grubs, with the highest increase recorded in boiled grubs. However, ADF contents of toasted, roasted and deep-fried grubs were statistically comparable.

Carbohydrate contents of raw, toasted and roasted grubs were statistically comparable. Although boiled and toasted grubs had statistically comparable carbohydrate contents, boiled grubs had significantly higher carbohydrate content than raw grubs. In addition, carbohydrate contents of boiled and toasted grubs were significantly higher than that of roasted and deep-fried grubs. Deep-fried grubs also had a significantly lower carbohydrate content than raw grubs.

3.2. Effect of cooking method on fatty acid composition

A total of 40 fatty acids were detected in the samples (Table 2). However, not all were detected in samples from each cooking method. Only four saturated fatty acids (SFA) (Numbers 1, 5, 14 and 26 in Table 2), and one monounsaturated fatty acid (MUFA) ((E)-9-Hexadecenoic acid) were detected in grubs across all the treatments, but at statistically different compositions. The content of tetradecanoic acid and hexadecanoic acid methyl esters were comparable in raw, toasted and roasted grubs, but they were significantly higher in boiled and deep-fried grubs. Although analysis of variance detected a significant effect of cooking method on the level of dodecanoic acid methyl ester, no mean differences between any treatments were detected using Tukey's test. The levels of octadecanoic acid methyl ester were significantly higher in samples from all the cooking methods than in raw grubs, with the highest increase recorded in the deep-fried grubs. Comparable levels of (E)-9-Hexadecenoic acid were recorded in all the grubs, except boiled grubs which contained a significantly higher level.

Five SFA (Numbers 9, 10, 13, 18 and 29), one MUFA (9-heptadecenoic acid methyl ester) and one polyunsaturated fatty acid (PUFA) (linoleic acid methyl ester) were present in only raw grubs. Fourteen SFA (Numbers 2–4, 6, 11–12, 19, 22–25, 27–28, and 30), three MUFA ((E)-2-Octadecenoic acid, (Z)-9-Hexadecenoic acid and (Z)-9-tetradecenoic acid*)* and one PUFA (gamma-linolenic acid methyl ester) were not detected in the raw grubs. Fourteen fatty acids were present in only one of the heat treatments. Six (Numbers 3, 6, 11, 25, 27 and 28), two (3-Methyltridecanoic acid and gamma-linolenic acid methyl esters), three (Numbers 4, 12 and 23) and one ((E)-2-Octadecenoic acid) in roasted, boiled, toasted and deep-fried grubs, respectively. Other fatty acids were detected in grubs from two or more treatments at statistically different levels.

Total SFA were most abundant in the boiled and deep-fried grubs, but were significantly lower in the raw, toasted and roasted grubs. Boiling and deep-frying also significantly increased the amount of MUFA unlike roasting and toasting which significantly reduced their levels. Interestingly, the MUFA levels in raw grubs were comparable to the content in all cooked grubs. Other than raw

Table 1
Dry matter basis proximate compositions (mean percentage ± SD) of *Oryctes* sp. grubs cooked differently.

Form	Dry matter	Ash	Crude protein	Crude fat	Crude fibre	NDF	ADF	Carbohydrates
Raw	96.8 ± 0.1^{b}	7.0 ± 0.6^{b}	38.9 ± 1.8^{b}	30.0 ± 1.2^{c}	0.35 ± 0.00^{a}	20.1 ± 0.7^{c}	15.7 ± 1.2^{a}	20.6 ± 1.3^{bc}
Toasted	97.1 ± 0.1^{c}	6.3 ± 0.5^{ab}	48.4 ± 1.1^{d}	19.9 ± 0.5^{a}	0.35 ± 0.01^{a}	18.7 ± 0.6^{bc}	18.4 ± 1.1^{b}	22.1 ± 1.6^{cd}
Boiled	95.5 ± 0.1^{a}	5.2 ± 0.5^{a}	43.7 ± 1.8^{c}	22.5 ± 0.9^{b}	0.34 ± 0.01^{a}	18.6 ± 1.4^{bc}	22.3 ± 1.2^{c}	23.8 ± 1.0^{d}
Roasted	97.3 ± 0.1^{c}	10.0 ± 1.0^{c}	47.0 ± 0.5^{cd}	21.7 ± 0.9^{ab}	0.36 ± 0.02^{a}	17.0 ± 0.9^{b}	18.3 ± 0.5^{b}	18.2 ± 0.3^{b}
Deep-fried	97.8 ± 0.1^{d}	7.5 ± 0.3^{b}	32.9 ± 0.2^{a}	49.0 ± 0.8^{d}	0.36 ± 0.01^{a}	14.1 ± 1.0^{a}	19.8 ± 0.1^{b}	8.0 ± 1.0^{a}
$F_{(4,10)}$	259.1	24.6	75.2	549.1	2.5	17.1	20.6	95.5
P	<0.001	<0.001	<0.001	<0.001	0.109	<0.001	<0.001	<0.001

Values bearing different letters within the same column are significantly different ($P < 0.05$).

Table 2
Dry matter basis fatty acid composition (mean μg/g ± SD) of oil extracted from *Oryctes* sp. grubs cooked differently.

Fatty acids	Raw	Toasted	Boiled	Roasted	Deep-fried	F value	df	P
Saturated fatty acids (SFA) methyl esters								
1. Tetradecanoic acid	0.49 ± 0.10^{a}	0.22 ± 0.02^{a}	1.86 ± 0.25^{b}	0.11 ± 0.003^{a}	2.01 ± 0.22^{b}	104.864	4, 10	<0.001
2.3-Methyltridecanoic acid	–	–	0.67 ± 0.18	–	–	–	–	–
3.3-Methyltetradecanoic acid	–	–	–	0.18 ± 0.02	–	–	–	–
4.12-Methyltridecanoic acid	–	0.33 ± 0.03	–	–	–	–	–	–
5. Dodecanoic acid	0.20 ± 0.06^{a}	0.15 ± 0.01^{a}	0.63 ± 0.48^{a}	0.63 ± 0.09^{a}	0.72 ± 0.09^{a}	4.438	4, 10	0.026
6.8-Methyldecanoic acid	–	–	–	0.17 ± 0.02	–	–	–	–
7.13-Methyltetradecanoic acid	0.03 ± 0.002^{a}	–	–	0.11 ± 0.007^{b}	0.32 ± 0.04^{c}	143.096	2, 6	<0.001
8. Pentadecanoic acid	0.07 ± 0.02^{a}	–	0.22 ± 0.01^{b}	–	–	127.975	1, 4	<0.001
9.10-Methyldodecanoic acid	0.007 ± 0.0001	–	–	–	–	–	–	–
10.3,7,11,15-Tetramethylhexadecanoic acid	0.02 ± 0.001	–	–	–	–	–	–	–
11. Octanoic acid	–	–	–	0.43 ± 0.01	–	–	–	–
12. Tridecanoic acid	–	0.18 ± 0.03	–	–	–	–	–	–
13.12-Methyltetradecanoic acid	0.18 ± 0.02	–	–	–	–	–	–	–
14. Hexadecanoic acid	11.87 ± 2.02^{a}	38.43 ± 1.12^{a}	715.9 ± 95.3^{b}	46.39 ± 16.99^{a}	701.55 ± 105.52^{b}	100.534	4, 10	<0.001
15.10-Methylhexadecanoic acid	1.17 ± 0.05^{b}	–	–	0.93 ± 0.04^{a}	1.12 ± 0.02^{b}	32.737	2, 6	0.001
16.14-Methylhexadecanoic acid	0.53 ± 0.13^{a}	–	2.02 ± 0.38^{b}	–	2.12 ± 0.27^{b}	30.214	2, 6	0.001
17. Decanoic acid	0.26 ± 0.02^{a}	39.12 ± 2.96^{b}	2.46 ± 0.25^{a}	0.15 ± 0.01^{a}	–	496.939	3, 8	<0.001
18.4,8,12-Trimethyltridecanoic acid	1.83 ± 0.19	–	–	–	–	–	–	–
19.15-Methylhexadecanoic acid	–	0.92 ± 0.04^{a}	–	0.88 ± 0.03^{a}	–	1.775	1, 4	0.254
20. Heptadecanoic acid	0.42 ± 0.04^{a}	0.83 ± 0.07^{b}	–		–	88.52	1, 4	0.001
21. Undecanoic acid	0.75 ± 0.04^{b}	0.12 ± 0.01^{a}	1.14 ± 0.16^{c}	–	–	86.838	2, 6	<0.001
22. Nonanoic acid	–	–	–	0.32 ± 0.03^{b}	0.19 ± 0.02^{a}	36.185	1, 4	0.004
23.5,9,13-Trimethyltetradecanoic acid	–	0.03 ± 0.003	–	–	–	–	–	–
24. Heptadecanoic acid	0.01 ± 0.003^{a}	–	0.30 ± 0.02^{b}	0.01 ± 0.001^{a}	–	488.265	2, 6	<0.001
25.5,9-Dimethyldecanoic acid	–	–	–	0.02 ± 0.002	–			
26. Octadecanoic acid	1.04 ± 0.37^{a}	16.64 ± 2.27^{b}	35.95 ± 9.91^{cd}	32.03 ± 1.03^{c}	41.90 ± 1.66^{d}	38.149	4, 10	<0.001
27.9-Methyltetradecanoic acid	–	–	–	0.03 ± 0.003	–	–	–	–
28.10-methylundecanoic acid	–	–	–	0.01 ± 0.002	–	–	–	–
29.11-methylnonadecanoic acid	1.75 ± 0.12	–	–	–	–	–	–	–
30.18-methylnonadecanoic acid	–	0.98 ± 0.14^{a}	2.80 ± 0.13^{b}	2.80 ± 0.13^{b}	4.36 ± 0.11^{c}	332.256	3, 8	<0.001
31. Arachidic acid	0.83 ± 0.04^{a}	–	–	–	1.76 ± 0.14^{b}	112.706	1, 4	<0.001
∑SFA	21.49 ± 2.46^{a}	97.91 ± 1.65^{a}	763.96 ± 89.64^{b}	85.18 ± 17.99^{a}	756.06 ± 106.60^{b}	109.798	4, 10	<0.001
Monounsaturated fatty acids (MUFA) methyl esters								
32. (E)-2-Octadecenoic acid	–	–	–	–	0.08 ± 0.02	–	–	–
33. (E)-9-Hexadecenoic acid	1.36 ± 0.13^{a}	0.34 ± 0.05^{a}	4.86 ± 1.55^{b}	1.83 ± 0.06^{a}	1.92 ± 0.24^{a}	17.026	4, 10	<0.001
34. (Z)-9-Hexadecenoic acid	–	–	0.90 ± 0.05^{b}	0.04 ± 0.003^{a}	–	862.997	1, 4	<0.001
35.9-heptadecenoic acid	0.72 ± 0.01	–	–	–	–	–	–	–
36. (Z)-9-tetradecenoic acid	–	–	–	0.84 ± 0.02	–	–	–	–
37. (E)-9-Octadecenoic acid	85.20 ± 2.89^{b}	38.29 ± 5.42^{a}	–	–	–	174.830	1, 4	<0.001
38. (Z)-9-Octadecenoic acid	32.42 ± 3.44^{a}	–	326.92 ± 90.95^{b}	89.81 ± 14.98^{ab}	209.13 ± 35.72^{b}	21.013	3, 8	<0.001
∑MUFA	119.71 ± 1.07^{ab}	38.64 ± 5.44^{a}	332.67 ± 91.85^{b}	92.53 ± 14.91^{a}	211.12 ± 35.94^{b}	20.035	4, 10	<0.001
Polyunsaturated fatty acids (PUFA) methyl esters								
39. Gamma-linolenic acid	–	–	0.34 ± 0.02	–	–	–	–	–
40. Linoleic acid	53.42 ± 2.33	–	–	–	–			
∑PUFA	53.42 ± 2.33^{b}	–	0.34 ± 0.02^{a}	–	–	1553.90	1, 4	<0.001

Values bearing the same letters within a row are not significantly different ($P < 0.05$).

grubs, PUFA were only detected in boiled grubs, but at significantly lower levels.

3.3. Effect of cooking method on amino acid composition

A total of 13 amino acids, 8 of them essential, were detected in the grubs (Table 3). Significant differences in the levels of the amino acids across treatments were only detected for lysine, histidine, arginine, valine and phenylalanine. Levels of lysine and arginine were lowest in raw grubs, and highest in the boiled grubs. Histidine levels were lowest in raw grubs and statistically comparable across the cooking methods; whereas valine levels were lowest in deep fried grubs, and comparable across other treatments. Phenylalanine levels were higher in toasted and boiled grubs than the rest of the treatments. Phenylalanine was the most abundant amino acid in the grubs followed by tyrosine; whereas the least abundant amino acid was threonine and methionine.

3.4. Effect of cooking method on microbial quality

The type and/or load of microbes analyzed were influenced by the cooking methods (Table 4). Compared to the raw grubs, all cooking methods significantly reduced TVC, with toasted grubs having the lowest counts. The counts of YMC in the grubs were comparable in all the treatments except in boiled grubs where they were significantly higher. Enterobacteriaceae was detected in only the substrate and raw grubs. Interestingly, the raw grubs contained significantly higher loads of TVC and Enterobacteriaceae than the substrate. The loads of *S. aureus* were comparable in the raw grubs and substrate samples, but thy were significantly reduced in all the heat cooking methods. *Salmonella* sp. were detected in all the samples except in the toasted and roasted grubs. *Shigella* sp. and *Campylobacter* sp. were only detected in the raw grubs and substrate, respectively.

4. Discussion

Our data show that ash contents of *Oryctes* sp. closely compare with the values (4–15 %) reported in roasted or dried *Oryctes rhinoceros* in Nigeria [38,49–51]. Contrastingly, lower ash contents (1.5–4.2 %) have been reported in *Oryctes boas* and other species of beetle grubs from related genera such as *Aphodius rufipe, Rhynchophorus phoenicis, Apomecyna parumpunctata* and *Analeptes trifasciata* [52]. Meanwhile, our data on protein and carbohydrate contents of the grubs corroborate the values reported in other studies [50,51], but Bolaji et al. [38] reported higher protein values (53–63 %) and lower carbohydrate values (0.1–6.6 %) in edible beetle grubs. Our data on dry matter content of the grubs corroborate the values reported by other authors [38,50], but Anaduaka et al. [51] reported a markedly lower dry matter content of 74 % in edible beetle grubs. On the other hand, all the three previous studies cited reported markedly lower fat contents of the grubs (9–15 %) than in our case. The crude fibre values in this study were slightly similar to those reported in *Oryctes rhinoceros* grubs (1.4 %) but considerably lower than that of other edible beetle grubs which contain 1.96–25.14 % crude fibre [33]. The NDF compositions of the grubs in this study (~14–20 %) is within the range (17–21 %) reported in *Tenebrio molitor*, *Zophobas atratus* and *Bombyx mori* [53]. The ADF composition of the grubs (~16–22 %) were remarkably higher than the ranges reported in *Tenebrio molitor* and *Hermetia illucens* (8–11 % and 5–9 %, respectively) [54]. Our studies also concur with findings of Chakravorty et al. [55] and Ghosh et al. [56] who reported higher proportions of saturated fatty acids than monounsaturated and polyunsaturated fatty acids in *Odontotermes* sp. and honey bee. However, Ghosh et al. [57] reported higher proportions of monounsaturated fatty acids in *Allomyrina dichotoma* larvae, *Protaetia brevitarsis* larvae and *Tenebrio molitor* larvae, which are used as food and feed in South Korea. The contradictions in nutritional profiles of the grubs compared to previous reports may be attributed to inter-species variation, cooking methods, and dietary sources, and hence the nutrient contents of the insects which are influenced by vegetation, climate and soil characteristics [58–60].

Our data indicate that toasting, roasting and deep-frying increased dry matter contents of the grubs, while boiling reduced the dry matter content relative to raw grubs. The most marked increase in dry matter content arose from deep-frying, probably as a result of markedly higher temperature during deep-frying (180 °C) than during boiling (100 °C) and roasting (150 °C). High temperatures cause

Table 3
Dry matter basis amino acid composition (mean mg/g ± SD) extracted from *Oryctes* sp. grubs cooked differently.

Amino acid	Raw	Toasted	Boiled	Roasted	Deep-fried	$F_{(4,10)}$	P
1. Alanine	0.30 ± 0.0^{a}	0.23 ± 0.06^{a}	0.23 ± 0.06^{a}	0.20 ± 0.0^{a}	0.23 ± 0.1^{a}	2.0	0.171
2. Lysine[a]	0.27 ± 0.06^{a}	0.60 ± 0.0^{bc}	0.67 ± 0.06^{c}	0.60 ± 0.0^{bc}	0.53 ± 0.1^{b}	36.7	<0.001
3. Histidine[a]	0.20 ± 0.0^{a}	0.27 ± 0.06^{ab}	0.30 ± 0.0^{b}	0.30 ± 0.0^{b}	0.30 ± 0.0^{b}	8.5	0.003
4. Arginine	0.30 ± 0.0^{a}	0.40 ± 0.0^{b}	0.50 ± 0.0^{c}	0.43 ± 0.06^{bc}	0.47 ± 0.1^{bc}	13.3	0.001
5. Threonine[a]	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	2.5	0.176
6. Glutamic acid	0.43 ± 0.06^{a}	0.40 ± 0.0^{a}	0.40 ± 0.0^{a}	0.37 ± 0.06^{a}	0.37 ± 0.06^{a}	1.2	0.382
7. Proline	0.57 ± 0.1^{a}	0.60 ± 0.0^{a}	0.53 ± 0.06^{a}	0.50 ± 0.0^{a}	0.50 ± 0.0^{a}	1.7	0.226
8. Valine[a]	0.33 ± 0.06^{ab}	0.40 ± 0.0^{b}	0.40 ± 0.0^{b}	0.33 ± 0.06^{ab}	0.30 ± 0.0^{a}	4.5	0.024
9. Methionine[a]	0.13 ± 0.06^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.07 ± 0.1^{a}	1.3	0.351
10. Tyrosine	0.90 ± 0.3^{a}	0.78 ± 0.06^{a}	0.78 ± 0.06^{a}	0.47 ± 0.06^{a}	0.67 ± 0.1^{a}	2.9	0.078
11. Isoleucine[a]	0.43 ± 0.06^{a}	0.50 ± 0.0^{a}	0.53 ± 0.06^{a}	0.43 ± 0.06^{a}	0.43 ± 0.1^{a}	2.5	0.109
12. Leucine[a]	0.57 ± 0.1^{a}	0.53 ± 0.06^{a}	0.60 ± 0.0^{a}	0.53 ± 0.06^{a}	0.50 ± 0.0^{a}	1.1	0.415
13. Phenylalanine[a]	2.27 ± 0.06^{a}	2.80 ± 0.1^{b}	2.70 ± 0.1^{b}	2.40 ± 0.1^{a}	2.33 ± 0.1^{a}	17.9	<0.001

[a] Essential amino acids.

Table 4
Effect of cooking method on microbial safety of *Oryctes* sp. grubs.

Product form	TVC (logcfu/g)	YMC (logcfu/g)	Enterobacteriaceae (logcfu/g)	*S. aureus* (logcfu/g)	*Salmonella* sp.	*Shigella* sp.	*Campylobacter* sp.
Substrate	5.95 ± 0.00^{b}	4.95 ± 0.00^{a}	5.43 ± 0.00^{a}	7.11 ± 0.22^{b}	+ +	- -	+ +
Raw	9.78 ± 0.04^{d}	5.26 ± 0.00^{a}	8.27 ± 0.00^{b}	7.05 ± 0.07^{b}	+ +	+ +	- -
Toasted	5.11 ± 0.22^{a}	4.95 ± 0.00^{a}	0	5.95 ± 0.00^{a}	- -	- -	- -
Boiled	6.95 ± 0.00^{c}	6.10 ± 0.04^{b}	0	6.20 ± 0.09^{a}	+ +	- -	- -
Roasted	5.95 ± 0.00^{b}	5.11 ± 0.22^{a}	0	5.95 ± 0.00^{a}	- -	- -	- -
Deep-fried	5.95 ± 0.00^{b}	4.95 ± 0.00^{a}	0	5.95 ± 0.00^{a}	+ +	- -	- -
F $_{(5,6)}$	667.16	48.53	3193741.80	60.97			
P	<0.001	<0.001	<0.001	<0.001			

Log cfu/g values are presented as Means ± S.D. Values bearing the same letters within a column are not significantly different ($p > 0.05$). + + indicates that the microorganisms were detected in all replicates while - - indicates the microorganisms were absent in all replicates. TVC-total viable count, YMC- yeast and mold count.

evaporation of water and a build-up of pressure within the food crust, causing cracks [61]. On the contrary, the reduction of dry matter contents of the grubs as a result of boiling could be due to imbibition of water by the grubs, raising moisture content [62,63].

Whereas toasting and deep-frying did not affect the ash content of the grubs relative to raw grubs, boiling reduced the ash content, while roasting increased it. This may be a result of leaching out of minerals into the boiling water while roasting could have markedly reduced the moisture content of the grub, hence concentrating the ash [64]. The high content of ash in the roasted grubs partly corroborates the report by Baek et al. [65] that roasted mealworm *Tenebrio molitor* contained higher ash contents than the deep-fried samples. On the other hand, our findings contradict that of Kouabitheu et al. [66] that fried termite *Macrotermes subhyalinnus* and saturniid *Imbrasia obscura*, contained higher ash contents than raw samples of the insects. The comparability of ash content in raw, toasted and deep-fried grubs coincides with our data on moisture contents which were comparable in grubs cooked by these methods. This may account for similar level of concentration of nutritional components [65].

With the exception of deep-frying, all the other cooking methods resulted in an increase in protein content of the grubs, with toasting causing the highest increase. These results corroborate other studies that toasted *Ruspolia differens* had higher protein content, while the deep-fried grasshoppers recorded the lowest protein content [67]. Nyangena et al. [68] also reported lower protein content in both boiled and roasted *Acheta domesticus*, *Hermetia illucens*, *Ruspolia differens* and *Spodoptera littoralis* compared to the toasted insect samples. The report by Ssepuuya et al. [64] that increase in protein content of *Ruspolia differens* is approximately proportional to loss in crude fat content corroborates our findings. Dry cooking of food at more than 100 °C results in moisture loss, thus concentrating the protein in the product [69]. Heat treatment (>100 °C) during food cooking may also result in loss of antioxidant protection within the food rendering the protein susceptible to attack by reactive oxygen species (ROS) leading to the formation of carbonyl derivatives and Schiff bases [70]. During boiling of food, some protein leaches into boiling water [71], which may partly explain our record of lower protein content in boiled grubs than toasted grubs. The statistical comparability of protein content of boiled grubs with roasted grubs but not with toasted grubs may be a result of dependence of the loss of protein on the heating temperatures, which were lower in toasting (150 °C) than roasting (180 °C), and varying heat transfer mechanisms like air currents velocity, flowrate, duration, and chamber pressure [72].

We recorded ~25–34 % reduction in fat content in the grubs due to toasting, boiling, and roasting, but >63 % increase in the same due to deep-frying. The former could be mainly attributed to the effect of heat [64], and leaching of the fat into the boiling water in case of boiling [62,63]. On the other hand, the observed increase in fat content of the grubs is consistent with reports on other deep-fried foods [73,74]. Deep-fried food take-up oil mostly during cooling after frying [73,74]. In most cases of immersion and during the frying process, the internal pressure in the food created by the pressure loss across the crust when steam escapes the product opposes the oil capillary pressure, preventing oil uptake [73,74]. The pressure gradient however reverses during cooling, causing oil to infiltrate the product. The elevated fat content of the deep-fried grub may therefore be that of the commercial frying oil rather than the innate fat of the grubs. Consumption of deep-fried grubs could therefore provide different nutritional outcomes to consumers than the grubs prepared using other methods. Deep-frying of food also has a limitation of increasing its calorie content which puts the consumer at risk of obesity and cardiovascular diseases [75].

We found that the crude fibre content of the grubs was not affected by any cooking method, which corroborates a report by Yisa et al. [76] on *G. bimaculatus*, *R. differens* and *B. alcinoe*. This implies that the influence of the cooking heat on the cell wall structures is similar. Meanwhile, all processing methods reduced NDF content of the grubs compared to the raw grubs. Similarly, all methods reduced ADF content compared to raw grubs, except boiling. These findings corroborate the report by Dhingra et al. [77] that heating decreases the amount of soluble components of fibre due to solubilization of the polysaccharides.

The carbohydrate contents of the grubs were increased by boiling, but decreased by deep-frying. This could be because water hydrates starch, leading to gelatinization which increases digestibility of starch [78]. On the other hand, deep-frying is known to reduce the content of digestible starch due to the formation of amylose–lipid complexes [79].

Several studies have reported anti-nutrients in edible insects, which are ingested after consuming plant matter, that inhibit the absorption of important nutrients [60]. For instance, Musundire et al. [80] reported presence of tannins, cyanogenic glycosides, alkaloids, oxalates, and saponins in varying quantities in *Henicus whellani* which may have both positive and negative effects. Trace values of tannins, phytates and oxalates that interfere with the biovailability of proteins and minerals have been reported in *Oryctes*

monoceros and *Oryctes boas* in Nigeria [81]. Boiling has been found to reduce the levels of anti-nutrients in edible insects to permissible levels [82], although drying has been reported to concentrate the levels of tannins, oxalic acid, phytic acid, hydrogen cyanide and trypsin inhibitor in *Acheta domesticus* [83]. There is therefore need for further exploration on the presence and quantities of anti-nutrients in edible insects.

The fatty acid profiles varied considerably across the grubs cooked differently, with only five of the forty detected fatty acids occurring in the grubs across all the treatments; and a variable number and amounts of fatty acids uniquely occurring in grubs cooked using the different methods. This is a clear indication of different chemical processes accompanying the different cooking methods, which require more detailed investigations. These chemical changes could be due to moisture loss during heat preparation which concentrates some fatty acids, lipid oxidation, loss of the fatty acid into the cooking medium, oozing of the fatty acids out of the food matrix or release of lipids bound to other nutritional components after the bonds are broken [84]. Differences in fatty acid profiles associated with cooking methods have also been reported in beef [84] and other edible insects like mealworms [85] and grasshoppers [64].

We detected 13 amino acids, of which eight were essential amino acids. A previous study on *Oryctes rhinoceros* larva in Nigeria detected 17 amino acids, seven of which were essential amino acids [49]. The differences between our results and those in previous findings could be attributed to differences in species and geographical locations [58–60]. Future studies to ascertain the species of the beetle grubs in western Kenya where the studied grubs were collected is warranted to confirm their effect on nutritional value. We detected methionine and threonine as the limiting amino acids in the grubs, which corroborates reports on other edible insects like termites, moth caterpillars and crickets [86]. Meanwhile, phenylalanine was the predominant amino acid, demonstrating that the protein of the grubs has high emulsification capacity [87]. The marked differences in levels of some amino acids across the different cooking methods may be attributed to oxidative damages, amino acid side chain modification, or modification of aromatic amino acids, the extent of which varies with cooking time and temperature [88].

Although all the heat cooking methods reduced the TVC compared to raw grubs, only toasted grubs contained about 5 log cfu/g, which is the safety limit set out in the standards on edible insect products in Uganda and Kenya [89,90]. Apart from boiling, the TVC in the grubs cooked using the rest of the methods were very close to 5.7 log cfu/g which is the limit for minced meat which is recommended as a benchmark where standards on edible insects are lacking [91,92]. This reduction in TVC in the grubs by the heat processing methods may be a result of the high temperatures which dehydrates the grubs, lowering their water activity [93]. The total viable counts in this study are comparable to those of other raw edible insects [92,94]. Interestingly, the grubs contained higher TVC than their breeding substrates. This might be due to the exposure of the substrate to ultraviolet radiation from the sun which are known to damage bacterial DNA by suppressing metabolic activities and inhibiting gene expressions [95]. Further research is necessary to provide insight on the exact effect of ultraviolet radiation on the DNA of the diverse microorganisms reported in this study.

Apart from boiling which increased yeast and moulds counts compared to raw grubs, all cooking methods had no effect on the counts of these microbes. This may be explained by the highest moisture (lowest dry matter) and carbohydrate contents of boiled grubs which favour microbial growth [93,96]. Unlike the report by Nyangena et al. [68] that both boiling and toasting eliminated yeast and moulds in other edible insects namely *H. illucens, A. domesticus, R. differens* and *S. littoralis,* complete elimination of these microbes was not achieved with the beetle grubs. This may be attributed to intrinsic differences brought about by different rearing environments [68]. The yeast and mold levels in the heat cooked grubs exceeded the limits for edible insects and minced meat (<3 log cfu/g) [34,89,90].

All the cooking methods eliminated Enterobacteriaceae which occurred in the raw grubs [and their substrate]. Enterobacteriaceae includes microorganisms like *Escherichia* sp., *Salmonella* sp. and *Campylobacter* sp. whose presence is usually an indicator of poor hygiene during food handling and processing [97]. Elimination of these organisms from the grubs could be attributed to the high treatment temperatures which inactivate the microorganisms by either lowering their water activity or denaturing their protein composition [93]. The *Enterobacteriaceae* counts in the raw grubs were comparable to those reported for raw *R. differens* (6.89–7.83 log cfu/g) [98].

All cooking methods reduced *S. aureus* counts, but the counts exceeded <1 log cfu/g safety limit set for edible insects-based food in Uganda and Kenya [89,90]. Complete elimination of *S. aureus* due to these treatments is expected as all thermal treatments were above the survival temperature range for *S. aureus* of 6.5–50 °C [99]. It is probable that the *S. aureus* detection in the grubs subjected to different heat treatment may have been because of handling after the treatment or exposure to air [8,94].

Only toasting and roasting eliminated *Salmonella* sp. in the grub samples, partly corroborating van Huis et al. [8] that although boiling is effective in eliminating enteric bacteria, some spore-forming bacteria prevail. *Campylobacter* sp. however was only detected in the substrate but not in the raw or processed products. According to Wynants et al. [100], when *Salmonella* sp. is present at low levels in the substrate, it may not be detected in the raw insect due to exclusion by intrinsic microorganisms within the insect. *Salmonella* sp., *Shigella* sp. and *Campylobacter* sp. are pathogenic bacteria and major causes of food poisoning that may cause human gastroenteritis, diarrhoea and dehydration, while *S. aureus* may cause nausea and vomiting [101]. No trace of these bacteria is therefore tolerated in standards of edible insects and/or 25 g of minced meat [89–91].

Notably, the sustainability of the insect value chain is dependent on several factors which also have an effect on the safety of insect products namely the biology of the species, entomophagy knowledge, changing efficiency of traditional methods, farming conditions, processing, transport, and storage conditions, and consumer awareness and acceptance [102].

5. Conclusion and recommendations

Cooking methods greatly affected the nutritional value and safety of edible *Oryctes* sp. larvae. Deep-frying markedly reduced

protein, NDF and carbohydrate content of the grubs, but remarkably elevated their fat content. Boiled grubs had the lowest dry matter and ash content, but with the highest ADF and carbohydrate composition. Roasting and toasting spiked the protein and ash content of the grubs, respectively. Only five out of the forty fatty acids detected occurred in grubs across all the treatments; and six, two, three and one fatty acids were uniquely detected in only raw, roasted, boiled, toasted and deep-fried grubs, respectively. Saturated fatty acids were most abundant in the boiled grubs, while levels of MUFAs were mostly elevated in boiled and deep-fried grubs. Other than raw grubs, PUFAs were only detected in boiled grubs, but at much lower levels. Levels of most amino acids were comparable across treatments, while some of them notably lysine and arginine were elevated in all the cooking methods compared to raw grubs. All the processing methods eliminated Enterobacteriaceae, *Shigella* sp. and *Campylobacter* sp. from the grubs. Aside from boiling, all methods reduced total viable count to safe levels. All grub products in this study had exceedingly high *Staphyloccus aureus* levels hence post-processing handling of both the personnel and equipment needs to be carefully observed. However, *Salmonella* sp. were only eliminated by toasting and roasting; while boiling promoted the growth of yeast and moulds. These findings will guide the choices on the type of method to employ under specific consumer needs.

The anti-nutrients present in the edible rhinoceros beetle larvae in this study were not quantified. It is important to note that anti-nutrients may hinder absorption of nutrients. Therefore, further investigation is warranted on the presence and quantity of anti-nutrients in *Oryctes* sp. and how different cooking methods may affect them.

Limitations of the study

The weaknesses of this study are (i) there were no independent biological replicates within cooking methods due to limited availability of the samples, and (ii) the chemical composition of the commercial cooking oil used in frying the grubs was not determined, hence limiting explanation of its effects on the nutritional value and microbial content of the grubs.

Data availability statement

The data associated with this study have not been deposited into a publicly available repository; but they will be made available by the corresponding author on request.

CRediT authorship contribution statement

Marliyn W. Muthee: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Fathiya M. Khamis:** Writing – review & editing, Validation, Supervision, Software, Methodology. **Xavier Cheseto:** Writing – review & editing, Validation, Software, Methodology. **Chrysantus M. Tanga:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Sevgan Subramanian:** Writing – review & editing, Validation, Resources, Funding acquisition. **James P. Egonyu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgments

The authors thank Jacktone Ooko Ongere, Onesmus Wanyama, Levi Ombura, Francine Amutallah, Judy Gitonga, Joshua Wambua, Dorothy Nyangena, Paul Karanja and Maureen Adhiambo for their technical support. We gratefully acknowledge the financial support for this research by the following organizations and Agencies: the Norwegian Agency for Development Cooperation (Grant Number: RAF-3058 KEN-18/0005); Australian Centre for International Agricultural Research (ACIAR) (ProteinAfrica–Grant No: LS/2020/154), the Rockefeller Foundation (WAVE-IN—Grant No: 2021 FOD 030); Bill & Melinda Gates Foundation (INV-032416); the Curt Bergfors Foundation Food Planet Prize Award; the Swedish International Development Cooperation Agency; the Swiss Agency for Development and Cooperation; the Australian Centre for International Agricultural Research; the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya.

References

[1] L.W. Filho, et al., Reinvigorating the sustainable development research agenda: the role of the sustainable development goals (SDG), Int. J. Sustain. Dev. World Ecol. 25 (2) (2018) 131–142, https://doi.org/10.1080/13504509.2017.1342103.
[2] United Nations, "World Population Prospects, Department of economic and social affairs, population division, Online Edition. Rev 1 (2019).
[3] A.T. Adesogan, A.H. Havelaar, S.L. McKune, M. Eilittä, G.E. Dahl, Animal source foods: sustainability problem or malnutrition and sustainability solution? Perspective matters, Global Food Secur. 25 (2020) (2020) 100325, https://doi.org/10.1016/j.gfs.2019.100325.
[4] H. El Bilali, C. Callenius, C. Strassner, L. Probst, Food and nutrition security and sustainability transitions in food systems, Food Energy Secur. 8 (2) (2019) 1–20, https://doi.org/10.1002/fes3.154.
[5] M. Chagunda, P. Løvendahl, Quantification and Mitigation Strategies to Reduce Greenhouse Gas Emissions from Livestock Production Systems, MDPI, 2020.

[6] D. Dobermann, J.A. Swift, L.M. Field, Opportunities and hurdles of edible insects for food and feed, Nutr. Bull. 42 (2017) 293–308, https://doi.org/10.1111/nbu.12291.
[7] S. Kelemu, et al., African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security, J. Insects as Food Feed 1 (2) (2015) 103–119, https://doi.org/10.3920/JIFF2014.0016.
[8] A. van Huis, et al., *Edible Insects: Future Prospects for Food and Feed Security*, No. 171, Food and Agriculture Organization of the United Nations, Rome, 2013.
[9] Y. Quah, et al., Bioactive peptide discovery from edible insects for potential applications in human health and agriculture, Molecules 28 (3) (2023) 28031233, https://doi.org/10.3390/molecules28031233.
[10] K.W. Lange, Y. Nakamura, Edible insects and their potential anti-obesity effects: a review, Food Sci. Anim. Prod. 1 (1) (2023) 9240008, https://doi.org/10.26599/fsap.2023.9240008.
[11] V.B. Meyer-Rochow, Can insects help to ease the problem of world food shortage? Search 6 (7) (1975) 261–262.
[12] C. Kipkoech, J.N. Kinyuru, S. Imathiu, V.B. Meyer-rochow, *In vitro* study of cricket chitosan's potential as a prebiotic and a promoter of probiotic microorganisms to control pathogenic bacteria in the human gut, Foods 10 (2021) 2310, https://doi.org/10.3390/foods10102310.
[13] M.G. Paoletti, R. Damini, Human gastric juice contains chitinase, Ann. Nutr. Metab. 51 (2007) 244–251, https://doi.org/10.1159/000104144.
[14] A. Parodi, et al., The potential of future foods for sustainable and healthy diets, Nat. Sustain. 1 (12) (2018) 782–789, https://doi.org/10.1038/s41893-018-0189-7.
[15] S. Govorushko, Global status of insects as food and feed source: a review, Trends Food Sci. Technol. 91 (July 2018) (2019) 436–445, https://doi.org/10.1016/j.tifs.2019.07.032.
[16] J. Tao, Y.O. Li, Edible insects as a means to address global malnutrition and food insecurity issues, Food Qual. Saf. 2 (1) (2018) 17–26, https://doi.org/10.1093/fqsafe/fyy001.
[17] A. van Huis, A. Halloran, J. Van Itterbeeck, H. Klunder, P. Vantomme, How many people on our planet eat insects : 2 billion, J. Insects as Food Feed 8 (1) (2022) 1–4, https://doi.org/10.3920/JIFF2021.x010.
[18] K. Das, Entomophagy in Africa, in: A.A. Mariod (Ed.), African Edible Insects as Alternative Source of Food, Oil, Protein and Bioactive Components, Ghibaish, Sudan, Springer Nature Switzerland, 2020, pp. 1–314.
[19] S. Ghosh, C. Jung, V.B. Meyer-Rochow, What governs selection and acceptance of edible insect species, in: A. H, R. F, P. V, N. R (Eds.), Edible Insects in Sustainable Food Systems, Springer International Publishing AG, 2018, pp. 331–351.
[20] R.T. Gahukar, Edible insects collected from forests for family livelihood and wellness of rural communities : a review, Global Food Secur. January (2020) 100348, https://doi.org/10.1016/j.gfs.2020.100348.
[21] A. van Huis, Importance of insects as food in Africa, in: M.A. A (Ed.), African Edible Insects as Alternative Source of Food, Oil, Protein and Bioactive Components, Springer, Cham, 2020.
[22] A. van Huis, Cultural significance of beetles in sub-Saharan Africa, Insects 12 (4) (2021), https://doi.org/10.3390/insects12040368.
[23] E.S. Kusia, C. Borgemeister, C.M. Tanga, S. Ekesi, S. Subramanian, Exploring community knowledge, perception and practices of entomophagy in Kenya, Int. J. Trop. Insect Sci. (2021), https://doi.org/10.1007/s42690-021-00469-9.
[24] M.N. Wanjala, et al., Socio-cultural practices on the use of beetle grubs as food and feed in western Kenya, Sci. Rep. 13 (1) (2023) 1–12, https://doi.org/10.1038/s41598-023-34264-y.
[25] M.A. Augustin, et al., Role of food processing in food and nutrition security, Trends Food Sci. Technol. 56 (2016) 115–125, https://doi.org/10.1016/j.tifs.2016.08.005.
[26] B. Abraha, H. Admassu, A. Mahmud, N. Tsighe, X.W. Shui, Y. Fang, Effect of processing methods on nutritional and physico-chemical composition of fish: a review, MOJ Food Process. Technol. 6 (4) (2018) 376–382, https://doi.org/10.15406/mojfpt.2018.06.00191.
[27] M.W. Muthee, J.O. Anyango, J.W. Matofari, Influence of potato variety on phenolic content, *in vitro* starch digestibility and predicted glycaemic index of crisps and chips from Nyandarua County, Kenya, J. Food Nutr. Sci. 9 (3) (2021) 64–72, https://doi.org/10.11648/j.jfns.20210903.11.
[28] M. Modzelewska-kapitu, D. Ewa, B. Jankowska, A. Kwiatkowska, M. Cierach, The effect of muscle, cooking method and final internal temperature on quality parameters of beef roast, J. Meat Sci. 91 (2012) 195–202, https://doi.org/10.1016/j.meatsci.2012.01.021.
[29] C. Huang, et al., Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (*Hermetia illucens* L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties, Eur. Food Res. Technol. 245 (1) (2019) 11–21, https://doi.org/10.1007/s00217-018-3136-y.
[30] T. Lautenschläger, C. Neinhuis, E. Kikongo, T. Henle, A. Förster, Impact of different preparations on the nutritional value of the edible caterpillar *Imbrasia epimethea* from northern Angola, Eur. Food Res. Technol. 243 (5) (2017) 769–778, https://doi.org/10.1007/s00217-016-2791-0.
[31] D.G.A.B. Oonincx, M.D. Finke, Nutritional value of insects and ways to manipulate their composition, J. Insects as Food Feed (November) (2020) 1–22, https://doi.org/10.3920/jiff2020.0050.
[32] F.A. Manditsera, P.A. Luning, V. Fogliano, C.M.M. Lakemond, Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects, Food Res. Int. 121 (2019) 404–411, https://doi.org/10.1016/j.foodres.2019.03.052.
[33] B.A. Rumpold, O.K. Schlüter, Nutritional composition and safety aspects of edible insects, Mol. Nutr. Food Res. 57 (5) (2013) 802–823, https://doi.org/10.1002/mnfr.201200735.
[34] S. Labu, et al., Microbial contaminants in wild harvested and traded edible long-horned grasshopper, *Ruspolia differens* (Orthoptera: tettigoniidae) in Uganda ,", J. Insects as Food Feed (2021) 1–12, https://doi.org/10.3920/jiff2020.0069.
[35] Y.O. Kewuyemi, H. Kesa, C.E. Chinma, Fermented edible insects for promoting food security in Africa, Insects 11 (2020) 1–16, https://doi.org/10.3390/insects11050283.
[36] S. Belluco, A. Mantovani, A. Ricci, Edible Insects in a Food Safety Perspective, Springer International Publishing AG, 2018.
[37] J.N. Kinyuru, G.M. Kenji, S.M. Njoroge, M. Ayieko, Effect of processing methods on the *in vitro* protein digestibility and vitamin content of edible winged termite (*Macrotermes subhylanus*) and grasshopper (*Ruspolia differens*), Food Bioprocess Technol. 3 (5) (2009) 778–782, https://doi.org/10.1007/s11947-009-0264-1.
[38] O. Bolaji, D. Bamidele, A. Adeboye, A. Tanimola, Influence of preparation and processing methods on the physico-chemical properties of *Oryctes rhinoceros* larvae, Croat. J. Food Sci. Technol. 13 (2) (2021) 148–154, https://doi.org/10.17508/CJFST.2021.13.2.02.
[39] R.V.A. Gayathri, D.A. Evans, Culex quinquefasciatus Say larva adapts to temperature shock through changes in protein turn over and amino acid catabolism, J. Therm. Biol. 74 (2018) 149–159, https://doi.org/10.1016/j.jtherbio.2018.03.016.
[40] M.W. Mmari, J.N. Kinyuru, H.S. Laswai, J.K. Okoth, Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper *Ruspolia differens* (Serville 1838) in Tanzania, J. Ethnobiol. Ethnomed. 13 (1) (2017) 1–11, https://doi.org/10.1186/s13002-017-0191-6.
[41] D. Nowak, E. Jakubczyk, The freeze-drying of foods—the characteristic of the process course and the effect of its parameters on the physical properties of food materials, Foods 9 (1488) (2020) 1–27.
[42] AOAC, in: K. Helrich (Ed.), "AOAC: Official Methods of Analysis (Volume 1, fifteenth ed. vol. 1, Association of Official Analytical Chemists, Inc., Arlington, Virginia USA, 1990.
[43] S. Boulos, A. Tannler, L. Nyström, "Nitrogen-to-protein conversion factors for edible insects on the Swiss market : *T. molitor, A. domesticus*, and L. migratoria" Front. Nutr. 7 (July) (2020) 1–12, https://doi.org/10.3389/fnut.2020.00089.
[44] P.J. van Soest, J.B. Robertson, B.A. Lewis, Polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (10) (1991) 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
[45] H. Hailu Kassegn, Determination of proximate composition and bioactive compounds of the Abyssinian purple wheat, Cogent Food Agric. 4 (1) (2018) 1421415, https://doi.org/10.1080/23311932.2017.1421415.
[46] N.O. Igiehon, O.O. Babalola, X. Cheseto, B. Torto, Effects of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress, Microbiol. Res. 242 (2021) 126640, https://doi.org/10.1016/j.micres.2020.126640.

[47] Libretexts, Introduction to Enumeration of Bacteria, General Microbiology Laboratory, Libretexts, 2021 52245.
[48] Remel, "SALMONELLA SHIGELLA (SS) AGAR," Remel, 2010.
[49] C.C. Okaraonye, J.C. Ikewuchi, Nutritional potential of *Oryctes rhinoceros* larva, Pakistan J. Nutr. 8 (1) (2009) 35–38, https://doi.org/10.3923/pjn.2009.35.38.
[50] C. Offiah, O. Fasalejo, A. Akinbowale, Evaluation of nutritional and anti-nutritional values of *Oryctes rhinoceros* larvae in Ondo State , Nigeria, J. Entomol. Zool. Stud. 7 (5) (2019) 204–207.
[51] E. Anaduaka, O. Uchendu, O. Osuji, N. Ene, O. Amoke, Nutritional compositions of two edible insects : *Oryctes rhinoceros* larva and *Zonocerus variegatus*, Heliyon 7 (2021) (2021) 1–8, https://doi.org/10.1016/j.heliyon.2021.e06531.
[52] Z.T. Hlongwane, R. Slotow, Nutritional composition of edible insects consumed in Africa : a systematic review, Nutrients 12 (2020) 2786, https://doi.org/10.3390/nu12092786.
[53] L. Kou, A. Adámková, Nutritional and sensory quality of edible insects, NFS J 4 (2016) 22–26, https://doi.org/10.1016/j.nfs.2016.07.001.
[54] S. Marono, et al., *In vitro* crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits, Ital. J. Anim. Sci. 14 (3) (2016) 3889, https://doi.org/10.4081/ijas.2015.3889.
[55] J. Chakravorty, S. Ghosh, K. Megu, C. Jung, V.B. Meyer-rochow, Nutritional and anti-nutritional composition of *Oecophylla smaragdina* (hymenoptera : formicidae) and *Odontotermes* sp. (isoptera : termitidae): two preferred edible insects of Arunachal Pradesh, India, J. Asia Pac. Entomol. 19 (3) (2016) 711–720, https://doi.org/10.1016/j.aspen.2016.07.001.
[56] S. Ghosh, C. Jung, V.B. Meyer-Rochow, Nutritional value and chemical composition of larvae, pupae, and adults of worker honey bee, *Apis mellifera* ligustica as a sustainable food source, J. Asia Pac. Entomol. 19 (2) (2016) 487–495, https://doi.org/10.1016/j.aspen.2016.03.008.
[57] S. Ghosh, S. Lee, C. Jung, Nutritional composition of five commercial edible insects in South Korea, J. Asia Pac. Entomol. 20 (2017) 686–694, https://doi.org/10.1016/j.aspen.2017.04.003.
[58] J.P. Egonyu, S. Subramanian, C.M. Tanga, T. Dubois, S. Ekesi, S. Kelemu, Global overview of locusts as food, feed and other uses, Global Food Secur. 31 (2021) 100574, https://doi.org/10.1016/j.gfs.2021.100574.
[59] H.J. Magara, et al., Edible crickets (Orthoptera) around the world: distribution, nutritional value, and other benefits—a review, Front. Nutr. 7 (2021) 257, https://doi.org/10.3389/fnut.2020.537915.
[60] V.B. Meyer-Rochow, R. Gahukar, S. Ghosh, C. Jung, Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method, Foods 10 (2021) 1036, https://doi.org/10.3390/foods10051036.
[61] S. Asokapandian, G.J. Swamy, H. Hajjul, Deep fat frying of foods : a critical review on process and product parameters, Crit. Rev. Food Sci. Nutr. 60 (20) (2020) 3400–3413, https://doi.org/10.1080/10408398.2019.1688761.
[62] R.C. Megido, et al., Effect of household cooking techniques on the microbiological load and the nutritional quality of mealworms (*Tenebrio molitor* L. 1758), Food Res. Int. 2017 (2018), https://doi.org/10.1016/j.foodres.2018.01.002.
[63] M. Juarez, S. Failla, A. Ficco, F. Pena, C. Aviles, O. Polvillo, Buffalo meat composition as affected by different cooking methods, Food Bioprod. Process. 88 (2010) (2010) 145–148, https://doi.org/10.1016/j.fbp.2009.05.001.
[64] G. Ssepuuya, et al., Effect of heat processing on the nutrient composition , colour , and volatile odour compounds of the long-horned grasshopper *Ruspolia differens* Serville, Food Res. Int. 129 (2020) 108831.
[65] M. Baek, et al., Effects of processing methods on nutritional composition and antioxidant activity of mealworm (*Tenebrio molitor*) larvae, Entomol. Res. 49 (2019) 285–294, https://doi.org/10.1111/1748-5967.12363.
[66] D. Kouabitheu, M. Lesage, S. Clément, A.L.I. Ahmed, M.B. Adji, Effect of culinary treatments on the nutritional and microbiological properties of *Macrotermes subhyalinnus* and *Imbrasia obscura* : two insects consumed in the Adamawa and Est regions of Cameroon, J. Environ. Sci. Toxicol. Food Technol. 15 (9) (2021) 35–46, https://doi.org/10.9790/2402-1509023546.
[67] B.O. Ochieng, et al., Dynamics in nutrients, sterols and total flavonoid content during processing of the edible long-horned grasshopper (*Ruspolia differens* Serville) for food, Food Chem. 383 (1–9) (2022), https://doi.org/10.1016/j.foodchem.2022.132397.
[68] D.N. Nyangena, et al., 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 (5) (2020) 1–16, https://doi.org/10.3390/foods9050574.
[69] R. Suleman, Z. Wang, R.M. Aadil, D.L. Hopkins, D. Zhang, Effect of Cooking on the Nutritive Quality, Sensory Properties and Safety of Lamb Meat: Current Challenges and Future Prospects Raheel, Meat Sci., 2020 108172, https://doi.org/10.1016/j.meatsci.2020.108172.
[70] M.M.C. Sobral, S.C. Cunha, M.A. Faria, I.M. Ferreira, Domestic cooking of muscle foods : impact on composition of nutrients and contaminants, Compr. Rev. Food Sci. Food Saf. 00 (Efsa 2014) (2017) 1–25, https://doi.org/10.1111/1541-4337.12327.
[71] J.W. Bell, B.E. Farkas, S.A. Hale, Effects of retorting and storage on liquid mass transfer in canned skipjack (*Katsuwonas pelamis*), J. Food Process. Preserv. 26 (4) (2002) 267–278.
[72] N.U. Sruthi, Y. Premjit, R. Pandiselvam, A. Kothakota, V. Ramesh, An overview of conventional and emerging techniques of roasting : effect on food bioactive signatures, Food Chem. 348 (2021) 129088, https://doi.org/10.1016/j.foodchem.2021.129088.
[73] J.C. Lumanlan, W.M.A.D.B. Fernando, V. Jayasena, Mechanisms of oil uptake during deep frying and applications of predrying and hydrocolloids in reducing fat content of chips, Int. J. Food Sci. Technol. 55 (4) (2020) 1661–1670, https://doi.org/10.1111/ijfs.14435.
[74] M. Touffet, G. Trystram, O. Vitrac, Revisiting the mechanisms of oil uptake during deep-frying, Food Bioprod. Process. 123 (2020) 14–30, https://doi.org/10.1016/j.fbp.2020.06.007.
[75] W. Kaguongo, G. Maingi, M. Rono, E. Ochere, Kenya Agricultural Value Chain Enterprises: Potato Market Survey Report, USAID-KAVES, 2014.
[76] N.K. Yisa, I.M. Osuga, S. Subramanian, S. Ekesi, M.N. Emmambux, K.G. Duodu, Effect of drying methods on the nutrient content, protein and lipid quality of edible insects from East Africa, J. Insects as Food Feed (2022) 1–13, https://doi.org/10.3920/JIFF2021.0124.
[77] D. Dhingra, M. Michael, H. Rajput, Dietary fibre in foods : a review, J. Food Sci. Technol. 49 (3) (2012) 255–266, https://doi.org/10.1007/s13197-011-0365-5.
[78] D.F. Apata, Effect of cooking methods on available and unavailable carbohydrates of some tropical grain legumes, Afr. J. Biotechnol. 7 (16) (2008) 2940–2945.
[79] M. Ghidurus, M. Turtoi, G. Boskou, P. Niculita, V. Stan, Nutritional and health aspects related to frying (I), Rom. Biotechnol. Lett. 15 (2010) 5675–5682.
[80] R. Musundire, C.J. Zvidzai, C. Chidewe, B.K. Samende, F.A. Manditsera, Nutrient and anti-nutrient composition of *Henicus whellani* (Orthoptera: stenopelmatidae), an edible ground cricket, in south-eastern Zimbabwe, Int. J. Trop. Insect Sci. 34 (4) (2014) 223–231, https://doi.org/10.1017/S1742758414000484.
[81] A.B. Idowu, E.O. Oliyide, K.O. Ademolu, J.A. Bamidele, Nutritional and anti-nutritional evaluation of three edible insects consumed by the Abeokuta community in Nigeria, Int. J. Trop. Insect Sci. 39 (2) (2019) 157–163, https://doi.org/10.1007/s42690-019-00021-w.
[82] Y. Kunatsa, C. Chidewe, C.J. Zvidzai, Phytochemical and anti-nutrient composite from selected marginalized Zimbabwean edible insects and vegetables, J. Agric. Food Res. 2 (November 2019) (2020) 100027, https://doi.org/10.1016/j.jafr.2020.100027.
[83] D. Turck, et al., Safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a Novel food pursuant to Regulation (EU) 2015/2283, EFSA J. 19 (8) (2021) 6779, https://doi.org/10.2903/j.efsa.2021.6779.
[84] C.M.M. Alfaia, et al., Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat, Meat Sci. 84 (4) (2010) 769–777, https://doi.org/10.1016/j.meatsci.2009.11.014.
[85] S. Mancini, S. Mattioli, S. Paolucci, F. Fratini, Effect of cooking techniques on the *in vitro* protein digestibility, fatty acid profile, and oxidative status of mealworms (*Tenebrio molitor*), Front. Vet. Sci. 8 (2021) 1–11, https://doi.org/10.3389/fvets.2021.675572.
[86] O.F. Inje, A.H. Olufunmilayo, J.A. Audu, S.A. Ndaman, E.E. Chidi, Protein quality of four indigenous edible insect species in Nigeria, Food Sci. Hum. Wellness 7 (2) (2018) 175–183, https://doi.org/10.1016/j.fshw.2018.05.003.
[87] T. Kim, Hae In Yong, H.H. Chun, M. Lee, Y. Kim, Y. Choi, Changes of amino acid composition and protein technical functionality of edible insects by extracting steps, J. Asia Pac. Entomol. 19 (2019) 17, https://doi.org/10.1016/j.aspen.2019.12.017.

[88] C. Xia, P. Wen, Y. Yuan, X. Yu, Y. Chen, H. Xu, RSC Advances Effect of roasting temperature on lipid and protein oxidation and amino acid residue side chain modification of beef patties, R. Soc. Chem. 11 (2021) 21629, https://doi.org/10.1039/d1ra03151a.
[89] UNBS, Kampala, Uganda, in: "Edible Insects – Specification: US 2146:2020, first ed., 2020, 1st editio., Kampala, Uganda, 2020.
[90] KEBS, Edible Insects – Specification Part 1 : Edible Insects ' Products, 2020.
[91] European Commission, "Commission regulation (EC) No 1441/2007, of 5 December 2007, amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs,", Off. J. Eur. Union 322 (1441) (2007) 12–29.
[92] R.C. Megido, et al., Microbiological load of edible insects found in Belgium, Insects 8 (2017) 12.
[93] E. Enache, R. Podolak, A. Kataoka, L. Harris, Persistence of Salmonella and other bacterial pathogens in low moisture foods, in: R. Podolak, D.G. Black (Eds.), Control of Salmonella and Other Bacterial Pathogens in Low Moisture Foods, John Wiley and Sons, Inc., Chichester, UK, 2017.
[94] E. Amadi, D. Kiin-kabari, Nutritional composition and microbiology of some edible insects commonly eaten in Africa, hurdles and future prospects : a critical review, J. Food, Microbiol. Hyg. 1 (1) (2016) 1–7, https://doi.org/10.4172/2476-2059.1000107.
[95] L. Li, et al., Postharvest biology and technology synergistic effects of ultraviolet light irradiation and high-oxygen modified atmosphere packaging on physiological quality, microbial growth and lignification metabolism of fresh-cut carrots, Postharvest Biol. Technol. 173 (August 2020) (2021) 111365, https://doi.org/10.1016/j.postharvbio.2020.111365.
[96] M. Laranjo, et al., Impact of salt reduction on biogenic amines , fatty acids , microbiota , texture and sensory profile in traditional blood dry-cured sausages, Food Chem. 218 (2017) 129–136, https://doi.org/10.1016/j.foodchem.2016.09.056.
[97] T.R. Murefu, L. Macheka, R. Musundire, F.A. Manditsera, Safety of wild harvested and reared edible insects: a review, Food Control 101 (2019) 209–224, https://doi.org/10.1016/j.foodcont.2019.03.003.
[98] G. Ssepuuya, et al., Microbial characterisation of the edible grasshopper *Ruspolia differens* in raw condition after wild-harvesting in Uganda, Food Microbiol. 77 (2019) 106–117, https://doi.org/10.1016/j.fm.2018.09.005.
[99] M.I. Halpin-Dohnalek, E. Marth, *Staphylococcus aureus*: production of extracellular compounds and behavior in foods - a review, J. Food Protect. 52 (4) (1989) 267–282, https://doi.org/10.4315/0362-028x-52.4.267.
[100] E. Wynants, L. Frooninckx, S. Van Miert, A. Geeraerd, J. Claes, L. Van Campenhout, Risks related to the presence of *Salmonella* sp. during rearing of mealworms (*Tenebrio molitor*) for food or feed: survival in the feeding substrate and transmission to the larvae, Food Control 100 (2019) 227–234.
[101] CFS, "Microbiological Guidelines for Ready-To-Eat Food," 66 Queensway, Hong Kong., 2007.
[102] N.T. Grabowski, et al., Review : insects — a source of safe and sustainable food ?— 'Jein' (Yes and No), Front. Sustain. Food Syst. 5 (January) (2022) 1–17, https://doi.org/10.3389/fsufs.2021.701797.