Food Chemistry 383 (2022) 132397

Contents lists available at ScienceDirect

Food Chemistry

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

Dynamics in nutrients, sterols and total flavonoid content during processing of the edible Long-Horned grasshopper (*Ruspolia differens* Serville) for food

Brian O. Ochieng [a,b,*], Joseph O. Anyango [b], John M. Nduko [b], Xavier Cheseto [a], Cynthia M. Mudalungu [a], Fathiya M. Khamis [a], Changeh J. Ghemoh [d], Peter J. Egonyu [a], Sevgan Subramanian [a], Dorothy Nakimbugwe [c], Geoffrey Ssepuuya [c], 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
[d] Centre for African Bio-Entrepreneurship (CABE), P.O. Box 25535-00603, Lavington, Nairobi, Kenya

ARTICLE INFO

Keywords:
Ruspolia differens
Tettigoniidae
Post-harvest processing
Total flavonoid
Sterols
Nutrients
Food and nutritional security

ABSTRACT

Long-horned grasshopper (*Ruspolia differens* Serville) is a tasty delicacy in over 20 African countries. This study evaluated the impact of diverse post-harvest thermal treatment (blanching, boiling, toasting, and deep-frying) on the nutrients, total flavonoid content and sterols preservation of *R. differens* products. Crude protein, ash, and fibre of *R. differens* was drastically reduced by deep-frying technique. There was increase in Omega-3 (α-linolenic acid), Omega-6 fatty acid (linoleic and arachidonic acids) and sterols [(22*Z*)-27-Norergosta-5,22-dien-3β-ol, cholesterol, campesterol, cholest-4-ene-3-one and β-sitosterol] and flavonoids (2–3 folds) during blanching compared to other techniques. The iron and zinc content increased significantly in blanched and boiled products of *R. differens*. Thus, losses of nutrients, total flavonoid content and sterols during processing of *R. differens* for food can be mitigated by employing blanching technique, which is cheaper and least time-consuming. The implications of these dietary and therapeutic compounds on human nutrition and health are discussed.

1. Introduction

Insects have contributed to human diet from time immemorial and have transcended to gain global attention marked by increased number of individuals joining the industry. The growing awareness on the use of insects for food and feed has been fostered by the splendid nutritional values, the benign ecological footprints they offer as compared to conventional sources of proteins and the need to feed an ever surging human population of 6 million per month in light of shrinking natural resources, climate change and inadequate food production (Kinyuru, Mogendi, Riwa, & Ndung'u, 2015; Veldkamp & Vernooij, 2020). Socioeconomics, gender participation, cultural beliefs and technology encompassing mass rearing, harvesting, processing and safety concerns are some of the factors for consideration in attempts to advance entomophagy. The traditional techniques adopted by indigenous communities in harvesting, preparing, consuming and preservation of edible insects, have set a formidable precedent for their use in human nutrition (Kinyuru et al., 2015).

Longhorn grasshopper (*R. differens*) is an edible insect belonging to Tettigoniidae family in the order Orthoptera and is native to the sub-Saharan Africa. The insect is a common delicacy to the communities living in Lake Victoria region e.g. the *Haya* of Tanzania, the *Luo* of Kenya and the *Baganda* of Uganda, from ancient times (Mmari, Kinyuru, Laswai, & Okoth, 2017) and still contributes 5–10% of protein intake in the region (Ng'ang'a et al., 2019).

The full utilization of *R. differens* in the region has been impeded majorly by seasonality, uneven regional availability, sustainability, safety risks and perishability (Ng'ang'a et al., 2019). The *R. differens* is traditionally considered nutritionally rich and medically useful delicacy. The nutritional benefit of *R. differens* has further been well researched and documented. *Ruspolia differens* is rich in protein (34.2–45.8%), fat (42.2–54.3%) and vital amino acids such as leucine (80.9–86.5 mg/g), lysine (54.0–69.8 mg/g) and valine (58.1–61.8 mg/g) which are identified as most abundant (Ssepuuya et al., 2019). The insect also furnishes copious amounts of essential vitamins; vitamin A (2.1–2.8 μg/g), vitamin E (201.0–152.0 μg/g), niacin (2.1–2.4 mg/100 g), riboflavin

* Corresponding authors at: 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.foodchem.2022.132397
Received 6 November 2021; Received in revised form 26 January 2022; Accepted 6 February 2022
Available online 12 February 2022

(1.2–1.4 mg/100 g), vitamin C (0.1 mg/100 g), folic acid (0.9 mg/100 g), and pyridoxin (0.2-0.04 mg/100 g) and minerals; K (446.0–673.0 mg/100 g), P (429.0–627.0 mg/100 g), Ca (34.9–128.0 mg/100 g), Fe (13.0–16.6 mg/100 g) and Zn (12.4–17.3 mg/100 g) (Kinyuru, Kenji, Muhoho, & Ayieko, 2010; Ssepuuya et al., 2019). Unsaturated fatty acids are also reportedly abundant in *R. differens*; oleic acid (38.4–42.7 g/100 g), linoleic acid (19.0–23.0 g/100 g), palmitoleic acid (26.6–27.8 g/100 g) and α-linolenic acid (0.96–1.5 g/100 g) (Ssepuuya et al., 2019).

Despite the high nutritional value, edible insects are commonly processed to ensure microbial safety, increase shelf-life and improve on the sensory appeal (Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019; Nyangena et al., 2020). In Africa, frying, drying, roasting, smoking, boiling, toasting and steaming are the common edible insect processing methods (Mutungi et al., 2019). These processing methods are critical in preservation and shelf-life extension of *R. differens* owing to its high spoilage and seasonality (Fombong, Van Der Borght, & Vanden Broeck, 2017). Previous investigations have established that processing methods cause loss and degradation of some nutrients besides enhancing the levels, digestibility and bioactivity of the others (Dobermann, Field, & Michaelson, 2019; Melgar-Lalanne et al., 2019; Mutungi et al., 2019). These changes are induced through solubilization, leakages, intra and inter-biochemical reactions. For instance, proteolysis, protein denaturation, amino acid destruction and alteration, as well as Maillard reactions, lipid and fatty acid oxidation, vitamin and mineral solubilization, are all invoked by heat processes (Melgar-Lalanne et al., 2019). Variations in the effect of different processing methods can be attributed to the severity and contact time of the treatment, and non-uniformity of the processing conditions (Dobermann, Field, & Michaelson, 2019). Detailed reports on the effect of the different processing methods applicable to *R. differens* on its nutritional contents is inevitable especially with recent findings revealing the significance of edible insects in the fight against malnutrition and food insecurity (Mmari, Kinyuru, Laswai, & Okoth, 2017). Further, information paucity exists on the effect of these processing methods on flavonoids and sterols components.

Study by Ssepuuya et al. (2020) only focused on the effects of two thermal processes on the nutritional composition, colour and aroma compounds of *R. differens*. Further, investigation by Nyangena et al. (2020) apprised on the effects of toasting, boiling and drying techniques on only the proximate composition and microbial quality of *R. differens* and other edible insects. This study sought to bridge this gap by comparing the effects of four common post-harvest thermal processing of *R. differens* on the nutritional composition, and the levels of sterols and flavonoids which have high potential of conferring certain therapeutic benefits to humans.

2. Materials and methods

2.1. Sample acquisition and preparation

Approximately 20 kg of *R. differens* were purchased from Kampala (0.3476° N, 32.5825° E) and Masaka (0°20′28.0″S 31°44′10.0″E), the prime sourcing and harvesting hubs in Uganda, during the October-December swarming season, in the year 2019. Of this quantity, about 10 kg of dead and sorted *R. differens* with appendages, wings, ovipositor removed were acquired from two commercial harvesters at Masaka while the remaining portion was obtained from three market sellers in Kampala. The batch of samples obtained from both Masaka and Kampala dominantly comprised of the green and brown polymorphs with the purple-coloured morph being infrequent. Basing on location of acquisition, the samples were stuffed into polyethylene sterile zip lock bags (SC Johnson brand, Size 13 × 15″) purchased from local market, Kampala, and labeled accordingly. The samples were ice-packed in cooler boxes and hermetically sealed. The samples were then transported to International Center of Insect Physiology and Ecology (*icipe*) laboratory, Nairobi, Kenya. A permit, provided by the Kenya's Kenya Plant Health Inspectorate Services (KEPHIS), Ministry of Agriculture, Livestock, Fisheries and Cooperatives (Permit No.: KEPHIS/21591/2019) and Uganda's Ministry of Agriculture, Animal Industries and Fisheries Plant Quarantine and Inspection Services (License No.: UQIS4269/93/PC (E)) facilitated the importation of the samples to Kenya. Samples from both Masaka and Kampala were thoroughly mixed into one lump then transferred into a 24 × 24″ 48 L polyethylene sterile sample bag (Thomas scientific). The samples were immediately stored in a deep freezer at −20 °C awaiting further processing and analysis.

2.2. Samples preparation and processing

Frozen *R. differens* (2 kg) was allowed to thaw overnight at 5 °C refrigeration temperature. The samples were then washed twice in fresh tap water at 18 °C to remove dirt, drained and apportioned into 4 lumps of approximately 500 g each. The first portion was blanched at 100 °C for 5 mins (Fombong, Van Der Borght, & Vanden Broeck, 2017) using a stainless steel pot on an electric coil cooker (Von, China) with frequent turning using a wooden spoon after every 1 min. The second portion was boiled at 100 °C for 15 min (Mmari, Kinyuru, Laswai, & Okoth, 2017) using a stainless steel pot on an electric coil cooker (Von, China) with a turning frequency of 3 mins. The third portion was toasted at approximately 150 °C for 10 min in a stainless steel pan (Gatheru et al., 2019), on a heated electric coil cooker (Von, China) with turning every 2 mins. The fourth portion was deep fried in a preheated vegetable cooking oil (Fresh Fri, Pwani Oil Ltd) at 175 °C (Bordin, Tomihe Kunitake, Kazue Aracava, & Silvia Favaro Trindade, 2013) with a 2 min turning until they attained a crunchy texture and dark brownish colour (Mmari, Kinyuru, Laswai, & Okoth, 2017). Blanched, boiled and deep-fried samples were drained using a metallic strainer of medium pore size 1/16 in. All the processed samples were evenly spread on aluminum foils followed by drying in an oven (SDO-225, Wagtech International, Thatcham, UK) at 60 °C for 24 h (Fombong, Van Der Borght, & Vanden Broeck, 2017). Processed samples were transferred to polyethylene sterile zip lock bags and frozen-stored at −20 °C awaiting chemical analyses at *icipe* and International Livestock Research Institute (ILRI) laboratories, Nairobi.

2.3. Proximate composition of processed R. differens

Proximate determinations were done following official methods of Association of Official Analytical Chemists (AOAC, 2012). Moisture and dry matter were determined as moisture loss on drying in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 105 °C for 3 h. Kjeldahl method was adopted for nitrogen analysis in an automatic Kjeldahl analyzer (Velp UDK 159, Velp Scientifica, Europe) after digestion in a concentrated Sulphuric acid. A 6.25 nitrogen-to-protein conversion factor was considered for crude protein computation (Finke, 2007). Soxhlet extraction method, with petroleum ether as an extractant, was employed for crude fat determination in a Soxhlet extractor (Velp SER 148, Velp Scientifica, Europe). Crude ash was done gravimetrically in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 550 °C for 3 h. Crude fibre was determined by acid digestion and loss on ignition in a fibre analyzer (FIWE, Velp Scientifica, Europe).

2.4. Fatty acid and sterols determination

2.4.1. Folch oil extraction method

Oil extraction from the processed *R. differens* was achieved through a modified previous method (Igiehon, Babalola, Cheseto, & Torto, 2021). Samples, 1 g each were mixed with 10 mL of dichloromethane and methanol (2:1 v/v) in 50 mL falcon tubes. The mixtures were vortexed for 1 min, sonicated at 20 kHz for 10 mins and centrifuged at 4200 rpm for 10 mins. The supernatants were carefully filtered (Whatman filter paper grade 1, diameter 90 mm, pore size 2.5 μm) into clean 250 mL round bottomed flasks and solvent evaporated *in vacuo* to yield approximately 400 mg of oil.

2.4.2. Gas chromatography analysis of fatty acids and sterols in the oil extracts

The fatty acid (FA) profiles and sterols of oil extracts (300 mg each) from processed *R. differens*. were determined as fatty acid methyl esters (FAMEs) and sterols, respectively according to modified preceding methods (Cheseto et al., 2015, 2020). To each sample, 1 mL of sodium methoxide solution (15 mg/mL) was added. The mixture was vortexed for 1 min, ultra-sonicated for 10 min and incubated in a 70 °C water bath for 1 h. distilled deionized water (100 μL) was added to quench the reaction then vortexed for another 1 min. One-milliliter of gas chromatography (GC)-grade hexane (Sigma–Aldrich, St. Louis, MO, USA) was added to extract the resulting FAMEs followed by a 20 min-centrifugation at 14,000 rpm. The supernatant was carefully dried over anhydrous sodium sulphate, filtered and analyzed (1.0 μL) by GC–MS on a 7890A gas chromatograph linked to a 5975C 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). The carrier gas was helium at a flow rate of 1.25 mL/min. The oven temperature, programmed from 35 °C to 285 °C with a rising rate of 10 °C/min had the initial and final temperatures set to hold for 5 min and 20.4 min, respectively. The ion source and quadrupole mass selective detector temperatures were maintained at 230 °C and 180 °C, respectively. Acquisition of spectral masses from electron impact (EI) were at acceleration energy of 70 eV. Fragment ions were analyzed over 40–550 *m/z* mass range in the full scan mode. The filament delay time was set at 3.3 min. Serial dilutions of authentic standard methyl octadecanoate (0.2–125 ng/μL) prepared from octadecanoic acid (≥95 % purity) (Sigma-Aldrich, St. Louis, MO) was analyzed by GC–MS in full scan mode to generate a linear calibration curve (peak area vs. concentration) with the following equation; [y = 5E+07x+2E+07] which gave R^2 =, 0.9997. The various fatty acids and sterols in the samples were quantified using this regression equation. ChemStation B.02.02 software was used for the data acquisition with the following set integration parameters; initial threshold = 3, initial peak width = 0.01, initial area reject = 1 and shoulder detection = on. The compounds were identified by comparison of mass spectral data and retention times with those of authentic standards and reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 05, 08, and 11. Determination of the FAMEs and sterols in all the processed samples were made in three different batches of insect samples.

2.5. Mineral analysis

Mineral analysis was conducted in consonance with official analytical methods (AOAC, 2000). Ground samples ~ 0.5 g were mixed with 8 mL concentrated nitric acid (67–69% w/v, VWR Chemicals, Fontenay-sous-Bois, France) and 2 mL 30 % hydrogen peroxide (w/w) (Sigma-Aldrich, USA) in digestion tubes and left to stand for 12 h in a fume hood. The samples were then subjected to a programmed temperature digestion of 75 °C, 120 °C, 180 °C and 200 °C with respective lapse times of 30, 20, 20 and 10 min in a block digester (TE007–A, TECNAL, São Paulo, SP, Brazil). Completely digested sample solutions (clear solutions) were cooled, transferred into 25 mL falcon tubes and topped to the mark with 2% nitric acid. The contents of minerals under investigation from the samples and standard solution were analyzed on an inductively coupled plasma optical emission spectrometry (ICP-OES) measurements (Optima 2100™DV ICP-OES, Perkin Elmer Massachusetts, USA). The working parameters of the ICP-OES were as follows: 1450 W for radio frequency power; 15 L min^{-1} for plasma gas flow rate; 0.2 L min^{-1} for auxiliary gas flow rate; 0.8 L min^{-1} for nebulizer gas flow rate; 1.5 L min^{-1} for sample flow rate; axial mode for view mode; the peak area for reads and 10 s read delay; 10 s Source equilibration time; Replicates set at 1; Background correction at 2-points (manual point correction); Scott type spray chamber; Flow GemTip Nebulizer (HF resistant) cross; CCD detector; Nitrogen as Purge and Plasma gas and Shear gas was air. The characteristic elemental spectrum emitted by individual minerals were measured at the following wavelengths: Mg-285.213 nm, Fe-259.939 nm, Mn-257.61 nm, Ca-317.933 nm, P-213.617 nm, Mo-202.031 nm, K-766.49 nm, Al-396.153 nm, Cu-224.7 nm, Co– 228.616 nm and Zn-213.857 nm. ICP-OES mix standard CatNo.43843 (Merck, Schnelldorf, Germany) prepared through serial dilution with 2% nitric acid to obtain calibration standards of 400, 800, 2000 and 4000 μg/L were also analyzed by the ICP-OES to yield linear calibration curves with elemental correlation coefficient of $R^2 = 0.999$ for all the minerals under study. Perkin Elmer Winlab 32 software (Perkin Elmer, USA) was used for the external standard calibration and data acquisition. The data obtained was used to quantify the final concentration of each element. The analysis was done in triplicate.

2.6. Flavonoid content determination

The total flavonoid content was determined following methods by Dewanto, Wu, Adom, & Liu, (2002). Summarily, 0.5 g of the sample was mixed with 10 mL of 80% methanol in propylene tubes. The mixture was shaken on a mechanical shaker at 25 °C for 24 h followed by centrifugation at 4000 rpm for 10 min. Approximately 20 μL of supernatant (sample extract) or standard solution of catechin (10, 20, 40, 60, 80 and 100 μg/mL) was mixed with 80 μL of distilled deionized water in a microtiter tube. Ten-microliters of 5% $NaNO_2$ was added and then gently mixed. After 5 min, 10 μL of 10 % $AlCl_3$ was added, left to stand for 5 min before adding 80 μL of 2 M NaOH and mixed gently. The reaction was then incubated for 30 min at room temperature. The absorbance was read against a reagent blank (80% methanol) using plate reader spectrophotometer (Bio Tek Instruments, Winooski VT, USA) at 510 nm wavelength in comparison with a standard calibration plot (0.01–0.02 – 0.04–0.06 – 0.08 – 0.1 mg/mL) curve of catechin in 80 % methanol.

2.7. Statistical analysis

R Studio software version 1.3.1093–1 (R Core Team, 2020) for windows was used to conduct statistical analysis at α level of 0.05. Each experiment was carried out in triplicates resulting into a total of 60 independent experiments. Data exploration using box plot and Shapiro wilks test ($p < 0.05$) indicated that the data assumed a normal distribution. Single factor one way ANOVA was then used to establish the effects of different processing techniques (blanching, boiling, toasting and deep frying) on the proximate composition, fatty acid, sterols, minerals and flavonoids content of *R. differens*. The generated means were differentiated using Tukey's multiple comparison option of the ANOVA test and principal component analysis (PCA) to evaluate the differences in the fatty acids.

3. Results

3.1. Proximate composition

Significant variations ($p < 0.05$) occurred in proximate compositions of differentially processed *R. differens* (Table 1). Deep fried *R. differens* had the highest levels of dry matter (DM) and fat followed by the toasted insects, while boiled *R. differens* had the lowest DM and fat content. Contrastingly, the deep-fried *R. differens* recorded lowest protein, ash and fibre contents. Toasted *R. differens* had significantly higher protein content compared to deep-fried and blanched *R. differens*, however no significant difference was noted when compared to boiled sample.

3.2. Fatty acids content of the processed R. differens

The fatty acids contents of processed *R. differens* oils are presented in Table 2 **and Suppl.** Fig. 1. A total of 32 fatty acids (FAs) were detected in all the samples. Of this, saturated fatty acids (SFAs), monounsaturated

Table 1
Proximate composition (% dry matter) of *R. differens* processed using different methods.

Proximate composition						
Processing method	Moisture	Dry Matter	Protein	Fat	Ash	Fibre
Blanching	1.8 ± 0.05^{b}	98.2 ± 0.05^{b}	40.1 ± 1.33^{b}	43.8 ± 0.41^{b}	2.2 ± 0.00^{b}	11.2 ± 0.01^{b}
Boiling	14.5 ± 0.10^{c}	85.6 ± 0.10^{a}	43.1 ± 1.60^{bc}	36.3 ± 1.06^{a}	2.3 ± 0.09^{b}	10.9 ± 0.19^{b}
Toasting	1.6 ± 0.06^{b}	98.4 ± 0.06^{b}	44.7 ± 1.03^{c}	46.0 ± 0.82^{b}	2.4 ± 0.17^{b}	9.0 ± 0.74^{a}
Deep-frying	0.8 ± 0.03^{a}	99.2 ± 0.03^{c}	7.8 ± 0.59^{a}	83.0 ± 1.54^{c}	1.2 ± 0.16^{a}	8.7 ± 0.39^{a}
F_{df}	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$
P-value	0.001	0.001	0.001	0.001	0.001	0.001

Same small superscript letters within columns indicate no significant differences of proximate composition at $p < 0.05$. All values are presented as mean ± SD.

fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) contributed 19, 10 and 3 components, respectively. Methyl hexadecanoate and methyl octadecenoate of the SFA, methyl (9Z)-hexadecenoate and methyl (11*Z*)-eicosenoate of the MUFAs and methyl (9*E*,12*E*)-octadecadienoate of the PUFAs were the most abundant FAs. Deep-frying significantly enhanced levels of methyl (9*E*)-octadecenoate (oleic acid) ($F_{(3,8)} = 371.4$, $p < 0.001$), toasting elevated the levels of methyl (9*Z*)-hexadecenoate (palmitoleic acid) ($F_{(3,8)} = 66.0$, $p < 0.001$) while blanching boosted the levels methyl (10*Z*)-nonadecenoate ($F_{(3,8)} = 58.3$, $p < 0.001$), methyl (10*Z*)-heptadecenoate ($F_{(3,8)} = 152.1$, $p < 0.001$) and methyl (9*E*)- tetradecenoate (myristoleic acid) ($F_{(3,8)} = 41.9$, $p < 0.001$). Of the PUFAs, the only omega 3 FA detected was methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate (*α*-linolenic acid) while omega 6 were methyl (9*E*,12*E*)-octadecadienoate (Linoleic acid) and methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate (Arachidonic acid). Blanching resulted into a significantly higher methyl (9*E*,12*E*)-octadecadienoate ($F_{(3,8)} = 423.1$, $p < 0.001$), methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate ($F_{(3,8)} = 89.9$, $p < 0.001$), methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate ($F_{(3,8)} = 49.3$, $p < 0.001$), total PUFAs ($F_{(3,8)} = 424.1$, $p < 0.001$), total MUFAs ($F_{(3,8)} = 68.1$, $p < 0.001$) and PUFAs/MUFAs ($F_{(3,8)} = 135.3$, $p < 0.001$) ratios than boiling, toasting and deep-frying.

Two-dimensional Principal Component Analysis (PCA) explained 68.3% of the variation based on influence of four processing methods on the levels of detected FAs (**Suppl.** Fig. 1**A**.) and grouping the FAs based on their differences in concentration from the four processing methods (**Suppl.** Fig. 1**B**.). The 1st and 2nd PC accounted for 42.6% and 36.1% of the total variance, respectively. Deep-frying and boiling had significantly same effect on the concentrations of FAs (Fig. 2A). The concentrations of methyl 15 methylhexadecanoate, methyl (5*Z*)-dodecanaote, methyl pentadecanoate, methyl (10*Z*) heptadecanoate, methyl (10*Z*)-nonadecanoate, methyl tridecanoate, methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate, methyl (9*Z*)-tetradecenoate, methyl (9*E*,12*E*)-octadecadienoate, methyl (11*Z*)-eicosenoate, methyl (9*Z*)-heptadecenoate and methyl dodecanoate were less different and contributed to variation in the first quadrant. Concentrations of methyl 3-methoxyoctadecanoate, methyl (9*Z*)-hexadecenoate, methyl undecanoate, methyl tetracosanoate, methyl 3-methyltridecanoate, methyl 12-methyltridecanoate, methyl nonadecanoate, methyl decanoate, methyl tetradecanoate, methyl hexadecanoate, methyl docosanoate, methyl (13*Z*)-docosenoate and methyl (11*Z*)-octadecenoate matched and led to the variation in the second quadrant. The levels of methyl 20-methylhexacosanoate, methyl octadecenoate, methyl tricosanoate, methyl eicosanoate and methyl (9*E*) octadecenoate were related and contributed to the variation in the third quadrant. The proportions of methyl heneicosanoate and methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate were indifferent and caused the variability in the fourth quadrant (**Suppl.** Fig. 1**B**.)

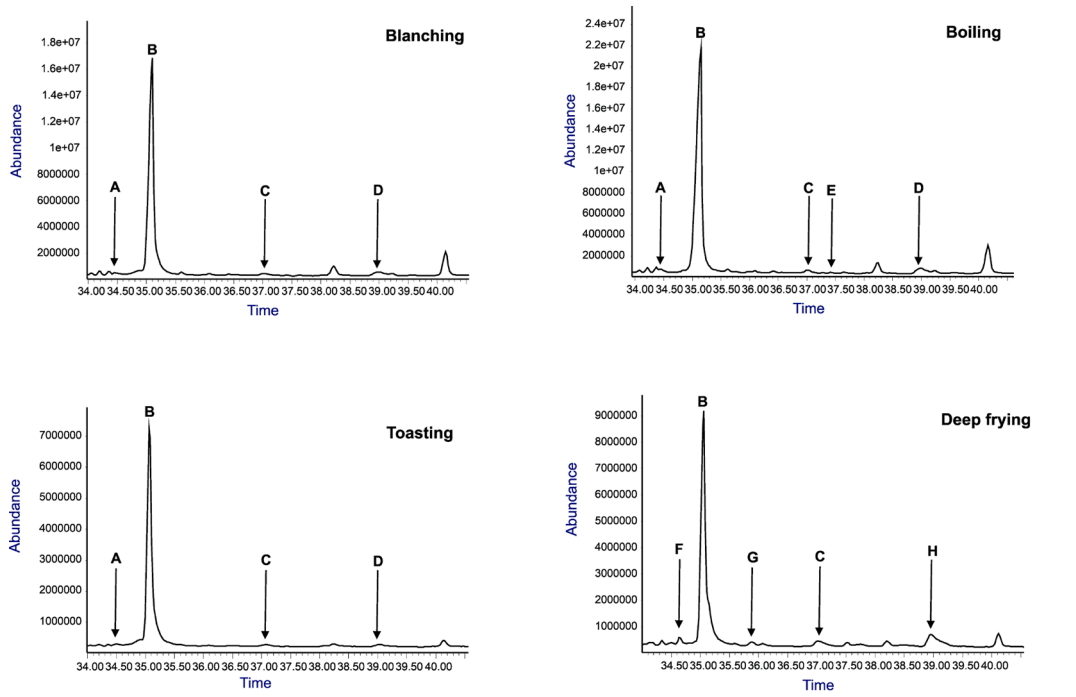
Table 2
Fatty acid composition (μg /g dry matter) of oil extracted from *Ruspolia differens* processed differently.

Processing method				
Fatty Acid	Blanching	Boiling	Toasting	Deep-frying
C10:0	0.1 ± 0.05^{a}	0.3 ± 0.03^{a}	1.3 ± 0.30^{b}	0.2 ± 0.02^{a}
C11:0	0.05 ± 0.02^{a}	0.06 ± 0.02^{a}	0.06 ± 0.008^{a}	0.04 ± 0.007^{a}
C12:0	17.2 ± 1.08^{b}	10.05 ± 2.69^{a}	15.8 ± 2.56^{b}	14.0 ± 1.89a^{b}
C13:0	0.2 ± 0.10^{b}	0.05 ± 0.01^{a}	0.09 ± 0.01^{a}	0.03 ± 0.002^{a}
isomethyl-C13:0	0.09 ± 0.03^{a}	0.09 ± 0.01^{a}	0.8 ± 0.24^{b}	0.006 ± 0.0005^{a}
isomethyl-C13:0	0.09 ± 0.02^{b}	0.01 ± 0.005^{a}	1.75 ± 0.04^{c}	0.008 ± 0.001^{a}
C14:0	73.2 ± 20.30 ^{a}	77.1 ± 4.03^{a}	162.3 ± 18.59^{b}	92.6 ± 24.16^{a}
C15:0	4.2 ± 1.98^{b}	0.9 ± 0.005^{a}	0.1 ± 0.02^{a}	0.1 ± 0.02^{a}
C16:0	1361.4 ± 107.90^{a}	1354.9 ± 235.84^{a}	1652.9 ± 80.27^{a}	1404.5 ± 97.18^{a}
isomethyl-C16:0	17.6 ± 3.51^{b}	48.6 ± 10.76^{c}	1.8 ± 0.23^{a}	0.4 ± 0.04^{a}
C18:0	271.1 ± 39.70^{a}	311.6 ± 10.52^{ab}	341.1 ± 30.28^{b}	350.9 ± 15.41^{b}
C19:0	5.9 ± 0.51^{b}	3.6 ± 0.19^{a}	17.9 ± 1.03^{c}	5.6 ± 0.22^{b}
isomethyl-C18:0	3.6 ± 0.82^{b}	4.4 ± 0.64^{b}	4.9 ± 0.85^{b}	0.5 ± 0.9^{a}
C20:0	54.3 ± 8.70^{a}	49.8 ± 0.55^{a}	57.8 ± 2.75^{a}	64.5 ± 7.66^{a}
C21:0	3.9 ± 1.06^{b}	0.2 ± 0.03^{a}	0.5 ± 0.15^{a}	2.9 ± 0.86^{b}
C22:0	3.5 ± 1.42^{a}	11.0 ± 0.44^{a}	28.8 ± 7.80^{b}	12.9 ± 1.49^{a}
C23:0	3.8 ± 0.99^{a}	5.08 ± 0.46^{ab}	5.6 ± 0.16^{b}	6.3 + 0.02^{b}
C24:0	4.5 ± 0.31^{a}	8.3 ± 1.47^{b}	8.7 ± 2.26^{b}	2.6 ± 0.52^{a}
isomethyl-C26:0	2.5 ± 0.28^{a}	3.9 ± 0.57^{b}	3.1 ± 0.25^{ab}	3.0 ± 0.57^{ab}
∑ SFA	1827.3 ± 92.24^{a}	1889.9 ± 242.93^{a}	2305.4 ± 89.85^{b}	1961.1 ± 114.16^{ab}
14:1n-5	2.3 ± 0.35^{c}	1.4 ± 0.16^{b}	1.4 ± 0.33^{b}	0.01 ± 0.002^{a}
C10:1n-5	1.7 ± 0.43^{b}	1.7 ± 0.21^{b}	0.05 ± 0.007^{a}	0.03 ± 0.01^{a}
C16:1n-7	130.3 ± 7.61^{b}	107.6 ± 5.54^{a}	159.9 ± 3.68^{c}	94.4 ± 6.90^{a}
C17:1n-7	41.5 ± 4.11^{c}	6.6 ± 0.45^{a}	14.3 ± 1.33^{b}	10.3 ± 0.98^{ab}
C17:1n-9	13.9 ± 0.99^{b}	11.9 ± 3.46^{b}	15.3 ± 1.74^{b}	2.4 ± 0.27^{a}
C18:1n-11	1.4 ± 0.07^{a}	2.7 ± 0.12^{ab}	4.7 ± 1.06^{c}	3.7 ± 0.45^{bc}
C18:1n-9	8.4 ± 0.79^{b}	1.8 ± 0.21^{a}	3.1 ± 0.59^{a}	42.1 ± 3.27^{c}
C19:1n-9	43.3 ± 6.11^{c}	14.9 ± 2.21^{ab}	20.9 ± 1.76^{b}	9.4 ± 0.49^{a}
C20:1n-9	50.7 ± 13.71^{a}	30.5 ± 4.00^{a}	53.5 ± 9.76^{a}	31.9 ± 10.27^{a}
C22:1n-9	0.6 ± 0.19^{a}	5.0 ± 0.86^{b}	5.7 ± 0.53^{b}	2.4 ± 1.07^{a}
∑ MUFA	294.0 ± 16.87^{b}	184.1 ± 5.52^{a}	278.9 ± 14.24^{b}	196.8 ± 5.95^{a}
C18:2n-6	2177.6 ± 42.67^{d}	1542.7 ± 33.59^{b}	1723.3 ± 80.41^{c}	678.4 ± 41.97^{a}
C18:3n-3	4.3 ± 0.33^{c}	2.5 ± 0.14^{b}	1.7 ± 0.27^{a}	2.7 ± 0.30^{b}
C20:4n-6	4.0 ± 0.22^{c}	2.1 ± 0.03^{a}	2.9 ± 0.08^{b}	1.7 ± 0.30^{a}
∑ PUFA	2185.9 ± 43.18^{d}	1547.3 ± 33.44^{c}	1727.9 ± 80.65^{b}	682.8 ± 41.37^{a}
∑ n-6 PUFA	2181.6 ± 42.89^{d}	1544.8 ± 33.56^{b}	1726.2 ± 80.46^{c}	680.1 ± 41.67^{a}
∑ n-3 PUFA	4.3 ± 0.33^{c}	2.5 ± 0.14^{b}	1.7 ± 0.27^{a}	2.7 ± 0.30^{b}
∑n-6/n-3	507.3	617.9	1015.3	251.9
∑PUFA/SFA	1.2 ± 0.04^{c}	0.8 ± 0.09^{b}	0.7 ± 0.03^{b}	0.3 ± 0.005^{a}

Fatty acids presented as a Mean ± SD (standard deviation) of triplicate determinations. SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

3.3. Sterol composition of the processed R. differens

The proportions of (22*Z*)-27-Norergosta-5,22-dien-3*β*-ol ($F_{(3,8)} = 71.3$, $p < 0.001$), cholesterol ($F_{(3,8)} = 34.8$, $p < 0.001$), campesterol ($F_{(3,8)} = 17.1$, $p < 0.001$), cholest-4-ene-3-one ($F_{(3,8)} = 14.4$, $p < 0.01$) and sitosterol ($F_{(3,8)} = 37.6$, $p < 0.001$) varied significantly across the processing methods. Blanching caused a significantly higher (22*Z*)-27-Norergosta-5, 22-dien-3*β*-ol, cholesterol, campesterol, cholest-4-en-3-one and *β*-sitosterol levels compared to other processing methods (Table 3).



A -(22*Z*)-27-Norergosta-5,22-dien-3β-ol, **B** - Cholesterol, **C** - Campesterol, **D** - β-Sitosterol, **E** -Cholest-4-en-3-one, **F** - Ergosta-4,6,22-triene-3-β-ol
G - Stigmasta-4,6,22-triene-3-β-ol, **H** - γ-Sitosterol

Fig. 1. Representative total ion Chromatograms of sterols from *R. differens* after subjecting to four different processing methods.

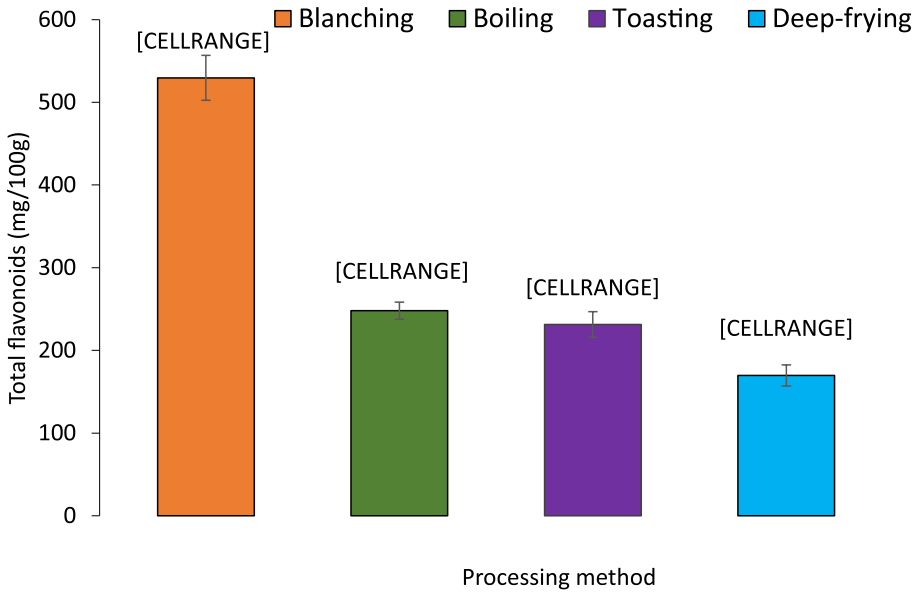


Fig. 2. Bar chart displaying the variability of total flavonoids (mg/100 g DM) in the different processed *R. differens* samples. Error bars indicate standard deviation of the means. For every processing method, the bars carrying the same small letters of b correspond to total flavonoid values that are not significantly different ($p < 0.05$).

The total ion chromatograms of the sterols and their thermally modified products from the four processed *R. differens* are indicated in the Fig. 1 below. Cholest-4-en-3-one levels were negligible and a clear peak could only be realized in boiled samples. In deep-frying, (22Z)-27-Norergosta-5,22-dien-3β-ol was transformed into ergosta-4,6,22-triene-3-β-ol and β-sitosterol was interchanged by γ-sitosterol isomer. Stigmasta-4,6,22-triene-3-β-ol was however detected in this sample for the first time as compared to the other processing methods.

3.4. Mineral profile

Calcium (Ca), Phosphorous (P), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn) varied significantly across the processed *R. differens* except for Copper (Cu) and Cobalt (Co) (Table 4.). P, Ca, Mg were the predominant macro-minerals while Fe, Zn and Mn were the predominant trace minerals detected in all the processed *R. differens*. Deep-fried *R. differens* showed significantly lower ($p < 0.05$) values of minerals in all the processed samples.

Table 3
Sterols content (μg/g dry matter) of oil extracted from *R. differens* processed differently.

Processing method				
Compound	Blanching	Boiling	Toasting	Deep-frying
(22*Z*)-27-Norergosta-5,22-dien-3*β*-ol	2.3 ± 0.05^{b}	2.3 ± 0.14^{b}	2.6 ± 0.19^{b}	1.2 ± 0.09^{a}
Ergosta-4,6,22-trien-3-*β*-ol	–	–	–	1.9 ± 0.04
Cholesterol	82.2 ± 6.04^{c}	63.2 ± 5.79^{b}	43.4 ± 6.61^{a}	41.8 ± 3.24^{a}
Stigmasta-4,6,22-triene-3- *β* -ol	–	–	–	2.5 ± 0.22
Campesterol	4.5 ± 0.37^{c}	4.4 ± 0.25b^{c}	2.8 ± 0.37^{a}	3.6 ± 0.34^{ab}
*Cholest-4-en-3-one	1.0 ± 0.16^{b}	1.1 ± 0.03^{b}	0.9 ± 0.06^{a}	0.7 ± 0.08^{a}
β Sitosterol	7.6 ± 0.59^{b}	4.2 ± 0.40^{a}	3.8 ± 0.79^{a}	–
γ Sitosterol	–	–	–	3.5 ± 0.13

Results are presented as mean ± standard deviation. Same small letters within rows indicate no significant differences of the minerals at $p < 0.05$. *Not a sterol.

Table 4
Mineral profiles (dry matter) of *R. differens* processed using different methods.

Processing method						
Minerals	Blanching	Boiling	Toasting	Deep-frying	F_{df}	*P-value*
Ca (mg/100 g)	47.9 ± 1.06^{b}	54.5 ± 1.92^{b}	55.0 ± 5.05^{b}	35.8 ± 0.47^{a}	$F_{(3,8)}$	0.001
P (mg/100 g)	427.8 ± 1.80^{b}	427.7 ± 14.36^{b}	496.8 ± 27.60^{c}	305.4 ± 18.02^{a}	$F_{(3,8))}$	0.001
Mg (mg/100)	52.8 ± 0.54^{b}	56.5 ± 1.57^{b}	53.2 ± 7.10^{b}	33.3 ± 1.15^{a}	$F_{(3,8)}$	0.001
Fe (mg/100 g)	140.9 ± 8.59^{b}	179.1 ± 14.18^{c}	22.2 ± 0.27^{a}	12.9 ± 2.48^{a}	$F_{(3,8)}$	0.001
Cu (mg/100 g)	1.9 ± 0.05^{b}	2.3 ± 0.18^{b}	2.2 ± 0.22^{b}	1.6 ± 0.04^{a}	$F_{(3,8))}$	0.01
Mn (mg/100 g)	5.3 ± 0.04^{c}	4.1 ± 0.0003^{b}	4.0 ± 0.0^{8b}	2.9 ± 0.70^{a}	$F_{(3,8)}$	0.001
Zn (mg/100 g)	16.8 ± 0.97^{c}	18.4 ± 0.08^{c}	13.2 ± 1.82^{b}	8.5 ± 0.15^{a}	$F_{(3,8)}$	0.001
Co (μg/100 g)	25.6 ± 4.49^{a}	33.1 ± 0.005^{a}	28.1 ± 5.71^{a}	29.9 ± 1.00^{a}	$F_{(3,8)}$	ns

3.5. Total flavonoid content

Fig. 2 represents the flavonoid composition of the differently processed *R. differens* pastes. The flavonoid contents of the processed *R. differens* were significantly different ($F_{(3,8)} = 248.6$; $p < 0.001$) . The total flavonoid contents analyzed varied in the following order, blanched (529.59 mg/100 g) > boiled (248.02 mg/100 g) > toasted (231.35 mg/100 g) > deep-fried (169.68 mg/100 g).

4. Discussion

Dry matter (DM) represents nutrients in a food devoid of moisture. The high deep-frying temperatures (150–200 °C) and immiscibility of oil and water cause a quick dehydrative effect to the food. Consequently, the oil quickly seeps to fill the voids in the food matrices left by moisture (Bordin et al., 2013) which resulted into the higher DM and fat content. On the other hand, the lower DM and fat shown in boiled *R. differens* was due to continuous loss of fat into the boiling water.

Toasted, boiled and blanched *R. differens* samples displayed higher protein content than the deep-fried one. Manditsera, Luning, Fogliano, & Lakemond (2019) however, reported remarkable protein loss in boiled beetles and crickets but no loss upon toasting. These losses have been linked to thermal hydrolytic breakdown of connective tissues, solubilization of soluble proteins into boiling water, declining nitrogen owing to amine and amide loss, and complexing with reactants in the food media, particularly lipid oxidation products (Nyangena et al., 2020). In this study, the masking impact of the substantial loss of important dry matter elements, especially fat, in boiled, blanched, and toasted *R. differens* may have been suppressed by these effects (Nyangena et al., 2020; Ssepuuya et al., 2020). This trend is clearly evident in Table 1, where the protein and fat content variations in boiled and blanched *R. differens* had a negative association. On the other hand, deep fat frying has been intimated to catalyze a series of chemical reactions in food which leads to changes in nutritional composition. For instance, proteins become denatured, amino acids are destroyed, amino groups of amino acids, peptides and proteins are involved in Mailliard reaction yielding flavour compounds (Bordin et al., 2013). This might explain the relatively lower protein content of the deep-fried *R. differens*.

The ash contents of blanched, boiled and toasted *R. differens* were comparable, but significantly higher than deep frying. This contradicts other studies that showed a decrease in ash concentration in boiled insects but an increase in toasted insect products (Nyangena et al., 2020; Ssepuuya et al., 2020). The notable ash decrease in boiled insects were due to leaching losses into water. Therefore, this effect may have been overshadowed by the proportional loss of other major components like fat in the boiled, blanched and toasted *R. differens* in the current study. The fibre content of the boiled and blanched *R. differens* was higher than toasted and deep-fried *R. differens*. These differences in fibre contents may be ascribed to process-induced alterations in the chemical constituents such as cellulose, hemicellulose, pectin, gums and lignin of the fibrous material (MarkManuel & Godwin, 2020). The processing methods used in this study differed in terms of duration, temperature, and medium used, and may have had varying degrees of influence on the fibre content.

The dominant SFAs, MUFAs and PUFAs observed in the processed *R. differens* mirrors the pattern of dominant FAs that have been detected in house flies, Turkestan cockroaches, mealworms, super worms, waxworms, crickets, tebo worms, locusts (Mohamed, 2015) and *R. differens* oil (Cheseto et al., 2020). The levels of FAs ranged 42.4–69.0% SFA, 5.1–6.9% MUFA and 24.0–50.8% PUFA across the processed *R. differens* which are consistent with the levels of 45% SFA, 20% MUFA and 34% PUFA previously reported in oil from *R. differens* (Cheseto et al., 2020). The levels of PUFAs *α*-linolenic acids (Omega 3), linoleic acid (Omega 6) and arachidonic acid (Omega 6) were 1.3–3.2-fold higher in blanched *R. differens* than in boiled, toasted and deep-fried samples. Furthermore, as the levels of PUFAs decreased from blanching > boiling > toasting > deep-frying, there was a proportional increase in the SFAs. This trend was also witnessed by Ali et al. (2013) where the proportions of palmitic, stearic and oleic acids increased with a proportional decrease in linoleic acid upon subjecting sunflower oil to frying temperatures of 185 °C. This can be accredited to decomposition of different PUFAs to SFAs during processing. Besides, the high temperature conduce the vulnerability of the double bonds in PUFAs to attack by free radicals, resulting into their significant decrease after heating (Ali et al., 2013). However, in blanching, the process was short-lived and could not promote a significant loss of the FAs. The levels of linoleic acid remained conspicuously higher than all the other FAs culminating into high PUFAs and ∑n-6/n-3 ratios surpassing the World Health Organization (WHO) daily intake ratio of 5:1 in all the processed samples (Cheseto et al., 2020). This may be ascribed to the detection of only one omega 3 FA despite Cheseto et al. (2020) revealing methyl (9*Z*,15*Z*)-octadecadienoate, methyl (9*Z*,11*E*,13*E*)-octadecatrienoate (α eleostearic acid), methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate (EPA) and methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoate (DHA) as main omega 3 FAs in *R. differens* oil, not detected in this study. This may be attributable to differences in dietary sources and other physiological parameters

(Oonincx & Finke, 2020). Nevertheless, linoleic acid has been reported an abundant PUFA in *R. differens* (Fombong, Van Der Borght, & Vanden Broeck, 2017; Ssepuuya et al., 2019). Linoleic acid is an essential FA, for the biosynthesis of arachidonic acid, which is a precursor for the synthesis of a number of hormones that regulate physiological processes in the body and prevent inflammatory skin diseases (Cheseto et al., 2020).

The significantly higher oleic acid in deep fried *R. differens* is due to the vegetable oil used, as the key ingredient was refined palm olein from palm oil reportedly comprising 40–56% oleic acid (de Almeida et al., 2021). Oleic acid confers health benefits such as lowering risk of coronary diseases, cancer and inflammation, promoting cell viability, serving as a precursor for linoleic acid synthesis and providing energy to humans (Cheseto et al., 2020). Interestingly, the PUFA/SFA ratios in all the processed *R. differens* remained higher than 0.4 as recommended by World Health Organization (WHO). The ratio was 1.5–4 folds higher in blanched *R. differens* than the other processed insects possibly due to the significantly higher amount of linoleic acid. A PUFA/SFA of<0.4 has been linked to promotion of atherosclerotic disorders while on the other hand, a PUFA/SFA value more than 0.4 has been associated with cholesterol lowering potential (Momenzadeh, Khodanazary, & Ghanemi, 2017).

A total of four sterols and an intermediary product of steroid transformation were detected in the processed *R. differens*. Sterols have previously been reported in desert locust (*Schistocerca gregaria*) (Cheseto et al., 2015) and crickets, mealworms and superworms (Finke, 2015). Despite insects' essential need of 7-Dehydrocholesterol for vitamin D3 and steroid hormone synthesis, they are metabolically incapable of synthesizing it *de novo*. They however acquire plant phytosterols for 7-Dehydrocholesterol synthesis (Sabolová et al., 2016). In the present study, the concentrations of (22*Z*)-27-Norergosta-5,22-dien-3β-ol, cholesterol, campesterol, cholest-4-ene-3-one and β sitosterol were influenced by the processing methods with blanched *R. differens* exhibiting higher concentrations of all sterols. Deep frying and toasting however showed lowest concentrations of all the sterols. These findings were consistent with the study by Dias-Martins et al. (2021) that demonstrated phytosterols decomposition at 60 °C and a subsequent soaring effect with proportional temperature rise to yield oxysterols, sterol fragments, volatiles and oligomers. The toasting and deep-frying were done at temperatures greater than 150 °C defining the low sterol values while blanching was conducted at 100 °C but for a shorter period than other processed *R. differens,* hence explaining the record high values of sterols. Nonetheless, deep-frying is noteworthy of the detected transformational products of sterols; ergosta-4,6,22-triene-3-β-ol, γ-sitosterol and stigmasta-4,6,22-triene-3-β-ol (Fig. 1) which were not identified in the other profiles. The transformed products may be hypothesized to emerge from (22*Z*)-27-Norergosta-5,22-dien-3β-ol, β-sitosterol and stigmasterol upon undergoing hydrolysis, oxidation, polymerization and isomerization reactions reportedly common in deep-frying (Kmiecik et al., 2021), however, this demands further investigation. Stigmasta-4,6,22-triene-3-β-ol may have resulted from chemical modification of stigmasterol present in the oil since it has been detected in palm oil (Hassanien, 2013). Phytosterols retard intestinal cholesterol absorption consequently declining plasma low density lipoprotein levels i.e. their consumption in amounts of 1.5–1.8 g/day result to 30–40% reduction in cholesterol absorption (Ogbe, Ochalefu, Mafulul, & Olaniru, 2015). Cholest-4-ene-3-one has been shown to be effective against liver disease, obesity and keratinization (Wu, Li, Song, & Li, 2015).

Of the minerals, P, Ca, Mg, Zn, Fe and Co were the major minerals recorded in *R. differens* which correspond to the major minerals detected in earlier studies (Fombong, Van Der Borght, & Vanden Broeck, 2017; Ssepuuya et al., 2020). Comparatively, P levels were higher in toasted *R. differens* than in the blanched, boiled and deep-fried samples. The lower levels of P in blanched, boiled and deep-fried *R. differens* could be as a result of leaching of the mineral into the process medium (Ssepuuya et al., 2020) however, future studies are highly recommended to confirm this speculation.

Mineral such as Ca, Mg and Zn levels were also significantly higher in boiled, blanched and toasted *R. differens* except for Fe which exhibited a lower level in the toasted insects. These results corroborate findings reported by other authors (Karimian-Khosroshahi et al., 2016; Mutungi et al., 2019; Ssepuuya et al., 2020). The stability of Ca and other micronutrients to diverse thermal processing in foods has been linked to their strong association to proteins and chitin in insects (Ssepuuya, et al., 2020). In the current study, deep frying resulted into very low protein levels. This might explain the low reported values of Ca and other micronutrients in deep-fried *R. differens*, since their levels reduced proportionally to the macromolecule's protein and chitin reduction. Blanching, boiling, toasting and deep frying had no influence on the Cu, Co and Mn levels. In fact, Cu, Mn and Co deviate from the normal trend of deep frying significantly reducing all the other minerals. This is in agreement with other similar studies where frying of fish significantly increased Mn and Cu levels (Karimian-Khosroshahi et al., 2016).

Thermal processing methods considerably influence the availability of flavonoids. Blanched *R. differens* in this study exhibited the highest total flavonoid content (TFC) while no significant difference was observed in the TFC of boiled and toasted with deep-fried insect recording the lowest value. Similar studies also reported steaming and microwaving to render meagre effects on flavonoids compared to boiling and high pressure cooking (Yadav et al., 2018). Boiling and pressure cooking soften and disintegrate cell walls of cells, promoting leach out of flavonoid compounds into the cooking medium. Moreover, these bioactive compounds are heat-labile and therefore, severe thermal processes greatly influence their availability (Yadav et al., 2018). Deep-frying in the current study could be labeled a severe heat treatment owing to the high temperature (175 °C) process and may explain the low levels of TFC.

The TFC of processed foods vary widely. This is subject to the time and magnitude of exposure to heat treatment, pH, phytochemical structure and oxygen presence. These variabilities stem from the impact of the processing conditions on the individual flavonoid compounds whose contents after processing directly influence the TFC. Therefore, processing conditions either enhance extraction or degradation of phenolic compounds in foods (Irina & Mohame, 2012). Flavonoids are polyphenolic compounds that confer an array of pharmacological benefits such as anti-inflammatory, antibacterial, antiviral, anti-allergenic, vasodilatory and anti-cancer properties in humans (Cheseto et al., 2020). These properties are derived from their antioxidant activities.

5. Conclusions

Processing techniques of *R. differens* for food has the potential retain essential nutrients and bioactive compounds that are beneficial for both human nutrition and health. Here, we demonstrated that thermal processing of *R. differens* for food has significant influence on the retention of nutrients, total flavonoid and sterols. Blanching was the most suitable technique with with minimal degradative effect on the quality of *R. differens* products. Deep frying had a devastating negative impact on nutrient retention in *R. differens* food products. Fatty acids, particularly PUFAs were severely affected by thermal processing, though the effects varied considerably and depended largely on exposure time and temperature change. The mineral status of the *R. differens* products was not significantly affected by blanching, boiling and toasting techniques. Overall, the nutrient profile of *R. differens* would offer a wide variety of potential positive nutritional and health implications through their consumption and there are various avenues available for further exploration into their inclusion as supplements in human diet in comparison to animal-based foods. However, additional research is warranted in this area to determine any possible risks or hazards that may be associated with human ingestion of *R. differens* products.

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 wish to thank Jackton Ooko Ongere, Joshua Wambua, Shem Ondiaka, Rachami Isaiah E. and Faith Nyamu Wamurango for their substantial contribution in providing technical support during data collection.

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), Canadian International Development Research Centre (IDRC) and the Australian Centre for International Agricultural Research (ACIAR) (INSFEED – Phase 2: Cultivate Grant No: 108866–001) and Norwegian Agency for Development Cooperation, the Section for research, innovation, and higher education grant number RAF–3058 KEN–18/0005 (CAP–Africa) through the International Centre of Insect Physiology and Ecology (*icipe*). We also gratefully acknowledge the support of *icipe*'s core funding provided by United Kingdom's Foreign, Commonwealth and Development Office (FCDO); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); 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.*

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132397.

References

Ali, M. A., Najmaldien, A. H. A., Latip, R. A., Othman, N. H., Majid, F. A. A., & Salleh, L. M. (2013). Effect of heating at frying temperature on the quality characteristics of regular and high-oleic acid sunflower oils. *Acta Scientiarum Polonorum Technologia Alimentaria, 12*.

AOAC. (2000). *Official Methods of AOAC International* (Horwitz W. (ed.); 17th ed.). AOAC International.

AOAC. (2012). *Official Method of Analysis of AOAC International* (W. Latimer (ed.); 19th ed.). AOAC International.

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

Cheseto, X., Baleba, S. B. 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*(6), 800. https://doi.org/10.3390/foods9060800

Cheseto, X., Kuate, S. P., Tchouassi, D. P., Ndung'u, M., Teal, P. E. A., Torto, B., & Mudiam, M. K. R. (2015). Potential of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) as an unconventional source of dietary and therapeutic sterols. *PLoS ONE, 10*(5), e0127171.

de Almeida, E. S., da Silva Damaceno, D., Carvalho, L., Victor, P. A., dos Passos, R. M., de Almeida Pontes, P. V., … Monteiro, S. (2021). Thermal and physical properties of crude palm oil with higher oleic content. *Applied Sciences (Switzerland), 11*(15), 7094. https://doi.org/10.3390/app11157094

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*, 3010–3014. https://doi.org/10.1088/0022-3727/31/22/002

Dias-Martins, A. M., Cappato, L. P., Trombete, F. M., Silva, T. L., Gamallo, O. D., Keller, L. M., … Saldanha, T. (2021). Effects of thermal processing on fatty acids, phytosterols and other chemical properties of black table olives. *Scientia Plena, 16*. https://doi.org/10.14808/sci.plena.2020.121501

Dobermann, D., Field, L. M., & Michaelson, L. V. (2019). Impact of heat processing on the nutritional content of *Gryllus bimaculatus* (black cricket). *Nutrition Bulletin, 44*(2), 116–122. https://doi.org/10.1111/nbu.2019.44.issue-210.1111/nbu.12374

Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo Biology, 26*(2), 105–115. https://doi.org/10.1002/(ISSN)1098-236110.1002/zoo.v26:210.1002/zoo.20123

Finke, M. D. (2015). Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology, 34*(6), 554–564. https://doi.org/10.1002/zoo.v34.610.1002/zoo.21246

Fombong, F. T., Van Der Borght, M., & Vanden Broeck, J. (2017). Influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect R*uspolia differens*. *Insects, 8*, 102. https://doi.org/10.3390/insects8030102

Gatheru, J. W., Khamis, F. M., Ombura, F. L. O., Nonoh, J., Tanga, C. M., Maina, J., … Fiaboe, K. K. M. (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*(3), 171–183. https://doi.org/10.3920/JIFF2018.0042

Hassanien, M. F. R. (2013). Plant sterols and tocols profile of vegetable oils consumed in Egypt. *International Journal of Food Properties, 16*(3), 574–585. https://doi.org/10.1080/10942912.2011.557758

Igiehon, N. O., Babalola, O. O., Cheseto, X., & Torto, B. (2021). Effects of *Rhizobia* and *Arbuscular mycorrhizal* fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress. *Microbiological Research, 242*, 126640. https://doi.org/10.1016/j.micres.2020.126640

Irina, I., & Mohame, G. (2012). Biological activities and effects of food processing on flavonoids as phenolic antioxidants. *Advances in Applied Biotechnology*, 101–124. https://doi.org/10.5772/30690.

Karimian-Khosroshahi, N., Hosseini, H., Rezaei, M., Khaksar, R., & Mahmoudzadeh, M. (2016). Effect of Different Cooking Methods on Minerals, Vitamins, and Nutritional Quality Indices of Rainbow Trout (*Oncorhynchus mykiss*). *International Journal of Food Properties, 19*(11), 2471–2480. https://doi.org/10.1080/10942912.2015.1039028

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., Mogendi, J. B., Riwa, C. A., & Ndung'u, N. W. (2015). Edible insects-A novel source of essential nutrients for human diet: Learning from traditional knowledge. *Animal Frontiers, 5*(2), 14–19. https://doi.org/10.2527/af.2015-0014

Kmiecik, D., Fedko, M., Rudzińska, M., Siger, A., Gramza-Michałowska, A., & Kobus-Cisowska, J. (2021). Thermo-oxidation of phytosterol molecules in rapeseed oil during heating: The impact of unsaturation level of the oil. *Foods, 10*, 50. https://doi.org/10.3390/foods10010050

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. *Food Research International, 121*, 404–411. https://doi.org/10.1016/j.foodres.2019.03.052

MarkManuel, D. P., & Godwin, J. (2020). Effects of culinary methods on the proximate composition of an edible insect (*Rhynchophorus phoenicis*) larvae obtained from bayelsa state, Nigeria. *European Journal of Agriculture and Food Sciences, 2*, 1–7. https://doi.org/10.24018/ejfood.2020.2.4.67

Melgar-Lalanne, G., Hernández-Álvarez, A., & Salinas-Castro, A. (2019). Edible insects processing: Traditional and innovative technologies. *Comprehensive Reviews in Food Science and Food Safety, 18*(4), 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

Mohamed, E. H. (2015). Fatty acids contents of the edible migratory locust *Locusta migratoria*, Linnaeus, 1758 (Orthoptera: Acrididae). *International Journal of Advances in Pharmacy, Biology and Chemistry, 4*, 746–750.

Momenzadeh, Z., Khodanazary, A., & Ghanemi, K. (2017). Effect of different cooking methods on vitamins, minerals and nutritional quality indices of orange-spotted grouper (*Epinephelus coioides*). *Journal of Food Measurement and Characterization, 11*(2), 434–441. https://doi.org/10.1007/s11694-016-9411-3

Mutungi, C., Irungu, F. G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., … Fiaboe, K. K. M. (2019). Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. *Critical Reviews in Food Science and Nutrition, 59*(2), 276–298. https://doi.org/10.1080/10408398.2017.1365330

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*(1). https://doi.org/10.1111/jfs.12549

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., … Fiaboe, K. K. M. (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*(5), 574. https://doi.org/10.3390/foods9050574

Ogbe, R. J., Ochalefu, D. O., Mafulul, S. G., & Olaniru, O. B. (2015). A review on dietary phytosterols: Their occurrence, metabolism and health benefits. *Pelagia Research Library Asian Journal of Plant Science and Research, 5*(4), 10–21. www.pelagiaresearchlibrary.com.

Oonincx, D. G. A. B., & Finke, M. D. (2020). Nutritional value of insects and ways to manipulate their composition. *Journal of Insects as Food and Feed, 7*(5), 639–659. https://doi.org/10.3920/JIFF2020.0050

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

Sabolová, M., Adámková, A., Kouřimská, L., Chrpová, D., & Pánek, J. (2016). Minor lipophilic compounds in edible insects. *Potravinarstvo, 10*, 400–406. https://doi.org/10.5219/605

Ssepuuya, G., Nakimbugwe, D., De Winne, A., Smets, R., Claes, J., & Van Der Borght, M. (2020). Effect of heat processing on the nutrient composition, colour, and volatile odour compounds of the long-horned grasshopper *Ruspolia differens* Serville. *Food Research International, 129*, 108831. https://doi.org/10.1016/j.foodres.2019.108831

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

Veldkamp, T., & Vernooij, A. (2020). "Insects for Africa": Opportunities for insect production in East Africa. *Inform, 31*, 24–26. https://doi.org/10.21748/inform.01.2020.24

Wu, K., Li, W., Song, J., & Li, T. (2015). Production, purification, and identification of cholest-4-en-3-one produced by cholesterol oxidase from *Rhodococcus sp*. in aqueous/organic biphasic system. *Biochemistry Insights, 8s1*, BCI.S21580. https://doi.org/10.4137/bci.s21580

Yadav, N., Kaur, D., Malaviya, R., Singh, M., Fatima, M., & Singh, L. (2018). Effect of thermal and non-thermal processing on antioxidant potential of cowpea seeds. *International Journal of Food Properties, 21*(1), 437–451. https://doi.org/10.1080/10942912.2018.1431659