LWT - Food Science and Technology 191 (2024) 115659

Contents lists available at ScienceDirect

LWT

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

Inhibiting post-harvest perishability of edible beetle grubs (*Oryctes* spp) by blanching and pre-treatment with sodium metabisulphite and ascorbic acid

Zipporah Wangari Maimba [a,b,#], John N. Kinyuru [b], George W. Wanjala [c], James P. Egonyu [a,1,*]

[a] International Centre of Insect Physiology and Ecology, P.O Box 30772, Nairobi, Kenya
[b] Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000, Nairobi, Kenya
[c] Kenya Industrial Research and Development Institute, P.O. Box 30650, Nairobi, Kenya

ARTICLE INFO

Keywords:
Amino acid composition
Colour
Microbial loads
Phenols
Protein quality

ABSTRACT

Although edible beetle grubs (*Oryctes* spp) are highly nutritious, post-harvest perishability limits their utilization in food processing. This study investigated the effects of blanching singly or in combination with either ascorbic acid or sodium metabisulphite or a blend of the two chemicals, on colour, microbial quality, protein quality, total phenol content and amino acid composition of the grubs after harvest. All the pretreatments effectively preserved the colour of the grubs, reduced protein oxidation and inhibited most harmful microbes to food safety limits. The treatments had insignificant effect on amino acid profiles of the larvae. Combining blanching with ascorbic acid eliminated *Staphylococcus aureus* from the larvae and outperformed the other treatments in enhancing the content of total phenols. The findings provide prospects for preservation of the edible beetle grubs using the affordable, readily available, and easy-to-apply heat and chemical pretreatments prior to processing into other palatable food products.

1. Introduction

The larvae or grubs of the rhinoceros beetles, *Oryctes* spp (Coleoptera: Scarabaeidae) such as *Oryctes monoceros* (Olivier), *Oryctes owariensis* (Palisot de Beauvois) and *Oryctes boas* (Fabricius) are consumed in Africa, South America, and Asia (Egonyu et al., 2022; Kelemu et al., 2015). Beetle grub consumption in Kenya ranks fifth, behind that of termites, grasshoppers, saturniid caterpillars, and crickets (Kusia, Borgemeister, Tanga, Ekesi, & Subramanian, 2021). Prior to being prepared for eating by boiling, toasting, roasting, or frying, the grubs are removed from farmyard compost, de-gutted, and washed (Wanjala et al., 2023). There are high prospects for mass rearing of the beetle grubs by many rural animal-keeping households rather than relying on scarce wild harvests (Wanjala et al., 2023).

Beetle grubs are highly nutritious although there are variations depending on the species and area of study. Rumpold & Schlüter (2013) reported that the protein and fat contents of *Oryctes* spp grubs from Nigeria were 26–58% and 1–34% on dry matter basis, respectively. Nutritional profiling of the grubs from Bungoma in Western Kenya where they are highly consumed indicates nutritional content of 40-46%, 13–23%, 10−11%, and 1.2%, in crude proteins, carbohydrates, ash, and fats, respectively (Egonyu, Olubowa, Tanga, Cheseto, & Subramanian, in press). The use of edible grubs as ingredients in known food products would be one way of increasing acceptability among diverse consumers where entomophagy is not traditionally practiced (Tang et al., 2019).

The use of edible grubs in food processing is constrained by their perishability after harvest. The grubs are susceptible to rapid degeneration due to intrinsic characteristics like high nutritional content, high moisture, high water activity, and an approximately neutral pH. Microbial spoilage, degradation processes such as lipid oxidation, enzymatic and non-enzymatic browning reactions, and proteolysis are the main causes of spoilage in edible insect larvae (Janssen, Vincken, van den Broek, Fogliano, & Lakemond, 2017). The process of lipid oxidation involves the oxidation of fatty acids, especially unsaturated fatty acids, to produce byproducts such as alkenals and malondialdehydes as well as volatile chemicals that change the taste and odor of insect products (Gray & Monahan, 1992). Color changes are the most visible quality deterioration indicators in edible insects after harvest (Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019). Color changes of particular

* Corresponding author.
E-mail addresses: jpegonyu@gmail.com, jegonyu.sci@busitema.ac.ug (J.P. Egonyu).
Present address: Kenya Bureau of Standards, P·O Box 54974- 00200 Nairobi.
1 Present address: Faculty of Science and Education, Busitema University, P. O. Box 236, Tororo, Uganda.

https://doi.org/10.1016/j.lwt.2023.115659
Received 1 August 2023; Received in revised form 12 December 2023; Accepted 13 December 2023
Available online 25 December 2023
0023-6438/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

importance in edible insects include melanization and browning reactions that involve cuticle darkening either because of enzymatic or non-enzymatic reactions (Nakhleh, El Moussawi, & Osta, 2017). Examples of non-enzymatic activities include the complexing of iron and polyphenols (Leni, Caligiani, & Sforza, 2019) and Maillard reactions (Larouche et al., 2019), which result in the formation of a dark compound called melanin. Polyphenol oxidases are mostly associated with color change in insects, crustaceans, and vegetables (Altunkaya & Gökmen, 2008; Janssen et al., 2017; Nirmal & Benjakul, 2009). In insects, polyphenols may serve as pigments and as a component of their chemical defense. The type and concentration of polyphenols in insects are influenced by their diet (Pyo, Kang, Jung, & Sohn, 2020). Some insects synthesize and store non-dietary phenolic compounds through the sclerotization process (Nino, Reddivari, Ferruzzi, & Liceaga, 2021). Many phenolic compounds are both good browning substrates and good antioxidants. Their role as substrates for oxidative browning is important for foods. A decrease in the total phenol content is linked to the formation of a dark-colored compound melanin in vegetables (Altunkaya & Gökmen, 2008). Janssen et al. (2017) also demonstrated that iron-polyphenol complexing causes the formation of melanin in black soldier fly larvae, causing their darkening. Protein breakdown results either from intrinsic enzymatic activity or microbial enzymatic activity. Proteases such as chymotrypsin and trypsin have been found to contribute to protein hydrolysis, leading to modification of the techno-functional properties of edible insect proteins (Janssen et al., 2017).

To enhance the potential of edible beetle grubs as a novel ingredient for food processing, it is necessary to ensure that they undergo post-harvest processing that maintains a good nutritional profile, ensures safety, enhances acceptability to consumers, and maintains good techno-functional properties. The effects of using affordable, readily available, and easy-to-apply larvae pretreatment methods using heat and chemical preservatives on the color, protein quality, and microbial quality of edible beetle grubs have not been evaluated. Various methods have been used for the stabilization of edible grubs after harvest, including, physical processes such as blanching (water or steam) and drying (Bolaji, Bamidele, Adeboye, & Tanimola, 2021; Purschke, Brüggen, Scheibelberger, & Jäger, 2018; Womeni et al., 2012). High hydrostatic pressure (HHP), microwave drying, and non-thermal techniques like pulsed electric fields, and cold atmospheric pressure plasma are some other new techniques used for this purpose (Sindermann, Heidhues, Kirchner, Stadermann, & Kühl, 2021). However, the choice of the stabilization methods is influenced by factors such as cost, technology availability, and the end use of the edible insect as a product or as an ingredient. Blanching is one of the most utilized stabilization methods in the edible insect industry (Melgar-Lalanne et al., 2019). According to Fombong, Van Der Borght, & Vanden Broeck (2017), this physical preservation strategy is used as a killing step and a microbial decontamination method to slow down or halt enzymatic activity, such as browning processes. It has been demonstrated that high-heat blanching, which involves boiling for 5 min, inhibits microbial activity and inactivates proteases that break down proteins (Fernandez-Cassi et al., 2019; Yan et al., 2023).

Chemical inhibitors are used to either slow down or eliminate the browning reactions in food products (Gonçalves & de Oliveira, 2016). Examples of such inhibitors are sulfites, reducing agents such as ascorbic acid, chelators such as ethylenediaminetetraacetic acid (EDTA) which reduce copper content, and antioxidants such as butylated hydroxytoluene (BHT) (Moon, Kwon, E.-B., Lee, & Kim, 2020). Chemical inhibitors are common in the horticultural industry, crustaceans, and meat products industries where effective levels of use of these inhibitors have been established. For example, Jiang, Xing, Sheng, & Zhan (2011) demonstrated that the application of 0.45% m/v ascorbic acid, 0.23% m/v sodium sulfite, and 2.27% m/v citric acid effectively inhibited polyphenol oxidase activity in the mollusk *Argopectin irradians*. Other studies have also shown that color stability and lowering of lipid oxidation in crustaceans, beef, and pork depended on the concentration of inhibitors such as sulfites and organic acids (Nirmal & Benjakul, 2009; Kim et al., 2020).

In this study, the combined effects of blanching and chemical pretreatments with ascorbic acid and sodium metabisulphite, singly or in a blend on the microbial loads, color, phenol content, and protein quality of fresh edible *Oryctes* spp larvae were evaluated.

2. Materials and method

2.1. Sample collection and preparation

Oryctes spp larvae were sourced from cattle compost in the backyards of households in Bungoma county and transported alive in plastic bins in cattle compost manure to the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The grubs were cleaned using potable water, drained, and inactivated by freezing at −20°C, for 60 min. The larvae were degutted by squeezing out gut content through an incision on the posterior gut and washed in potable water. Part of the larvae was preserved as untreated (TU), whereas others were blanched for 5 min in boiling water (Fombong et al., 2017), and divided into four groups during the cooling stage: i.e., T1 (heat treated and cooled in chilled water only), T2 (heat treated and cooled in chilled water containing 0.5 g/100 ml of sodium metabisulphite), T3 (heat treated and cooled in chilled water containing 0.25 g/100 ml sodium metabisulphite and 0.25 g/100 ml ascorbic acid) and T4 (heat treated and cooled in 0.5 g/100 ml of ascorbic acid water solution) at a 1:2 (w/v) insect: water ratio for 15 min. The study followed a completely randomized experimental design with four replications.

The differentially treated larvae were stored frozen at −20°C after being drained, during which color change was monitored after one, seven, and fourteen days. Guided by the color monitoring trend, microbial loads were analyzed after one and 14-day storage period. The grubs were then dried using a FD 115 oven drier (WTC Binder, Tuttlingen, Germany) at 60°C for 24 h and subjected to subsequent protein quality, moisture, pH, amino acid composition, and total phenol analyses.

2.2. Color measurements

The pretreated larvae were pulverized using a 12-speed laboratory grinder (Oster BLSTFE, FL, USA), and their color was assessed using a DC65 cell colorimeter (NR 200 Colorimeter, 3NH, Shenzhen, China), which was calibrated against white reference plates supplied by the manufacturer. The color was expressed as L*(lightness), a*(redness), and b*(yellowness) (Pathare, Opara, & Al − Said, 2013). The estimation of color changes was done using fresh larvae as a reference. At each measuring point, color was compared with the reference color and differences in L*, a* and b* were compiled into the total color differences (ΔE^*_{lab}). In addition, for each measuring point the whitening index (WI) was determined. The parameters were calculated using the following expressions derived from Pathare et al. (2013).

$$WI = 100\sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

Where, WI is the whitening index, L* values indicate lightness, a* values measure colors from green to red, and b* values measure colors from blue to yellow.

$$\Delta E^*_{lab} = \sqrt{\left(L^{*\,*}_{ref} - L^*\right)^2 - \left(a^*_{ref} - a^*\right)^2 - \left(b^*_{ref} - b^*\right)^2} \quad (2)$$

In which, ΔE^*_{lab} stands for overall color change indicator. L* values indicate lightness, a* values measure colors from green to red, and b* values measure colors from blue to yellow.

2.3. Microbial counts

Microbial analysis was done for aerobic mesophilic total viable counts (TVC), coliform, and the pathogens *Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* and *Bacillus cereus,* one day and fourteen days after pretreatment for samples stored at −20 °C. A Stomacher 400 circulator blender (Seward, West Sussex, U.K.) was used to blend 10 g of pulverized sample with 90 ml of 0.1% sterile peptone, then used to make 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} serial dilutions. Dilutions 10^{-2}, 10^{-3} and 10^{-4} were plated for TVC while other tests used dilutions 10^{-1} and 10^{-2}. Tests were done in four replicates. Table 1 shows a summary of the test methods and testing conditions applied.

2.4. Crude protein determination

The Kjeldahl method was used to evaluate protein content according to ISO standard method 5983 part 1:2005.0. Three grams of ground larvae and one Kjeldahl tablet (Thompson 7 capper Ltd, Cheshire WA7 1 PH, United Kingdom) comprising 0.4 g copper sulphate, 3.5 g potassium sulphate, and concentrated sulphuric acid (95%, VWR Chemicals) were put into a digestion tube. The samples were digested in a Digester (Velp Scientifica DKL 20 Usmate MB, Italy) and then cooled to room temperature. After cooling, the digestion tube was connected to the distillation unit (Vapodest 20 S, Gerhardt, Leystand, The Netherlands) where a 33% sodium solution was added, and the ammonia was distilled automatically into a 4% boric acid solution. Using a standardized 0.1 M hydrochloric acid solution, the nitrogen concentration was measured by titrating the ammonium borate generated. The endpoint was established using bromocresol green. A factor of Notrogen (N) = 4.76 was used to compute the crude protein content according to the following expressions (Janssen et al., 2017).

$$\text{Crude protein } (\%) = \%\text{ N} \times \text{F } (4.76) \tag{3}$$

$$\%\text{ Crude protein (DM)} = \frac{\text{Crude protein } (\%)}{\text{DM content}}. \tag{4}$$

2.5. Dry matter content determination

In order to calculate the dry matter, the AOAC (1990), Method 930.15 was used. Along with the crucible, a 1 g ground sample was weighed and added to the crucible's weight. The samples were dried in an oven (WTC Binder, FD 115, Tuttlingen, Germany) for 2 h at 135 °C before being desiccated to room temperature. The result was obtained as oven dried weight. The percentage of dry matter was calculated according to equation (5):

Table 1
Standard methods for the evaluation of practical cell counts and their growth parameters.

Target Microbe	Analytical method	Culture medium	Growth conditions.
Total viable counts	ISO 4833–2:2013	Plate count agar (PCA)	30 °C, 72 h, aerobic.
E. coli	ISO 16649–2:2004	Nutrient agar Tryptone bile X-glucuronide (TBX)	24 h nutrient agar, 24 h TBX at 44°C in aerobic conditions.
***Staphylococcus* spp.**	ISO 6888–1:2003Amd.	Baird Parker agar (BP)	48 h, 37°C in aerobic conditions.
B. cereus	ISO 7932:2005–03	MYP Confirmation is done using blood agar.	48 h, 37°C in aerobic conditions.
***Salmonella* spp**	ISO 6579:2007–10	Xylose-Lysine deoxycholate agar (XLD)	24 h, 37°C in aerobic conditions

$$\text{Dry matter } (\%) = \frac{\text{Oven dried weight } - \text{ Crucible weight}}{\text{Crucible and Sample weight } - \text{ Crucible weight}} \times 100 \tag{5}$$

2.6. Composition of amino acids

The analysis of the amino acid composition followed the methodology outlined in Cheseto, Baleba, Tanga, Kelemu, and Torto (2020). Briefly, the insect powder (100 mg) was transferred into a 5-ml micro-reaction vial into which 2 ml of 6N HCl were added. The sample was hydrolyzed for 24 h at 110 °C. Following the hydrolysis, 1 ml of deionized water and acetonitrile (95:5) were added. The mixture was then vortexed for 30 s, sonicated for 30 min, and centrifuged at 14,000 rpm. The supernatant was then subjected to analysis using chromatographic separation. The LC−MS operating conditions were as follows: a quaternary LC pump (Model 1200) coupled to Agilent MSD 6120-Single quadruple MS with electrospray source (Palo Alto, CA, USA). The chromatographic separation was completed on an (LC-MS -Agilent system 1100 series (Agilent, MA, USA) running at 40 °C using ZORBAX SB-C18, 4.6 × 250 mm, 3.5 μm column. For the mobile phases, acetonitrile (B) and water (A) containing 0.01% formic acid each were utilized. 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 3 μL. A mass spectrometer with a quadrupole was connected to the LC. At a mass range of m/z 50–600 and a cone voltage of 30 eV, the mass spectrometer was run in ESI–positive mode. Similar LC-MS analyses were performed on serial dilutions of standard, which contained 14 amino acids (1–105 g/l, Sigma-Aldrich, St. Louis, MO, USA), to produce linear calibration curves (peak area vs. concentration) that were utilized for external quantification.

2.7. Proteolysis index

Total nitrogen was determined using the Kjeldahl method, according to ISO standard method 5983 part 1:2005 as earlier described in 2.4 above. Grub sample (0.3 g) was used in the determination. Non-protein nitrogen was measured using the procedure described by Lorenzo, Fontán, Franco, & Carballo, (2008), in which 2.5 g of sample was homogenized with 25 ml of deionized water, followed by the addition of 10 ml of 20% TCA and thorough stirring. The mixture was centrifuged for 10 min at 4200 rpm after stabilizing for 60 min at room temperature. Following the Kjeldahl procedure, the supernatant was filtered, and 15 ml of the filtrate was used to determine the nitrogen level. The equation below was used to compute the proteolysis index.

$$\text{Proteolysis index} = \frac{\text{Non Nitrogen Protein}}{\textit{Total Nitrogen}} \times 100 \tag{6}$$

2.8. Total phenols

Analysis of total phenols was done using the Folin ciocalteau (FC) method as described by Nino et al. (2021). Diethyl ether (20 ml) was added to the insect sample (2 g), which was then shaken for 25 min before being centrifuged for 10 min at 3000 rpm. The residue was allowed to dry, and 50 ml of cold methanol was added, vortexed for 1 min, and incubated for 72 h at 25 °C in darkness. The extract was then filtered, and centrifuged at 13,000 rpm for 10 min. Two milliliters of 10% v/v FC reagent were added to 1 ml of the methanolic extract. Four milliliters of a 7.5% sodium carbonate solution were added after 8 min, and the mixture was left to stand at room temperature for 60 min. The absorbance of the mixture was measured using a UV–Vis spectrophotometer (Shimadzu UV-1700, PharmasSpec, Tokyo, Japan) at 765 nm. The gallic acid solution was used as a standard. A stock solution was prepared by diluting 0.5 g of gallic acid in 10 ml of ethanol and topping

up to 100 ml using distilled water. Standard solutions were prepared with 0, 1,2,4,6,8, and 10 ml of stock solution to make 0, 50, 100, 200, 300,400 and 500 mg/l of gallic acid.

2.9. pH

A digital pH-ORP meter (H12209, Hanna Instruments, RI, USA) was used to measure the samples' pH using the method described by Lee et al. (2017), after homogenizing 5 g of insect sample with 20 ml of distilled water.

3. Statistical analysis

A one-way analysis of variance was used to examine the effects of treatments on color, microbial loads expressed as Log cfu/g, and physiochemical parameters. Parameters with significant differences were subjected to Tukey's mean separation. The normality and homogeneity of the collected data were evaluated using Bartlett's test. R statistics program version R.4.2.2 (R Core Team 2022) was used to run all the statistical analyses, using a significance level of 0.05.

4. Results

4.1. Effects of blanching and pretreatment with sodium metabisulphite and ascorbic on the color of Oryctes spp grubs

All the heat and chemical pretreatments of the grubs caused significant improvements in all larval color parameters compared to untreated larvae, except in the overall color change (ΔE) where the reverse was true (Table 2).

Within treated samples, a significantly lower a* value at 14 days was recorded in the larvae treated by a combination of blanching and ascorbic acid than in those which were only blanched; whereas combining blanching with sodium metabisulphite and ascorbic acid blend yielded significantly higher b* values on day one than the samples which were only blanched. At 7 days, L* was significantly higher in the samples treated by a combination of blanching with either sodium metabisulphite or ascorbic acid than in those subjected to the other treatments tested. However, at 14 days, L* was significantly higher in the samples which were blanched followed by ascorbic acid treatment and its blend with sodium metabisulphite than the samples which were only blanched. After 7 days, the whitening index was significantly higher in the samples treated by blanching only or blanching followed by a blend of sodium metabisulphite and ascorbic acid than in those which were blanched followed by treatment with either chemicals singly; but after 14 days, it was higher in the larvae which were blanched and treated with either ascorbic acid or its blend with sodium metabisulphite than in those which were only blanched. At seeven days, a combination of blanching and a blend of sodium metabisulphite and ascorbic acid recorded a significanlty higher overall color change in the grubs than the other treatments; but at 14 days, the overall color change was significantly lower in the samples treated by a combination of blanching and sodium metabisulphite than in those which were blanched followed by ascorbic acid treatment.

4.2. Effect of blanching and pretreatment with sodium metabisulphite and ascorbic on the microbial quality of edible Oryctes spp grubs

Bacillus cereus counts were significantly lower in all the treated larvae than the control after one day of incubation, but they were not affected by any treatment after 14 days (Table 3). Total viable counts were significantly lower in the samples from all the treatments than in untreated larvae. Counts of coliforms were lower in the larvae which were blanched and those which were blanched followed by sodium metabisulphite treatment than untreated larvae after one day; and in the larvae which were blanched and treated with only ascorbic acid after 14 days. *Escherichia coli* was detected in the untreated larvae after one and 14 days, but it was eliminated by all the pretreatments. *Staphylococcus aureus* was eliminated by combining blanching with ascorbic acid treatment, but its counts were unaffected by the rest of the treatments.

4.3. Effect of blanching and pretreatment with sodium metabisulphite and ascorbic on the physicochemical properties of edible beetle grubs

All pre-treated samples had comparable crude protein, non-protein nitrogen, and proteolysis index, which were significantly lower than in untreated larvae (Table 4). The pH of the grubs ranged from ~6.3 to ~6.8 and differed significantly across the treatments. The lowest pH was recorded in the untreated grubs, while the highest was recorded in the grubs treated with sodium metabisulphite after blanching. The concentration of total phenols was statistically comparable in the untreated and blanched grubs but was significantly higher in the grubs subjected to the chemical treatments after blanching than untreated larvae. There were significant differences in the moisture contents of the grubs treated differently, being lowest in the blanched grubs and highest in the grubs treated with ascorbic acid after blanching.

Table 2
Effect of blanching and pretreatment with sodium metabisulphite and ascorbic on the color of *Oryctes* spp grubs.

Cielab value	Day	Treatment					df	F	*p*
		TU	T1	T2	T3	T4			
Cielab a*	1	-0.36 ± 0.12^{a}	3.14 ± 0.21^{b}	3.25 ± 0.13^{b}	3.63 ± 0.16^{b}	3.39 ± 0.18^{b}	4,55	100.4	<0.001
	7	-0.41 ± 0.13^{a}	3.55 ± 0.52^{b}	3.55 ± 0.40^{b}	3.92 ± 0.75^{b}	3.37 ± 0.67^{b}	4,55	118.7	<0.001
	14	-0.33 ± 0.31^{a}	3.75 ± 0.81^{c}	3.48 ± 0.81^{bc}	3.27 ± 0.93^{bc}	2.84 ± 0.53^{b}	4,51	49.73	<0.001
Cielab L*	1	28.74 ± 0.27^{a}	43.83 ± 0.43^{b}	43.98 ± 0.29^{b}	44.11 ± 0.51^{b}	44.14 ± 0.40^{b}	4,55	306.9	<0.001
	7	27.58 ± 0.21^{a}	42.04 ± 0.67^{b}	44.85 ± 0.29^{c}	42.42 ± 0.91^{b}	45.05 ± 0.25^{c}	4,55	180.3	<0.001
	14	27.41 ± 0.26^{a}	41.79 ± 0.87^{b}	43.89 ± 0.31^{bc}	44.96 ± 0.80^{c}	46.19 ± 1.10^{c}	4,51	76.38	<0.001
Cielab b*	1	-0.600 ± 0.11^{a}	6.06 ± 0.21^{b}	6.18 ± 0.20^{bc}	6.89 ± 0.20^{c}	6.50 ± 0.21^{bc}	4,55	276.1	<0.001
	7	-0.706 ± 0.16^{a}	6.26 ± 0.24^{b}	6.59 ± 0.16^{b}	6.88 ± 0.43^{b}	6.34 ± 0.28^{b}	4,55	141	<0.001
	14	-0.631 ± 0.20^{a}	6.45 ± 0.33^{b}	6.68 ± 0.29^{b}	6.35 ± 1.32^{b}	5.96 ± 0.91^{b}	4,51	76.38	<0.001
WI	1	28.74 ± 0.27^{a}	43.40 ± 0.41^{b}	43.53 ± 0.29^{b}	43.56 ± 0.51^{b}	43.65 ± 0.37^{b}	4,55	301.4	<0.001
	7	27.58 ± 0.21^{a}	41.58 ± 0.64^{c}	44.34 ± 0.27^{b}	41.85 ± 0.84^{c}	44.57 ± 0.27^{b}	4,55	193	<0.001
	14	27.09 ± 0.26^{a}	41.30 ± 0.85^{b}	43.37 ± 0.27^{bc}	44.48 ± 0.77^{c}	45.77 ± 1.07^{c}	4,55	110.7	<0.001
ΔE	1	16.44 ± 0.24^{b}	$1.80 \pm 0.18a$	$1.59 \pm 0.18a$	$2.33 \pm 0.38a$	$1.70 \pm 0.30a$	4,55	602	<0.001
	7	17.57 ± 0.13^{c}	2.62 ± 0.48^{a}	1.94 ± 0.28^{a}	3.97 ± 1.75^{b}	1.97 ± 0.30^{a}	4,55	881.1	<0.001
	14	17.92 ± 0.21^{c}	3.44 ± 1.82^{ab}	1.71 ± 0.32^{a}	2.94 ± 0.50^{ab}	3.97 ± 0.79^{b}	4,55	172.9	<0.001

TU- untreated sample, TI blanched only, T2 - blanched and treated with (0.5 g/100 ml) sodium metabisulphite, T3 - blanched and treated with 1:1 (0.25 g/100 ml each) of sodium metabisulphite and ascorbic acid and T4 - blanched and treated with (0.5 g/100 ml) ascorbic acid. Color was expressed as L*(lightness), a*(redness), and b* (yellowness), total color differences ΔE represents overall color difference in reference to original color and (WI) the whitening index. All values are of four replicates. Values within a row bearing different letters are statistically different at $p < 0.001$.

Table 3
Microbial counts (Log $_{10}$ cfu/g) of edible *Oryctes* spp grubs incubated at −20 °C for one and 14 days after pretreatments.

Microbe	Day	Treatments					df	F	*p*
		TU	T1	T2	T3	T4			
Total viable counts	1	6.44 ± 0.31^{c}	5.21 ± 0.23^{ab}	5.27 ± 0.34^{ab}	5.64 ± 0.12^{b}	4.83 ± 0.21^{a}	4,15	22.41	<0.001
	14	5.72 ± 0.81^{b}	4.56 ± 0.25^{a}	4.31 ± 0.11^{a}	4.32 ± 0.33^{a}	4.38 ± 0.81^{a}	4,15	33.74	<0.001
Coliforms	1	4.64 ± 0.41^{b}	3.20 ± 0.86^{a}	3.03 ± 0.69^{a}	3.79 ± 0.08^{ab}	2.69 ± 0.82^{ab}	4,15	6.86	0.0054
	14	3.95 ± 0.18^{b}	2.12 ± 0.48^{ab}	2.22 ± 0.99^{ab}	2.22 ± 0.80^{ab}	1.72 ± 1.13^{a}	4,15	4.05	0.0201
Escherichia coli	1	3.05 ± 0.63	nd	Nd	nd	nd	–	–	–
	14	1.78 ± 0.91	nd	Nd	nd	nd	–	–	–
Staphylococcus aureus	1	2.40 ± 0.15^{a}	1.00 ± 0.00^{a}	1.72 ± 1.01^{a}	1.39 ± 0.55^{a}	nd	3,5	2.863	0.143
	14	1.65 ± 0.31^{a}	1.48 ± 0.00^{a}	1.95 ± 0.00^{a}	1.39 ± 0.55^{a}	nd	3,4	0.536	0.682
Bacillus cereus	1	3.29 ± 0.14^{b}	2.30 ± 0.03^{a}	2.28 ± 0.06^{a}	2.14 ± 0.38^{a}	2.22 ± 0.05^{a}	4,15	31.24	<0.001
	14	3.26 ± 0.26a	2.63 ± 0.57a	2.28 ± 0.50a	2.32 ± 0.57a	2.51 ± 1.13a	4,15	2.488	0.0878

TU- untreated sample, TI blanched only, T2 blanched and treated with (0.5 g/100 ml) sodium metabisulphite, T3 blanched and treated with 1:1 (0.25 g/100 ml) of sodium metabisulphite and ascorbic acid, and T4 blanched and treated with (0.5 g/100 ml) ascorbic acid. All values are of four replicates. Different letters superscripts in the same row signify significant differences between treatments at $p < 0.001$. nd- Not detected.

Table 4
Chemical analysis of pretreated beetle grubs after 14 days of storage at −20 °C.

Chemical parameter.	TU	T1	T2	T3	T4	df	F	p
Crude protein (% DMB)	49.52 ± 1.37^{b}	41.93 ± 0.20^{a}	41.49 ± 1.37^{a}	41.59 ± 1.37^{a}	41.50 ± 0.22^{a}	4,15	15.74	<0.001
Non-Nitrogen protein (%)	1.88 ± 0.07^{b}	0.76 ± 0.03^{a}	0.72 ± 0.03^{a}	0.70 ± 0.00^{a}	0.73 ± 0.03^{a}	4,10	555.1	<0.001
Proteolysis Index (%)	18.99 ± 0.68^{b}	8.90 ± 0.33^{a}	8.40 ± 0.68^{a}	8.26 ± 0.25^{a}	8.53 ± 0.23^{a}	4,10	284.5	<0.001
pH	6.28 ± 0.03^{a}	6.66 ± 0.02^{c}	6.75 ± 0.02^{d}	6.65 ± 0.01^{c}	6.30 ± 0.01^{b}	4,10	303.8	<0.001
Total Phenols (mg/100 g)	1.976 ± 0.01^{a}	2.00 ± 0.01^{ab}	2.00 ± 0.01^{b}	2.01 ± 0.01^{b}	2.01 ± 0.00^{b}	4,10	6.119	<0.001
Moisture content (%)	69.22 ± 1.17^{bc}	66.04 ± 0.58^{a}	67.02 ± 0.83^{a}	67.68 ± 0.42^{ab}	69.97 ± 0.38^{c}	4,15	16.46	<0.001

Note. TU- untreated sample, TI blanched only, T2 blanched and treated with (0.5 g/100 ml) sodium metabisulphite, T3 blanched and treated with 1:1 (0.25 g/100 ml) of Sodium metabisulphite and ascorbic acid, and T4 blanched and treated with (0.5 g/100 ml) ascorbic acid. All values are of four replicates. Different letters superscripts in the same row signify significant differences between treatments at $p < 0.001$.

4.4. Effects of blanching and pretreatment with metabisulphite and ascorbic on the amino acid composition of Oryctes spp larvae

Fourteen amino acids were detected in the beetle larvae, of which only the levels of tyrosine recorded a detectable effect of blanching and the chemical pretreatments (Table 5). The level of tyrosine was however significantly higher in the larvae treated by blanching followed by ascorbic acid than in those treated by blanching only.

5. Discussion

Aesthetic condition of food is frequently associated with its deterioration and the ensuing decline in economic worth. It frequently comes with changes to the food flavor, lower nutritional value, and sensory, and functional qualities. In this study, treatment of edible *Oryctes* spp grubs by blanching or its combination with sodium metabisulphite and ascorbic acid, either singly or in a blend resulted in improved levels of a*, L*, b* and whitening index, while lowering total color difference when compared to the untreated larvae. All the treated samples showed ΔE in the range of 1.70–3.97, which is close to 3 which is considered narrowly visible while the untreated sample had a range of 16.44–17.97, way above 6 which is considered a measure of clearly visible color change (Wibowo et al., 2015). The whitening index which is used to show the tendency to paleness as opposed to darkening of food (Pathare et al., 2013), was almost twice higher in the larvae subjected to any of the treatments than in the untreated larvae. This is a clear indication that the use of blanching singly or in combination with either 0.5% ascorbic acid or 0.5% sodium metabisulphite or a blend of the two

Table 5
Effects of blanching and pretreatment with sodium metabisulphite and ascorbic acid on the amino acid composition of edible *Oryctes* spp grubs (g/100 g *on* dry matter basis).

	TU	T1	T2	T3	T4	df	F	*p*
Methionine	0.527 ± 0.10	0.55 ± 0.16	0.59 ± 0.11	0.57 ± 0.11	0.67 ± 0.05	4,10	1.039	0.434
Alanine	0.23 ± 0.03	0.24 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.02	4,10	0.304	0.869
Arginine	2.34 ± 0.39	2.45 ± 0.22	2.81 ± 0.03	3.03 ± 0.25	3.24 ± 0.60	4,10	2.137	0.150
Glutamic acid	0.89 ± 0.04	1.00 ± 0.09	0.91 ± 0.05	0.98 ± 0.04	0.96 ± 0.02	4,10	0.778	0.564
Glycine	1.03 ± 0.03	1.18 ± 0.70	1.24 ± 0.02	1.15 ± 0.08	1.26 ± 0.02	4,10	3.132	0.065
Histidine	3.71 ± 0.30	3.47 ± 0.09	3.58 ± 0.06	3.98 ± 0.09	3.86 ± 0.26	4,10	1.186	0.314
Isoleucine	0.60 ± 0.14	0.84 ± 0.35	0.85 ± 0.20	1.46 ± 0.08	1.37 ± 0.08	4,10	3.621	0.050
Leucine	1.75 ± 0.52	1.27 ± 0.31	1.47 ± 0.37	1.92 ± 0.46	2.43 ± 0.11	4,10	1.386	0.307
Lysine	4.90 ± 0.50	5.95 ± 0.22	5.86 ± 0.51	6.74 ± 0.21	6.60 ± 1.20	4,10	1.294	0.336
Phenylalanine	0.89 ± 0.28	0.96 ± 0.0.80	0.66 ± 0.06	1.18 ± 0.03	1.06 ± 0.07	4,10	1.44	0.290
Proline	2.21 ± 0.15	2.68 ± 0.12	2.74 ± 0.24	2.54 ± 0.06	3.12 ± 0.32	4,10	2.686	0.094
Threonine	0.46 ± 0.06	0.56 ± 0.06	0.48 ± 0.03	0.53 ± 0.02	0.61 ± 0.11	4,10	1.029	0.439
Tyrosine	2.91 ± 0.08^{ab}	1.93 ± 0.41^{a}	3.14 ± 0.33^{ab}	3.00 ± 0.72^{ab}	4.14 ± 0.12^{b}	4,10	3.788	0.0409
Valine	2.70 ± 0.64	3.58 ± 0.33	2.98 ± 0.75	3.50 ± 0.38	4.01 ± 0.12	4,10	1.076	0.418

Note. TU- untreated sample, TI blanched only, T2 blanched and treated with (0.5 g/100 ml) sodium metabisulphite, T3 blanched and treated with 1:1 (0.25 g/100 ml) of Sodium metabisulphite and ascorbic acid, and T4 blanched and treated with (0.5 g/100 ml) ascorbic acid. All values are of four replicates. Different letters superscripts in the same row signify significant differences between treatments at $p < 0.05$.

chemicals at 0.25% each provided sufficient preservation of the color of the edible grubs. Nonetheless, the color preservation activity of the treatments differed statistically. For instance, while blanching improved the whitening index of the grubs, its combination with the two chemical pretreatments resulted in better stability of the color over the storage period monitored. Consideration of the cost and level of desired color preservation is therefore necessary for choosing the type of treatment of the edible larvae to prevent darkening during processing.

The difference in the activity of the used inhibitors and heat treatment could be associated with their different mode of action and with different causes of browning in the edible insects. Sodium metabisulphite works by irreversibly inactivating the activity of polyphenol oxidase, whereas ascorbic acid acts by reducing formed quinones to diphenols thus preventing their polymerization into melanin (Yoruk & Marshall, 2003). On the other hand, blanching preserves food color through the denaturation of enzymes such as polyphenol oxidase. The activity of polyphenol oxidases is linked to the darkening of protein fractions of mealworms *Tenebrio molitor* as a result of oxidation of phenolic compounds (Janssen et al., 2017). Additionally, darkening can result from iron phenolic complexing reactions or non-enzymatic browning from heat treatment such as blanching which leads to the formation of melanin (Leni et al., 2019).

Our data show that blanching *Oryctes* spp larvae or its combination with treatments with sodium metabisulphite and ascorbic acid treatments, either singly or in a blend markedly reduced total viable counts compared to untreated larvae and eliminated *E. coli* in the larvae. Additionally, combining blanching with ascorbic acid treatment eliminated *S. aureus*. These results partly corroborate previous reports on edible insects such as mealworms (*Tenebrio molitor*) (Yan et al., 2023) and the house crickets (*Acheta domesticus*) (Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012), in which blanching markedly reduced TVC and/or *E. coli* counts. Yan et al. (2023) underscored the importance of blanching for microbial decontamination in food preservation and storage through microbial inactivation. The elimination of *S. aureus* by a combination of blanching with ascorbic acid treatment demonstrates enhancement of safety of the edible larvae through the combination of the heat and chemical treatments. This confirms inhibitory activity of ascorbic acid against *S. aureus* and other microbes like *Enterococcus faecalis* (Golonka et al., 2017). Therefore, besides blanching, ascorbic acid treatment may be required to enhance the safety of edible *Oryctes* spp larvae during preservation depending on the cost implications and desired level of safety. The pretreatments largely resulted in microbial loads that were within the Kenyan regulatory limits of KS 2922–1:2020 which put the maximum allowable limits for edible insects' products that will undergo further processing as 10^5 cfu/g of total viable counts, 10^2 cfu/g *E. coli* counts and 10 cfu/g for coagulase-positive *Staphylococcus aureus*.

All blanched larvae contained lower levels of crude protein, non-protein nitrogen, and proteolysis index than untreated larvae, with no indication of the extra effect of the chemical treatments on these values. Edible insect proteins consist of hemolymph proteins, cuticular proteins and muscle proteins (Janssen et al., 2017). The hemolymph proteins include fluids that circulate in the interior of arthropods, and they contain a high concentration of free amino acids (Janssen et al., 2017). The reduction of protein content in *Oryctes* spp larvae upon blanching could be attributed to the leaching of these soluble solids and further denaturation during heat treatment. On the other hand, non-protein nitrogen is associated with the breakdown of protein into other compounds such as peptides, urea, and free amino acids due to factors such as enzymatic processes, microbial activity, and heat processing (Pérez-Santaescolástica et al., 2018). The proteolysis index reflects the level of protein degradation upon hydrolysis (Pérez-Santaescolástica et al., 2018). Our results suggest that the larval protein in the untreated samples was more exposed to protein oxidation than the blanched larvae. This could be related to the denaturation of protein oxidation enzymes (proteases) by the blanching heat (Estévez & Xiong, 2021).

Although we recorded a narrow range of slightly acidic pH (6.3–6.8) in the larvae, it was statistically lower in the untreated than the treated larvae and highest in the grubs treated with sodium metabisulphite after blanching. Variations in pH can impact different aspects of a food product such as flavor, color, and protein solubility (Kim et al., 2020). The pH ranges recorded in this study are within neutral pH ranges associated with other edible insects such as black soldier fly larvae, meal worms and crickets (Janssen et al., 2017; Kim et al., 2020; Queiroz et al., 2023). An increase in pH following blanching is consistent with observations made by pretreatment by blanching in sweet corn (Zin et al., 2017). Elevation of pH due to blanching can be as a result of extraction of soluble compounds and precipitation due to heat (Cacchiarelli et al., 2022; Lee et al., 2023). The low pH in the untreated grubs on the other hand could be attributed to higher microbial activity, lowering of pH to slightly acidic (Cacchiarelli et al., 2022).

We found that the use of chemical inhibitors (sodium metabisulphite and ascorbic acid) recorded the highest content of total polyphenols in *Oryctes* spp larvae, which is attributable to their inhibitory effect on polyphenol oxidase enzymes leading to reduced browning in foods (Altunkaya & Gökmen, 2008). This finding is consistent with the reported high retention of total phenolic compounds in tomatoes (Mwende, Owino, & Imathiu, 2018) and lettuces, (Altunkaya & Gökmen, 2008), when treated with 0.5% sodium metabisulphite and ascorbic acid, respectively.

Apart from tyrosine, we recorded no alteration of levels of amino acids in the edible beetle grubs as a result of blanching or its combination with sodium metabisulphite and ascorbic acid pretreatments. This observation is closely consistent with the report by Ssepuuya et al. (2020) that amino acid profile of *Ruspolia differens* (Servile) was not altered by boiling at 100 °C for 30 min and roasting at 165 °C for 45 min. Similarly, heat treatment by boiling had no effect on amino acid profile of edible caterpillar *Imbrasia epimethea;* and blanching and sodium metabisulphite pretreatment did not alter the amino acid composition of mealworms, *Alphitobius diaperinus* and black soldier fly larvae (Janssen et al., 2017). This finding thus shows that the pretreatments done in this study maintain the protein quality by preserving the amino acid composition of the edible beetle grubs.

Tyrosine levels were higher in the larvae which were blanched followed by ascorbic acid treatment than in those treated by blanching only. Tyrosine is a polar, hydroxyl amino acid which plays an important role in signaling and is a precursor of melanin formation (Janssen et al., 2017). Tyrosine hydroxylation is catalyzed by tyrosinase enzymes such as polyphenol oxidase in the first step of melanin formation. In this process, tyrosine is converted to L dopamine which are then converted to dopa quinones, which are further non-enzymatically polymerized to melanin. Melanization in insects occurs as an immune response to environmental stresses and also during wound healing process.

6. Conclusion

The use of blanching singly or in combination with either 0.5 g/100 ml ascorbic acid or 0.5 g/100 ml sodium metabisulphite or a blend of the two chemicals at 0.25 g/100 ml each provided sufficient preservation of the color of the edible grubs, reduction of protein oxidation and inhibition of most harmful microbes to food safety limits, with insignificant effect on amino acid integrity of the edible *Oryctes* spp grubs.

Funding

We gratefully acknowledge the financial support for this research by the following organizations and Agencies through the International Centre of Insect Physiology and Ecology (*icipe*): the Norwegian Agency for Development Cooperation (grant number 222005/Z/20/Z); the Australian Centre for International Agricultural Research; the Swedish International Development Cooperation Agency; the Swiss Agency for Development and Cooperation; the Federal Democratic Republic of

Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors.

CRediT authorship contribution statement

Zipporah Wangari Maimba: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **John N. Kinyuru:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **George W. Wanjala:** Methodology, Resources, Supervision, Validation, Writing – review & editing. **James P. Egonyu:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

We acknowledge Kenya Industrial Research and Development Institute, for the use of their food texture and sensory evaluation laboratory; Kenya Bureau of standard Food and microbial laboratory and Xavier Cheseto, Peter Musembi, Jacktone Ooko Ongere, George Mathenge, Shem Ondiaka, Faith Nyamu, Eric Ibrahim, Bernard Kirui, John Kimani, and William Mutwiri for their technical support.

References

Altunkaya, A., & Gökmen, V. (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chemistry, 107*(3), 1173–1179. https://doi.org/10.1016/j.foodchem.2007.09.046
AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis (Volume 1), 15th ed., K. Helrich, Ed. Arlington, Virginia USA: , Inc.
Bolaji, O., Bamidele, D., Adeboye, A., & Tanimola, A. (2021). Influence of preparation and processing methods on the physico-chemical properties of *Oryctes rhinoceros* larvae. *Croatian Journal of Food Science and Technology, 13*(2), 148–154. https://doi.org/10.17508/CJFST.2021.13.2.02
Cacchiarelli, C., Fratini, F., Puccini, M., Vitolo, S., Paci, G., & Mancini, S. (2022). Effects of different blanching treatments on colour and microbiological profile of *Tenebrio molitor* and *Zophobas morio* larvae. *LWT, 157*, 113112. https://doi.org/10.1016/j.lwt.2022.113112.
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). https://doi.org/10.3390/foods9060800. Article 800.
Egonyu, J. P., Baguma, J., Martínez, L. C., Priwiratama, H., Subramanian, S., Tanga, C. M., Anankware, J. P., Roos, N., & Niassy, N. (2022). Global advances on insect pest management research in oil palm. *Sustainability, 14*. https://doi.org/10.3390/su142316288. Article 16288.
Zin, Z. M., Man, P. Y. A. N., Nasution, Z., Hui, C. K. A. H., & Zainol, M. K. (2017). effect of pre-treatment on physical properties, ascorbic acid and β-carotene content of frozen sweet corn kernels hibrimas (Zea mays var saccharata bailey) variety. *Malaysian Applied Biology, 46*(3), 23–31.
*Egonyu, J. P., Olubowa, R.R., Tanga C.M., Cheseto, X., & Subramanian, S. . *Oryctes* sp larvae as a nutrient rich delicacy: a comparison of nutrient profiles from four sites in western Kenya. Journal of Insects as Food and Feed. https://doi.org/10.1163/23524588-20230004.
[This paper demonstrates that edible rhinoceros beetle grubs consumed in Kenya are highly nutritious with 18, 42, 2, 1, and 19 per cent moisture, crude protein, crude fiber, crude fat and carbohydrates on dry matter basis, respectively. The larvae also contain appreciable levels of nine minerals namely magnesium, iron, zinc, calcium, copper, aluminium, cobalt, manganese and sodium; flavonoids; and vitamins A, E, nicotinic acid, nicotinamide, B2, B5, B6 and B9. Due to the high perishability of the larvae after harvesting, it is therefore important to device ways of inhibiting their perishability and preserving their nutritional composition during processing].
Estévez, M., & Xiong, Y. L. (2021). Protein oxidation in foods: mechanisms, consequences, and antioxidant solutions. *Foods, 10*(10), 2346. MDPI.
Fernandez-Cassi, X., Supeanu, A., Vaga, M., Jansson, A., Boqvist, S., & Vagsholm, I. (2019). The house cricket (*Acheta domesticus*) as a novel food: a risk profile. *Journal of Insects as Food and Feed, 5*(2), 137–157. https://doi.org/10.3920/JIFF2018.0021
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 *Ruspolia differens*. *Insects, 8*(3). https://doi.org/10.3390/insects8030102. Article 102.
Golonka, I., Oleksy, M., Junka, A., Matera-Witkiewicz, A., Bartoszewicz, M., & Musiał, W. (2017). Selected physicochemical and biological properties of ethyl ascorbic acid compared to ascorbic acid. *Biological and pharmaceutical bulletin, 40*(8), 1199–1206. https://doi.org/10.1248/bpb.b16-00967
Gonçalves, A. A., & de Oliveira, A. R. M. (2016). Melanosis in crustaceans: A review. *LWT-Food Science and Technology, 65*, 791–799. https://doi.org/10.1016/j.lwt.2015.09.011
Gray, J. I., & Monahan, F. J. (1992). Measurement of lipid oxidation in meat and meat products. *Trends in Food Science & Technology, 3*, 315–319. https://doi.org/10.1016/S0924-2244(10)80019-6
Janssen, R. H., Vincken, J.-P., van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor, Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry, 65*(11), 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471
Jiang, J., Xing, J., Sheng, X., & Zhan, W. (2011). Characterization of phenoloxidase from the bay scallop *Argopecten irradians*. *Journal of Shellfish Research, 30*(2), 273–277. https://doi.org/10.2983/035.030.0212
Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonnang, H., Maniania, N. K., & Ekesi, S. (2015). African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed, 1*(2), 103–119. https://doi.org/10.3920/JIFF2014.0016
Kim, T.-K., Yong, H. I., Chun, H. H., Lee, M.-A., Kim, Y.-B., & Choi, Y.-S. (2020). Changes of amino acid composition and protein technical functionality of edible insects by extracting steps. *Journal of Asia-Pacific Entomology, 23*(2), 298–305.
* Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food Control, 26*(2), 628–631. https://doi.org/10.1016/j.foodcont.2012.02.013.
[This study demonstrated that blanching of edible mealworm larvae and house crickets eliminated Enterobacteriaceae, but not some spore forming bacteria in cooked insects. For this reason, our study was designed to combine blanching, with other chemical inhibitors to enhance preservation of edible rhinoceros beetle larvae].
Kusia, E. S., Borgemeister, C., Tanga, C. M., Ekesi, S., & Subramanian, S. (2021). Exploring community knowledge, perception, and practices of entomophagy in Kenya. *International Journal of Tropical Insect Science, 41*, 2237–2246. https://doi.org/10.1007/s42690-021-00469-9
Larouche, J., Deschamps, M.-H., Saucier, L., Lebeuf, Y., Doyen, A., & Vandenberg, G. W. (2019). Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. *Animals, 9*(4). https://doi.org/10.3390/ani9040182. Article 182.
Lee, C.-W., Kim, T.-K., Hwang, K.-E., Kim, H.-W., Kim, Y.-B., Kim, C.-J., & Cho, Y.-S. (2017). Combined effects of wheat sprout and isolated soy protein on quality properties of breakfast sausage. *Korean Journal for Food Science of Animal Resources, 37*(1). https://doi.org/10.5851/kosfa.2017.37.1.52. Article 52.
Lee, J. H., Kim, T.-K., Park, S.-Y., Kang, M.-C., Cha, J. Y., Lim, M.-C., & Choi, Y.-S. (2023). Effects of blanching methods on nutritional properties and physicochemical characteristics of hot-air dried edible insect larvae. *Food Science of Animal Resources, 43*(3), 428–440. https://doi.org/10.5851/kosfa.2023.e4.
Leni, G., Caligiani, A., & Sforza, S. (2019). Killing method affects the browning and the quality of the protein fraction of Black Soldier Fly (*Hermetia illucens*) prepupae: A metabolomics and proteomic insight. *Food Research International, 115*, 116–125. https://doi.org/10.1016/j.foodres.2018.08.021
Lorenzo, J. M., Fontán, M. C. G., Franco, I., & Carballo, J. (2008). Biochemical characteristics of dry-cured lacón (a Spanish traditional meat product) throughout the manufacture, and sensorial properties of the final product. Effect of some additives. *Food Control, 19*(12), 1148–1158. https://doi.org/10.1016/j.foodcont.2007.12.005
* 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.
[This review highlights the traditional and modern methods used to process edible insects, among which is blanching which was selected for use in this study].
Moon, K. M., Kwon, E.-B., Lee, B., & Kim, C. Y. (2020). Recent trends in controlling the enzymatic browning of fruit and vegetable products. *Molecules, 25*(12). https://doi.org/10.3390/molecules25122754. Article 2754.
Mwende, R., Owino, W., & Imathiu, S. (2018). Effects of pretreatment during drying on the antioxidant properties and color of selected tomato varieties. *Food Science & Nutrition, 6*(2), 503–511. https://doi.org/10.1002/fsn3.581
Nakhleh, J., El Moussawi, L., & Osta, M. A. (2017). The melanization response in insect immunity. *Advances in Insect Physiology, 52*, 83–109. https://doi.org/10.1016/bs.aiip.2016.11.002
Nino, M. C., Reddivari, L., Ferruzzi, M. G., & Liceaga, A. M. (2021). Targeted phenolic characterization and antioxidant bioactivity of extracts from edible *Acheta domesticus*. *Foods, 10*(10). https://doi.org/10.3390/foods10102295. Article 2295.
Nirmal, N. P., & Benjakul, S. (2009). Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chemistry, 116*(1), 323–331. https://doi.org/10.1016/j.foodchem.2009.02.054

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology, 6*, 36–60. https://doi.org/10.1007/s11947-012-0867-9

Pérez-Santaescolástica, C., Carballo, J., Fulladosa, E., Garcia-Perez, J. V., Benedito, J., & Lorenzo, J. M. (2018). Effect of proteolysis index level on instrumental adhesiveness, free amino acids content and volatile compounds profile of dry-cured ham. *Food Research International, 107*, 559–566. https://doi.org/10.1016/j.foodres.2018.03.001

* Purschke, B., Brüggen, H., Scheibelberger, R., & Jäger, H. (2018). Effect of pre-treatment and drying method on physico-chemical properties and dry fractionation behavior of mealworm larvae (*Tenebrio molitor* L.). *European Food Research and Technology, 244*(2), 269–280. https://doi.org/10.1007/s00217-017-2953-8.

[The study reported that post-harvest processing (including blanching) significantly affects physical-chemical properties of dried mealworm larvae. It informed the choice of blanching as post-harvest treatment of edible beetle grubs and some attributes such as colour which we measured].

Queiroz, L. S., Silva, N. F. N., Jessen, F., Mohammadifar, M. A., Stephani, R., de Carvalho, … Casanova, F. (2023). Edible insect as an alternative protein source: a review on the chemistry and functionalities of proteins under different processing methods. *Heliyon, 9*, Article e14831. https://doi.org/10.1016/j.heliyon.2023.e14831.

R Core Team. (2022). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. URL https://www.R-project.org/.

Pyo, S.-J., Kang, D.-G., Jung, C., & Sohn, H.-Y. (2020). Anti-thrombotic, antioxidant, and haemolysis activities of six edible insect species. *Foods, 9*(4). https://doi.org/10.3390/foods9040401. Article 401.

Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research, 57*(5), 802–823. https://doi.org/10.1002/mnfr.201200735

Sindermann, D., Heidhues, J., Kirchner, S., Stadermann, N., & Kühl, A. (2021). Industrial processing technologies for insect larvae. *Journal of Insects as Food and Feed, 7*(5), 857–875. https://doi.org/10.3920/JIFF2020.0103

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.

Tang, C., Yang, D., Liao, H., Sun, H., Liu, C., Wei, L., & Li, F. (2019). Edible insects as a food source: a review. *Food Production, Processing and Nutrition, 1*(1), 1–13. https://doi.org/10.1186/s43014-019-0008-1.

* Wanjala, M. N., Orinda, M., Nyongesah, J. M., Tanga, C. M., Subramanian, S., Kassie, M., & Egonyu, J. P. (2023). Socio-cultural practices on the use of beetle grubs as food and feed in western Kenya. *Scientific Reports, 13*(1). https://doi.org/10.1038/s41598-023-34264-y. Article 7805.

[This paper demonstrated that edible rhinoceros beetle grubs were used as food and feed in ~ 39% and 78% of the households in study sites in western Kenya, respectively. The perceived benefits of the grubs for human consumption were nutritiousness and no linkage to allergies; and for animal feed were enhancing weight gain and increasing poultry egg laying. The significance of the insects informed their choice for further studies on post-harvest handling].

Wibowo, S., Grauwet, T., Santiago, J. S., Tomic, J., Vervoort, L., Hendrickx, M., & Van Loey, A. (2015). Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. *Food Chemistry, 187*, 140–151. https://doi.org/10.1016/j.foodchem.2015.03.131

Womeni, H. M., Tiencheu, B., Linder, M., Nabayo, C., Martial, E., Tenyang, N., Tchouanguep Mbiapo, F., Villeneuve, P., Fanni, J., & Parmentier, M. (2012). Nutritional value and effect of cooking, drying and storage process on some functional properties of Rhynchophorus phoenicis. *International Journal of Life Science and Pharma Research, 2*(3), 203–219.

Yan, X., Laurent, S., Hue, I., Cabon, S., Grua-Priol, J., Jury, V., Federighi, M., & Boué, G. (2023). Quality of *Tenebrio molitor* Powders: Effects of Four Processes on Microbiological Quality and Physicochemical Factors. *Foods, 12*(3). https://doi.org/10.3390/foods12030572. Article 572.

Yoruk, R., & Marshall, M. R. (2003). Physicochemical properties and function of plant polyphenol oxidase: a review. *Journal of Food Biochemistry, 27*(5), 361–422. https://doi.org/10.1111/j.1745-4514.2003.tb00289.x