BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED (2023) DOI:10.1163/23524588-20230124

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Low energy electron beam to support safe whole dried insect products

D.A. Peguero[1,2], *M. Gold*[1], *T. Duewell*[1], *A. Waser*[3], *B. Dubovcova*[3], *D. Vandeweyer*[4], *C. Zurbrügg*[2] *and A. Mathys*[1]*

[1]Sustainable Food Processing Laboratory, Institute of Food, Nutrition and Health, ETH Zürich, 8092 Zürich, Switzerland; [2]Department Sanitation, Water and Solid Waste for Development (Sandec), Eawag: Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland; [3]Digital Technologies, Data Analytics and Services Business Unit, Bühler AG, 9240 Uzwil, Switzerland; [4]Department of Microbial and Molecular Systems (M²S), Research Group for Insect Production and Processing, KU Leuven, Geel Campus, 2440 Geel, Belgium; *alexander.mathys@hest.ethz.ch

Received 8 June 2023 | Accepted 8 August 2023 | Published online 20 September 2023

Abstract

Product safety is a major concern when using edible insects and insect-derived products due to insects' diverse microbial community. Therefore, development of reliable post-processing treatments are required. Commonly used thermal treatments are effective against microorganisms but can have negative effects on product quality and nutritional value. Low-energy electron beam (LEEB) is an emerging non-thermal surface treatment technology for microbial decontamination of low water activity goods while preserving product quality. However, its potential application as an insect post-processing treatment has not been explored. To assess the effectiveness of LEEB treatment (250 keV and 12 kGy), three separate experiments were conducted with dried black soldier fly larvae (BSFL) and yellow mealworm (YMW). First, to assess LEEB's potential in inactivating microorganisms in insect products, LEEB treatment was conducted on dried BSFL inoculated with *Escherichia coli* K-12. Secondly, the effect of LEEB treatment on reducing naturally occurring microbial populations after microwave drying was evaluated. Finally, a six-month controlled shelf-life study (24 °C, 65% RH) was conducted to assess the long-term efficacy of LEEB treatment by monitoring physical, chemical and microbiological parameters. LEEB achieved a 4-$\log_{10}$ reduction of inoculated *E. coli* K-12 on dried BSFL and was effective in reducing numbers of all microbiological parameters (aerobic and anaerobic counts) in YMW. Specifically, in non-inoculated samples, aerobic and anaerobic total viable counts (TVC) were reduced by approximately 4-$\log_{10}$ colony forming units per gram (cfu/g) in YMW. In contrast, LEEB treatment moderately reduced microbial numbers in BSFL, with aerobic and anaerobic TVC reduced by approximately 1–2-$\log_{10}$ cfu/g following LEEB treatment. Microbial counts in both BSFL and YMW remained lower than the control throughout the shelf-life. LEEB treatment did not have an influence on the peroxide value. Therefore, LEEB can be an effective and gentle processing technique to support safe dried insect products.

Keywords

non-thermal treatment – microbial inactivation – *Hermetia illucens* L. – *Tenebrio molitor* – food safety

1 Introduction

Edible insects and insect-derived products have gained increased attention in recent years as potential alternatives to traditional protein sources due to their nutritionally dense profiles and potentially lower environmental impacts (Smetana *et al.*, 2019; Spykman *et al.*, 2021). Two of the most promising insects for food and feed are the larvae of the yellow mealworm (YMW), *Tenebrio molitor* L., and black soldier fly (BSFL), *Hermetia illucens* L. Due to their high protein content of 51-62 % (dry mass, DM) for YMW (Finke, 2002; Zhao *et al.*, 2016) and 30-50% DM for BSFL (Gold *et al.*, 2018; Wang and Shelomi, 2017), whole insects or insect-derived products are a promising protein source for human food (Bessa *et al.*, 2020; Zhao *et al.*, 2016) or feed for pets (Bosch and Swanson, 2021), livestock (e.g. poultry and pigs) (De Marco *et al.*, 2015; Hong *et al.*, 2020) and different fish in aquaculture (Gasco *et al.*, 2023).

YMWs and BSFL have a rich and diverse microbial community including fungi and bacteria. Freshly harvested BSFL and YMW can have total viable counts (TVCs) ranging from 8-10 $\log_{10}$ colony forming unit (cfu)/gram (Mancini *et al.*, 2019; Raimondi *et al.*, 2020; Stoops *et al.*, 2016; Wynants *et al.*, 2019; Zhen *et al.*, 2020). These microbial communities can include heat-resistant spores and foodborne pathogens, such as *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp., *Listeria* spp. and mycotoxin-producing fungi (Kashiri *et al.*, 2018; Raimondi *et al.*, 2020; Vandeweyer *et al.*, 2020, 2021; Wynants *et al.*, 2019). Microbial communities in the intestinal tract of BSFL and YMW are highly variable depending on the diet they were fed as well as rearing conditions (Bruno *et al.*, 2019; Wynants *et al.*, 2019). Microbiologically contaminated insects fed to livestock or consumed directly by humans can lead to foodborne illnesses (Heredia and García, 2018). Spore-forming bacteria, such as *B. cereus* and *C. perfringens* are pathogenic and highly resistant causing foodborne diseases (Delbrück *et al.*, 2021). For example, globally, *B. cereus* represents 1-12% of cases for foodborne illnesses (Grutsch *et al.*, 2018). To ensure prevention of such outbreaks from insect-based products, post processing steps are required.

Blanching, boiling and drying are common thermal treatments to reduce or stabilise the microbial load of insects (Saucier *et al.*, 2022; Vandeweyer *et al.*, 2017). Although thermal treatments are effective in microbial inactivation, specific pathogens such as *Salmonella* spp. and bacterial spores are heat resistant and can remain in heat treated products (Lang *et al.*, 2016; Zhang *et al.*, 2018). For instance, Vandeweyer *et al.* (2017) observed 1.3-1.9 $\log_{10}$ cfu/g aerobic bacterial spores in dried YMW after blanching (100 °C, 40 s) and microwave drying (maximum 80 °C, 8-20 min). Multiple studies have found pathogens such as *Salmonella* spp., *E. coli* (Kashiri *et al.*, 2018), and *B. cereus* in BSFL and YMW after drying (Grabowski and Klein, 2017; Wynants *et al.*, 2019). Microbial inactivation and thereby product safety could be increased by longer drying times and/or higher drying temperatures. However, this is typically associated with reductions in nutritional value, affecting product quality. Longer drying times can result in discoloration of the product which can be an indicator for reduction of vitamin and mineral contents (Ratti, 2001) and induce lipid oxidation (Larouche *et al.*, 2019). Alternative treatments such as high hydrostatic pressures (Kashiri *et al.*, 2018; Larouche *et al.*, 2019), and cold plasma (Rumpold *et al.*, 2014) have been investigated but were not effective in reducing TVCs. Therefore, it is important to identify effective treatments that can reduce microbial loads without compromising product quality (Berk, 2013).

Electron-beam, a non-thermal treatment, could be a promising technology for the decontamination of edible insects and insect-based products. The key advantage over thermal treatments is its potential to reduce microbial numbers without reducing product quality or addition of chemicals, in addition to potentially further extending the shelf-life. Electron beam has been used for various food products such as dry spices and herbs, meat and seafood or fresh produce to reduce the presence of pathogens (Clemmons *et al.*, 2015). Similar to gamma-rays and x-rays, the main inactivation mechanism for electron beam is the damage to the microbial DNA (Hertwig *et al.*, 2018; Moeller *et al.*, 2008). Inactivation occurs mainly through direct interactions of electrons with microbial DNA, RNA, enzymes and membrane molecules (Hertwig *et al.*, 2018) or indirect interactions due to the formation of free radicals (Tahergorabi *et al.*, 2012). The penetration depth of the electron beam treatment is dependent on the kinetic energy of the electrons and the density of the treated material (Helt-Hansen *et al.*, 2010). Electron beam can be classified into two categories based on the kinetic energy of electrons. High energy electron beam (HEEB) has a kinetic energy of >300 kiloelectronvolts (keV) and can penetrate products up to 8-10 centimetres (i.e. density 1 g/cm^3). Low energy electron beam (LEEB) has a kinetic energy of <300 keV resulting in a lower penetration

depth of micrometres, leading mainly to surface decontamination of the product (Hertwig *et al.*, 2018).

An advantage of LEEB compared to other ionising radiation treatments such as HEEB and gamma-ray is the lower energy. This allows for compact radiation protection, reduces potential health risks and saves physical space (Schopf *et al.*, 2022), making it feasible for use in a continuous process and integration within an existing production pipeline. Although industrial application of LEEB remains relatively new within the food industry, it has shown potential for treatment of herbs and spices (Murdoch *et al.*, 2022). Over 50 countries have approved the use of irradiated foods, particularly in the United States of America (USA) and certain parts of Asia (Kume *et al.*, 2009). However, maximum treatment dose measured in kiloGray (kGy) is country, product, and region specific. For example, for herbs and spices, the maximum dose in the USA is 30 kGy vs 10 kGy in the European Union (EU, CFR, 2023; European Commission (EC), 2017). In regions of the world where irradiated foods are gaining increased acceptance, LEEB could be a suitable option for ensuring the microbiological safety of whole dried insect products.

The use of LEEB has yet to be investigated as a potential post treatment method for insects. Therefore, the objective of this study was to assess the efficacy of LEEB for microbial decontamination for whole dried insects, specifically BSFL and YMW, in an industrial application. This research aimed to contribute to the understanding of this technology's potential use for the insect industry in addition to providing more knowledge on using an industrial-scale LEEB unit and its effects on potentially extending the shelf-life. Thereby, this research is working towards improving the food safety of insects and insect-derived products by LEEB treatment.

2 Materials and methods

Experimental overview

The microbial treatment efficacy of the industrial LEEB unit for whole dried insect products was evaluated in three independent experiments. In experiment 1 (see section "Experiment 1"), the inactivation efficacy of LEEB treatment was evaluated on decontaminated dried whole BSFL inoculated with *E. coli* K-12, a common non-pathogenic surrogate for foodborne pathogens. Following, in experiment 2 (see section "Experiment 2"), the inactivation efficacy of LEEB treatment was evaluated on naturally contaminated (i.e. non-inoculated) microwave dried whole BSFL and YMW. In experiment 3 (see section "Experiment 3"), this was repeated with oven-dried BSFL and YMW and complemented by a six-month shelf-life test comparing control (i.e. without LEEB treatment) and LEEB-treated insects.

Source of insects

To mimic real-life conditions, YMW and BSFL were sourced from commercial European insect producers (Table 1). The insects were processed before LEEB treatment according to the commercial procedures of the insect companies (images shown in Supplementary Figure S1). Processing consisted of blanching and drying with different temperature and time combinations and two drying methods (i.e. oven drying and microwave drying) (Table 1). All insects were stored at 4 °C until use.

Industrial-scale LEEB unit

Whole dried insects were treated with an industrial LEEB unit (Laatu™, Bühler AG, Uzwil, Switzerland). The unit was designed for the treatment of low moisture foods (i.e. <12% moisture content) and can continuously process up to 1,000 kg/h. However, the processing capacity was not tested in this study as the quantities used in the experiments were significantly below the commercial throughput. A schematic of the LEEB is shown in Figure 1 and was previously described in detail by Murdoch *et al.* (2022). Briefly, the product to be treated is placed in a loose form into the inlet hopper (Figure 1a). From the inlet hopper, the product to be treated falls onto shaking feeders, which aim to evenly distribute the product through the following treatment zone. The treatment zone contains two electron beam lamps (Figure 1b) which treats the product within a few milliseconds as it falls from the feeders to the outlet beyond the treatment zone, exiting the LEEB.

Dosimetry for LEEB treatment

The LEEB processor was characterized following ISO (2020) with radiochromic dosimetry film (B3 film, GEX Corp., Palm City, FL, USA). During each experiment, routine dosimetry was performed to ensure that the beam parameters were within an expected range of performance established during beam characterization. The expected range was set at ± 10%, as the overall uncertainty (k = 2) was determined at 10.6% during B3 system calibration. To estimate the dose on the whole dried insects, cylinders (20 mm length, 4 mm width) were wrapped with B3 film and irradiated at the same beam parameters. High-speed video recording of the product flow confirmed that the cylinders and both dried BSFL

TABLE 1 Thermal treatment conditions of BSFL and YMW used in experiments 1-3

Experiment	Drying method	BSFL	YMW	Biological replicates used (n =)	Source
1	Oven	Blanched: 1-2 min, 95-97 °C Dried: 16 h, 70 °C	–	3	Hermetia Baruth GmbH, Germany
2	Microwave	Dried: 20 min, max. 80 °C	Dried: 20 min, max. 80 °C	2	Inagro, Belgium and microwave dried by MEAM, Belgium
3	Oven	Blanched: 2 min, 75-85 °C Dried: 27 h at 80 °C	Blanched: 3 min, 95 °C Dried: 3.5 h at 90 °C	3	BSFL: Hermetia Baruth GmbH, Germany YMW: Essento, Switzerland

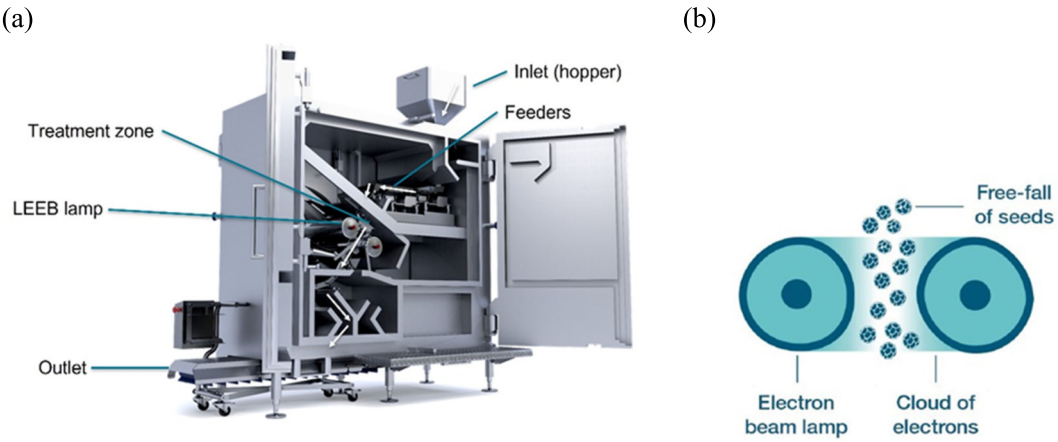

FIGURE 1 (a) Illustration of industrial scale LEEB unit and (b) schematic of the treatment zone consisting of two electron beam lamps treating the product with electrons as it passes from the feeders to the outlet (source: Bühler AG, Uzwil, Switzerland).

and YMW had the same velocity within the treatment zone, resulting in the same treatment time within ± 4%. The difference in velocity was not considered for measurement uncertainty, as it was less than the overall uncertainty of the B3 film during calibration. The LEEB treatment parameters were set at 250 keV at 18 mA with all other machine parameters (e.g. air flows) kept constant and ran at ambient temperature (approximately 24 °C). The resulting surface dose on the cylinders (Dμ) was 12 ± 2 kGy for 250 keV and 18 mA. These LEEB treatment parameters were used for all three experiments.

Estimating LEEB penetration depth of insects

The electron beams were characterized by conducting depth-dose distribution measurements on B3 film strips (18 μm thick) which were stacked in 30 layers and LEEB-treated at 250 keV and done according to ISO (2020). The LEEB depth-dose correlation was then determined by measuring the dose on each B3 film layer. The depth-dose was then calculated using the apparent density (i.e. 1.12 g/cm^3) of the B3 film. To roughly estimate the LEEB penetration depth of the insects, the apparent density (g/cm^3) was measured using an envelope and density analyser (GeoPyc 1360, Micromeritics, Norcross, GA, USA). The density measurement procedure involved determining the weight of the sample, followed by placing it in a bed of DryFlo® granular medium within a cylindrical chamber. The granular medium was then carefully consolidated around the sample using a piston, that was gradually pushed into the rotating cylindrical chamber until the consolidation force (28 Newtons) was reached (Siavashani *et al.*, 2020). This method allows for determining the density of the insect samples, including the air within the pores of the insects but excluding the air between insects. Once the insect density was obtained, a correction factor was calculated by dividing the density of the B3 film strips by the density of the insects (equation 1 shown in Supplementary

Material). The depth at which 50% of the dose penetrated the B3 film was 289 μm. To estimate the depth at which 50% of the LEEB dose could have penetrated the insects, the correction factor was multiplied by 289 μm (equation 2 shown in Supplementary Material). It is important to note, these are simplified estimates to give a general indication of the potential depth-dose distribution as there is currently no direct way to measure depth-dose in insects. To give an understanding of the depth-dose in relation to how deep it could have penetrated the insects, the thickness (mm) and length (mm) of 10 insects was measured using a caliper and ruler, respectively. The thickness and length were both important physical properties to measure as the beam could have targeted the insect horizontally or vertically depending on its position during the fall through the treatment zone.

Experiment 1: inactivation of a single foodborne pathogen indicator organism by LEEB

Since LEEB was not yet used for insects, the first experiment aimed to assess the inactivation efficacy of LEEB on a single microbial indicator organism on an insect product. *Escherichia coli* K-12 strain MG 1655 obtained from DSMZ (German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) was chosen as the model organism as it is a non-pathogenic strain and has a high radiation resistance making it a suitable surrogate for common foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. (Rodriguez *et al.*, 2006). Whole dried BSFL were intensely decontaminated by HEEB treatment (10 MeV with 27 kGy, Leoni AG, Daniken, Switzerland). Decontamination was confirmed by enumeration of TVCs (detection limit: 2-$\log_{10}$ cfu TVC/g dried BSFL). Decontaminated BSFL were then inoculated with approximately 8-$\log_{10}$ cfu *E. coli* K-12/g dried BSFL as described below. The inactivation efficacy was estimated by enumerating *E. coli* K-12 on Lysogeny broth (LB) agar plates (37 °C, 24 h) before and after LEEB treatment.

Additionally, HEEB treatment was used to determine the D_{10} value, an estimate of radiation resistance for the *E. coli* K-12 strain, to assess whether it was indeed a suitable model organism for resident bacteria. The D_{10}-value is the radiation dose required for one $\log_{10}$ reduction of microorganisms at a given energy level and is calculated from the negative inverse of the slope of a semi-logarithmic microbial inactivation curve (Blank and Corrigan, 1995). Whole dried BSFL were intensely decontaminated by HEEB treatment and then inoculated with approximately 8-$\log_{10}$ cfu *E. coli* K-12/g dried BSFL as described below. The whole dried BSFL were packaged in heat-sealed sterile plastic bags, which were then positioned flat on a tray. The insects were spread out in a single layer within the bags and HEEB treated at 0, 1, 3, 5 and 10 kGy. Following HEEB treatment, the number of *E. coli* K-12 was enumerated.

Escherichia coli K-12 was cultivated from a –80 °C glycerol stock and first grown in 10 ml of LB-broth (Sigma-Aldrich, Buch, Switzerland) in an incubating shaker for 24 hours at 37 °C at 180 revolutions per minute (rpm). Subsequently, 0.1 ml of the previous culture was propagated into 10 ml of fresh LB-broth and again incubated for another 24 hours at 37 °C and 180 rpm. After the second 24 hours, 1 ml of the culture was propagated into a sterile flask with 500 ml of LB broth and incubated overnight. To ensure no contamination, a control served alongside with 10 ml of LB-broth.

Before inoculation, 50 ml of the overnight *E. coli* K-12 culture (approximately 10^8 cfu/ml) was aseptically dispensed into ten 50 ml centrifuge tubes and centrifuged at 7.280 × g for 15 minutes at 4 °C. Following this, 49.5 ml of the supernatant was discarded. The remaining 0.5 ml of the cell suspension was vortexed. Then, 50 droplets of 100 μl of the cell suspension were inoculated onto 100 grams of sterilised whole dried BSFL, which were placed in a sterile aluminium tray and mixed using a sterile spoon. The inoculated BSFL was stored to dry under a biosafety bench at ambient temperature for approximately six hours, until the initial moisture content was reached. After inoculation, the dried BSFL had 8-$\log_{10}$ cfu *E. coli* K-12/g dried BSFL.

Experiment 2: inactivation of naturally contaminated BSFL and YMW by LEEB

The second experiment assessed the efficacy of LEEB treatment on naturally contaminated whole dried BSFL and YMW after gentle microwave drying. 150 grams of dried insects were either LEEB-treated in duplicates or handled in parallel as an untreated control (i.e. without LEEB treatment).

LEEB-treated samples and controls were immediately taken to the laboratory for physical, chemical and microbiological analyses. Physicochemical analyses included moisture content and water activity of pulverised insect samples. Moisture content was calculated from the difference in weight of three grams of insect sample after overnight oven drying at 105 °C. Water activity was determined with a water activity meter at 25 °C (LabMaster a_w, Novasina, Lachen, Switzerland).

Chemical analysis included primary lipid oxidation estimated with the peroxide value by an external laboratory (Eurofins, Schonenwerd, Switzerland) (AOCS, 2009).

Aerobic and anaerobic microbial numbers were enumerated on insect samples by plate counts including aerobic and anaerobic TVC, aerobic and anaerobic bacterial spores as well as yeast and moulds. For all microbial analyses, samples were plated the same day. A 60-gram insect sample was pulverised in sterilised beakers with a hand blender for one minute (Stoops *et al.*, 2016). Then, a five-gram subsample was transferred into a sterile stomacher bag and mixed with 45 ml of sterile maximum recovery diluent (0.85% (w/v) NaCl, 0.1% (w/v) peptone; Sigma-Aldrich) and homogenized for one minute with a stomacher and then ten-fold serially diluted and plated on agar plates (Stoops *et al.*, 2016). Aerobic TVC were enumerated on nutrient agar (30 °C, 48 h), yeasts and moulds on potato glucose and lactic acid agar (30 °C, 120 h). Aerobic bacterial spores were enumerated following incubation on nutrient agar (30 °C, 48 h) after heat shocking 2 ml of the pulverised sample mixed with max recovery diluent (80 °C, 5 min) in a water bath according to Klunder *et al.* (2012). Anaerobic TVC and anaerobic bacterial spore count (30 °C, 72 h) was enumerated according to ISO (2013) in anaerobic conditions at the same external laboratory (Eurofins, Schonenwerd, Switzerland). The detection limit for all aerobic microbial counts was 2-$\log_{10}$ cfu/g dried insect and 1-$\log_{10}$ cfu/g dried insect for all anaerobic counts. The detailed composition of all media is included in the Supplementary Table S1.

Experiment 3: shelf-life of LEEB-treated insect products

Experiment 3 was completed in the same manner as experiment 2 but with 500 g insects and conducted in triplicates. Following LEEB treatment, control and LEEB-treated samples were analysed for physical, microbiological, and chemical parameters (as described above). Control and LEEB-treated samples were packaged in airtight bags and stored in a dark climate-controlled chamber for a six-month shelf-life test (at 24 °C, relative humidity: 65%). Every month, insects were sampled by removing three bags from both the treatment and control. These samples were analysed for physical, and microbiological parameters as described above in triplicate. In addition, chemical analyses included primary and secondary lipid oxidation estimated with the peroxide and p-anisidine value, respectively, by the same external laboratory (Eurofins, Schonenwerd, Switzerland) according to AOCS (2009) and were performed for BSFL and YMW at the start of the experiment and after one, three and six months of storage. Chemical analysis for BSFL was conducted in triplicate. Since chemical analysis for YMW was only conducted for one single biological replicate per time point, the results can be found in the Supplementary Table S6.

Data analyses

Data was analysed using R in RStudio (R Core Team 2022, version 4.2.0, USA). We abstained from statistical analyses due to the small sample size ($n \leq 3$). The results were compared using mean and standard deviation ($n = 3$).

3 Results and discussion

LEEB penetration depth of dried insect products

Based on the measured densities of the dried insect products (see Supplementary Table S2), it was estimated that 50% of the LEEB dose could penetrate between 400 (oven dried) and 1,000 μm (microwave) for BSFL and between 500 (oven dried) and 800 μm (microwave) for YMW (see Supplementary Table S2). This confirms that LEEB is mainly a surface decontamination technology. Aisala *et al.* (2021) reported the penetration depth for pumpkin seeds ranged from 295-340 μm (density 1.06-1.22 g/cm^3), while for flax seeds, it was found to be between 330-360 μm (density 1.0-1.1 g/cm^3). Additionally, Gryczka *et al.* (2021) reported LEEB could penetrate the outer layer of pepper grains to 160-380 μm (density 0.9 g/cm^3). These results align with this study's findings, considering the product densities and the range of electron beam energy used (200-300 keV). In general, with the increasing density of the material, the penetration depth decreases (Ghomi *et al.*, 2005). Given that BSFL and YMW are around 2 mm in length and 2-4 mm in thickness, the majority of the LEEB dose did not likely penetrate into the larval digestive tract that includes most microorganisms (Bruno *et al.*, 2019) but only penetrated up to a certain layer. Further research should investigate whether LEEB can penetrate the larval exoskeleton and penetrate the digestive tract to contribute to a more comprehensive understanding of the depth-dose distribution of LEEB on edible insects. A previous study with peppercorns highlighted that the ability of LEEB to penetrate different materials is highly variable, with LEEB penetrating the thickness of the external layer of the white peppercorn but not the black peppercorn (Gryczka *et al.*, 2021). This may also be the case for edible insects. Interpretation of the LEEB pen-

etration depth requires caution due to the many influencing factors, particularly for insects, such as, size, shape, density and other surface properties (Gryczka *et al.*, 2021). Further research should explore the best approach for understanding LEEB dosimetry of different products as there are still limitations to LEEB depth-dose distribution measurements.

Experiment 1: inactivation of single foodborne pathogen indicator organism by LEEB

This is the first study to assess the efficacy of non-thermal LEEB treatment on inactivating microbial numbers in whole insect products. Our first experiment assessed the efficacy of LEEB treatment on a single bacterium, *E. coli* K-12. Before LEEB treatment, the radiation resistance of *E. coli* K-12 was quantified by an inactivation curve from HEEB treatment (shown in Supplementary Figure S2). Based on the inactivation curve, the D_{10} of *E. coli* K-12 was 0.90 kGy (r^2 = 0.99), which is consistent with the previously reported value of 0.88 kGy by Rodriguez *et al.* (2006). These findings are also similar to the reported D_{10} of *E. coli* K-12 for mung bean, clover, and fenugreek seeds, which were 1.11, 1.21, 1.4 kGy, respectively (Fan *et al.*, 2017). In contrast, on fresh-cut cabbage, the D_{10} value was calculated at 0.56 kGy (Grasso *et al.*, 2011), while on blueberries it was even lower at 0.37 kGy (Kong *et al.*, 2014). This difference in D_{10} value highlights how different materials, such as fresh produce versus low water activity goods, can influence the radiation resistance of the microorganisms. This suggests, that dried BSFL may have a more similar radiation resistance to low water activity goods, such as seeds, herbs and spices. Given the high radiation resistance of *E. coli* K-12 inoculated on dried BSFL, this strain was used in the following LEEB inactivation trial as a good surrogate for common foodborne pathogens, such as *Salmonella* or *E. coli*.

LEEB (250 keV, 12 ± 2 kGy) treatment of *E. coli* K-12 inoculated at a concentration of approximately 8-$\log_{10}$ cfu/g whole dried BSFL led to a 4-$\log_{10}$ reduction (shown in Supplementary Figure S3). The initial high level of *E. coli* K-12 inoculated on the dried BSFL, suggests that dried BSFL have a rough surface similar to red clover and fenugreek seeds (Fan *et al.*, 2017). Surface morphology and size of the material can influence initial inoculation levels, as seeds of mung beans had lower inoculated *E. coli* K-12 concentrations of around 6-$\log_{10}$ cfu/g due to their smooth surface and larger size (Fan *et al.*, 2017). Although a high reduction of *E. coli* K-12 was observed on inoculated dried BSFL, complete inactivation was not achieved. These findings are similar to Fan *et al.* (2017) who reported complete inactivation of *E. coli* K-12 was not achieved at doses of 4-12 kGy and 200 keV for fenugreek, mung bean and red clovers seeds. The author attributed this to a potential tailing effect, where bacterial inactivation does not follow a linear pattern but levels off at higher doses. The tailing effect has been observed for *E. coli* O157:H7 subjected to HEEB on lower water activity foods (Black and Jaczynski, 2008). However, LEEB is not intended as a sterilization technology, but rather a surface decontamination technology.

Therefore, given the effectiveness of LEEB treatment in reducing *E. coli* K-12, this suggests LEEB could be an effective method in reducing bacterial contamination on BSFL. Previous studies have shown that dried BSFL could still have *E. coli* concentrations ranging from 3-6-$\log_{10}$ (Kashiri *et al.*, 2018; Saucier *et al.*, 2022), while Kashiri *et al.* (2018) found *Salmonella* at 6-$\log_{10}$. Regulations on microbiological safety of insect-based products intended for animal feed state that *Salmonella* should be absent in 25 grams in the final product (European Commission (EC), 2011). Additionally, IPIFF (2019) recommends 1-$\log_{10}$ cfu/g of *E. coli* as the targeted limit. Therefore, LEEB could be effective in achieving the regulations and targeted limit for *Salmonella*, and *E. coli*, because of their lower radiation resistance compared to *E. coli* K-12. However, to confirm this, additional LEEB tests should be conducted with the pathogen inoculated at similar concentrations that have been previously identified in BSFL (Kashiri *et al.*, 2018; Saucier *et al.*, 2022). Based on the results, the same LEEB process parameters were used for experiments two and three.

Experiment 2: inactivation of naturally contaminated BSFL and YMW by LEEB

BSFL

Initial microwave dried BSFL had a moisture content of 6% and water activity below 0.6 (see Supplementary Table S3) and therefore microbial growth was unlikely to occur (Bonazzi and Dumoulin, 2011). As expected, LEEB treatment had no influence on the water activity and moisture content, as all trials were conducted at ambient temperature. Microwave dried BSFL had no detectable anaerobic TVC, anaerobic bacterial spores, and yeast and moulds. These results are to be expected, as the heat generated from microwave drying primarily achieves microbial inactivation by protein denaturation within the cells, independent of the cell wall structure (Alp and Bulantekin, 2021; Woo *et al.*, 2000). However, aerobic TVC and bacterial spores were 5.5 and 5 $\log_{10}$ cfu/g dried BSFL, respectively, suggesting that a large portion of TVC were bacterial spores (Table 2). TVC

were consistent with previous studies that reported concentrations ranging from 5-5.5 $\log_{10}$ cfu/g after boiling and drying (Campbell *et al.*, 2020; Saucier *et al.*, 2022) but lower than the 7-$\log_{10}$ cfu/g dried BSFL reported by Kashiri *et al.* (2018). Such findings are to be expected as bacterial spores exhibit high heat resistance. Several bacteria belonging to the orders Bacillales and Clostridiales can survive adverse environmental conditions by forming spores (Delbrück *et al.*, 2021). Within this range (5-8 $\log_{10}$ cfu/g) foodborne diseases can be caused by *B. cereus* (EFSA, 2005), thus indicating the need to ensure these concentrations are reduced.

LEEB treatment reduced aerobic TVC and bacterial spores by 2-$\log_{10}$ (Table 4). This demonstrates the efficacy of LEEB treatment in reducing microbial numbers in low water activity materials. However, the treatment was unable to reduce microbial numbers below the detection limit. Because LEEB treatment is likely most effective on or just below the surface, this could be due to the microorganisms in the larval digestive tract that were not affected by LEEB due to the limited penetration depth of the treatment. Additionally, different bacterial spores have varying levels of resistance to irradiation (Zhang *et al.*, 2018). For example, the spore-forming pathogen *B. cereus* was found to be more resistant than *B. subtilis* (De Lara *et al.*, 2002). Although the bacterial spores were not identified in this study, *B. cereus* was previously detected in BSFL and could have been present (Wynants *et al.*, 2019). Further research should investigate whether varying LEEB process parameters can lead to complete inactivation of bacteria in BSFL.

The fat-rich BSFL (15-39% DM) (Gold *et al.*, 2018) were also evaluated for primary lipid oxidation (Table 2). This is because lipid oxidation can reduce nutritional value, cause rancidity, and potentially pose human health risks, and is a crucial parameter when evaluating product quality (Johnson and Decker, 2015). Before LEEB treatment, BSFL had approximately 8 milliequivalents of active oxygen/kg fat (meq./kg fat), which is consistent with previous studies reporting peroxide values between 3-11 meq./kg fat (Hurtado-Ribeira *et al.*, 2023; Kathumbi *et al.*, 2022; Tome *et al.*, 2021). Interestingly, LEEB treatment did not appear to influence peroxide value, which could be attributed to the short treatment time. LEEB treatment has the potential to promote lipid oxidation through production of free radicals and/or reactive oxygen species reacting with unsaturated fatty acids (Barden and Decker, 2016). However, no lipid oxidation in BSFL after LEEB treatment may be due to the higher concentration of saturated fatty acids compared to unsaturated fatty acids in BSFL. The fatty acid profile of BSFL is highly variable but typically has 45-75% saturated fatty acids and 7-32% mono- and polyunsaturated fatty acids (Ewald *et al.*, 2020; Hurtado-Ribeira *et al.*, 2023; Kroeckel *et al.*, 2012), with the latter being more susceptible to oxidation. In case of edible oils, the fatty acid composition is considered a strong predictor of oxidation stability, particularly at the early oxidation stage (Yun and Surh, 2012). The fatty acid profiles of BSFL can differ depending on the rearing substrate used, thus potentially influencing the oxidation stability (Ewald *et al.*, 2020). Therefore, the effect of LEEB treatment on lipid oxidation needs to be further evaluated individually for different rearing substrates as various factors such as, fatty acid profile, the product type, presence of antioxidants and storage conditions can affect it (Aisala *et al.*, 2021). Overall, the peroxide value for BSFL was below a threshold peroxide value established for animal fats (max 10 meq/kg) for human consumption (FAO and WHO, 2001) but was above the threshold peroxide value for fish oils (5 meq./kg fat) (FAO and WHO, 2017). However, since the microwave dried BSFL had a peroxide value on the higher end compared to previous studies, this could mean the product would oxidize more quickly. As the peroxide value is affected by various factors it could be further reduced by optimizing killing and BSFL post-processing as well as defatting methods (Hurtado-Ribeira *et al.*, 2023; Larouche *et al.*, 2019; Zhen *et al.*, 2020).

YMW

Similar to BSFL, YMW had a moisture content of 6% and water activity below 0.6 (see Supplementary Table S3) which saw no effect from LEEB treatment. Before LEEB treatment, aerobic and anaerobic bacterial spores, and yeast and moulds were not detected in dried YMW. YMW had 4-$\log_{10}$ aerobic TVC and 3-$\log_{10}$ anaerobic TVC cfu/g dried YMW (Table 2). Aerobic TVC were fourfold higher compared to those reported by Vandeweyer *et al.* (2017) who reported 1-$\log_{10}$ cfu/g blanched and microwave dried YMW (20 min). The difference may be attributed to the effect of blanching, which has been shown to reduce microbial counts compared to processing without blanching. Interestingly, aerobic and anaerobic TVC were similar to one another in this study which is consistent with a previous study by Vandeweyer *et al.* (2020), where aerobic and anaerobic TVC for YMW were found to be comparable. This suggests that a substantial portion of TVC in YMW consists of facultative species, which are capable of thriving under both aerobic and anaerobic conditions. More specifically, *Bacillus* spp. are known to be facultatively anaerobic (Sperber and Doyle,

2009). While bacterial spores were not detected in this study, this could be a result of the relatively high detection limit (i.e. 2-$\log_{10}$ cfu/g dried YMW). For example, Vandeweyer *et al.* (2017) reported bacterial spore counts ranging from 1.3-1.9 $\log_{10}$ cfu/g dried YMW which are below the detection limit of this study. Additionally bacterial spores can be quite variable between rearing batches. LEEB treatment reduced aerobic and anaerobic TVC below the detection limit (Table 2) indicating that LEEB treatment led to a 2-$\log_{10}$ reduction for aerobic and anaerobic TVC in low water activity YMW after drying (Table 4).

Similar to BSFL, YMW are typically rich in fat (30-40 % DM) and were analysed for their peroxide value before and after LEEB treatment (Dreassi *et al.*, 2017; Ravzanaadii *et al.*, 2012; Son *et al.*, 2020). Similar to BSFL, LEEB appeared not to affect the peroxide value for YMW. The peroxide value of YMW before and after LEEB treatment was 2.2 and 2.6 meq./kg fat (Table 2), respectively. This is similar to 1.6-3.5 meq./kg fat reported previously (Lenaerts *et al.*, 2018; Son *et al.*, 2020). In general, the high unsaturated fatty acid content of YMW (e.g. 66-77%) could increase susceptibility of fat oxidation. Additionally, the treatment process can have a high influence on the oxidative stability of the product, with freeze-dried YMW having higher peroxide values of 19-125 meq./kg fat (Jeon *et al.*, 2016; Lenaerts *et al.*, 2018). However, fat oxidation may also be partially inhibited by natural antioxidants such as vitamin E. YMW contain approximately 144 mg/kg fat of tocopherol, a form of vitamin E (Son *et al.*, 2020). Such antioxidants can prevent free radicals from attacking fatty acids causing oxidation (Barden and Decker, 2016). Further studies are required to fully understand the effect of LEEB treatment on fatty acid composition, antioxidant profiles and sensory attributes since YMW are often intended for human consumption. Overall the peroxide values for YMW in this study fell well below the limits established for animals fats (10 meq./kg fat) as well as fish oils (5 meq./kg fat) (FAO and WHO, 2017).

Experiment 3: shelf-life of LEEB-treated insect products

BSFL

Initial water activity before and after LEEB treatment was around 0.3 and moisture content was below 3% (see Supplementary Table S4). Similar to experiment 2, LEEB treatment did not influence these physicochemical parameters.

Initial microbial counts were above the detection limit for all parameters except for yeast and moulds (Figure 2). In comparison to experiment 2, which only detected aerobic TVC and bacterial spore counts, in experiment 3, aerobic and anaerobic TVC and aerobic and anaerobic bacterial spore count were detected. The initial aerobic TVC (i.e. 4-$\log_{10}$ cfu/g dried BSFL) was slightly lower than in previous studies, where TVC ranged between 5-5.5 $\log_{10}$ cfu/g dried BSFL (Campbell *et al.*, 2020; Saucier *et al.*, 2022). Additionally, aerobic TVC was lower (i.e. 4-$\log_{10}$ cfu/g dried BSFL) compared to the results of experiment 2. However, overall results in experiment 3 had more microbiological parameters detected than in experiment 2.This could be due to many factors including different BSFL rearing conditions (e.g. substrate, duration, larval densities, product cleaning) resulting in a higher initial microbial load before post-processing, in addition to the different drying methods (microwave vs oven) and thermal conditions (Table 1), as well as potentially different product storage between experiment 2 and 3. However, systematic information on these factors was not recorded. In accordance with EU regulations for BSFL intended as animal feed, there is not a limit for aerobic TVC but it is required that producers ensure the absence of *Salmonella*, with a maximum allowable limit of 2.5-$\log_{10}$ cfu/g for *Enterobacteriaceae* (EC, 2005). A limitation to this study, is that these specific microorganisms were not identified.

LEEB treatment decreased all microbiological parameters below the detection limit, except for TVC (3-$\log_{10}$ cfu/g dried BSFL) (Figure 2). Specifically, there were reductions of at least 1-$\log_{10}$ for aerobic and anaerobic TVC, and 1-$\log_{10}$ for anaerobic bacterial spore counts (Table 4). The largest reduction was observed in aerobic bacterial spore counts by 2-$\log_{10}$ (Table 4). Surprisingly, LEEB was able to reduce aerobic bacterial spore counts to the detection limit, but aerobic TVC still remained around 3-$\log_{10}$ cfu/g dried BSFL. As mentioned in experiment 2, it is important that aerobic bacterial spore counts remain below the 5-8 $\log_{10}$ cfu/g, as this is the concentration responsible for foodborne diseases (EFSA, 2005). LEEB treatment had a higher $\log_{10}$ reduction of aerobic TVC in experiment 2 compared to experiment 3 (2-$\log_{10}$ vs 1-$\log_{10}$) (Table 4). Trinetta *et al.* (2011) found that LEEB treatment of different seeds resulted in different levels of microbial inactivation efficiency of *Salmonella*. These findings suggested that the treated surface morphology influenced the inactivation efficiency, demonstrating that a complex surface structure can reduce the antimicrobial effectiveness of LEEB. The difference in reduction of TVC in experiment 2 compared to 3 could be due to the surface structure differences between microwave-dried BSFL and oven-dried.

TABLE 2 Microbial counts and primary oxidation (peroxide value) of microwave dried whole BSFL and YMW before and after LEEB treatment. Data displayed are mean values and range (n = 2)

Insect	Treatment	Primary lipid oxidation	Microbial counts (log_{10} cfu/g dried insect)				
		Peroxide value meq./kg fat	Aerobic TVC	Anaer. TVC	Bacterial Spores	Anaer. bacterial spores	Yeast and moulds
MW dried BSFL	Control	8.1 ± 0.2	5.5 ± 0.6	<1	4.8 ± 0.2	<1	<2
	LEEB-treated	8.8 ± 1.7	3.5 ± 0.1	<1	3.2 ± 0.2	<1	<2
MW dried YMW	Control	2.2 ± 0.3	3.7 ± 0.1	2.8 ± 0.5	<2	<1	<2
	LEEB-treated	2.6 ± 0.2	<2	<1	<2	<1	<2

1 MW = microwave dried; BSFL = black soldier fly larvae; YMW = yellow mealworm; LEEB = Low energy electron beam; TVC = Total viable counts; Anaer. = anaerobic.

Upon visual inspection, the oven-dried BSFL appeared more shrivelled with more crevices (see images in Supplementary Material) in addition to having a higher density compared to the microwave dried BSFL, which lowers the LEEB penetration depth. Furthermore, the low reduction of aerobic TVC could be due to the initial intense (80 °C, 27 h) thermal treatment before LEEB treatment resulting in low initial microbial counts and a low water activity of 0.3. Vegetative cells in a dehydrated state can become more resistant to ionising radiation, comparable to that of spores (Barkai-Golan and Follett, 2017). This is because when cells are in a dehydrated state, the water within the cells becomes immobilized and the cellular metabolism is slowed down, thus limiting the movement of the cells and potentially reducing overall damage caused by radiation (Barkai-Golan and Follett, 2017). Overall LEEB treatment was effective in reducing initial microbial counts compared to the control. However, further research should investigate the impact of LEEB treatment on dried BSFL using a lower drying temperature (e.g. 60 °C) and shorter treatment time.

Throughout the entire shelf-life study, microbial counts were lower in LEEB-treated BSFL than in the non-LEEB-treated control (Figure 2). LEEB-treated anaerobic microbial counts remained at or below the detection limit (1-log_{10} cfu/g dried BSFL). Yeast and mould which typically indicate spoilage was only detected in low counts (3-log_{10} cfu/g dried BSFL) in the control after five months of storage, whereas for LEEB-treated samples they remained at or below the detection limit throughout the shelf-life study. Currently the EU regulation does not limit yeast and moulds but to obtain a high-quality certification of BSF as animal feed <6-log_{10} cfu/g of yeast and moulds is required (GMP + International, 2020) which was achieved with and without LEEB treatment. Overall, the findings suggest that LEEB treatment improved the shelf-life of BSFL as microbial counts remained at or below the detection limit.

Peroxide value in experiment 3 (2 meq./kg fat, Table 3) were lower than in experiment 2 (8 meq./kg fat, Table 2) and below the limit for animals fats (10 meq./kg fat) and fish oils (5 meq./kg fat) (FAO and WHO, 2017). LEEB treatment increased the peroxide value by 2 meq./kg fat, compared to the control. One possible explanation for the observed difference compared to experiment 2 could be the variation in the initial unsaturated fatty acid profile, which is influenced by factors such as the larvae's feed composition. However, since the fatty acid profile was not measured, it is difficult to draw a definitive conclusion. The p-anisidine value remained lower than the peroxide value. Within the first month of the shelf-life study, the initial peroxide and p-anisidine value in the LEEB-treated BSFL did not change. In contrast, the control peroxide value appeared to increase (Table 3). After three months of storage, the peroxide and p-anisidine value increased in both the control and LEEB-treated BSFL, exceeding the limit for animal-based fats (10 meq./kg fat). The similar results between control and LEEB-treated BSFL suggest that LEEB treatment has little influence on long-term lipid oxidation.

YMW

The initial water activity of dried YMW before LEEB treatment was 0.4 and the moisture content was below 6% (see Supplementary Table S5). While water activity remained below 0.6, it did increase to 0.5 after four months of storage in both the control and LEEB-treated YMW (see Supplementary Table S5).

Aerobic TVC in YMW were approximately 6-log_{10} cfu/g dried YMW (Figure 3). This is high relative to previously reported results after a thermal treatment (e.g. 1–4 log_{10} cfu/g dried YMW) (Mancini *et al.*, 2019; Vandeweyer *et al.*, 2017) and were higher than what was

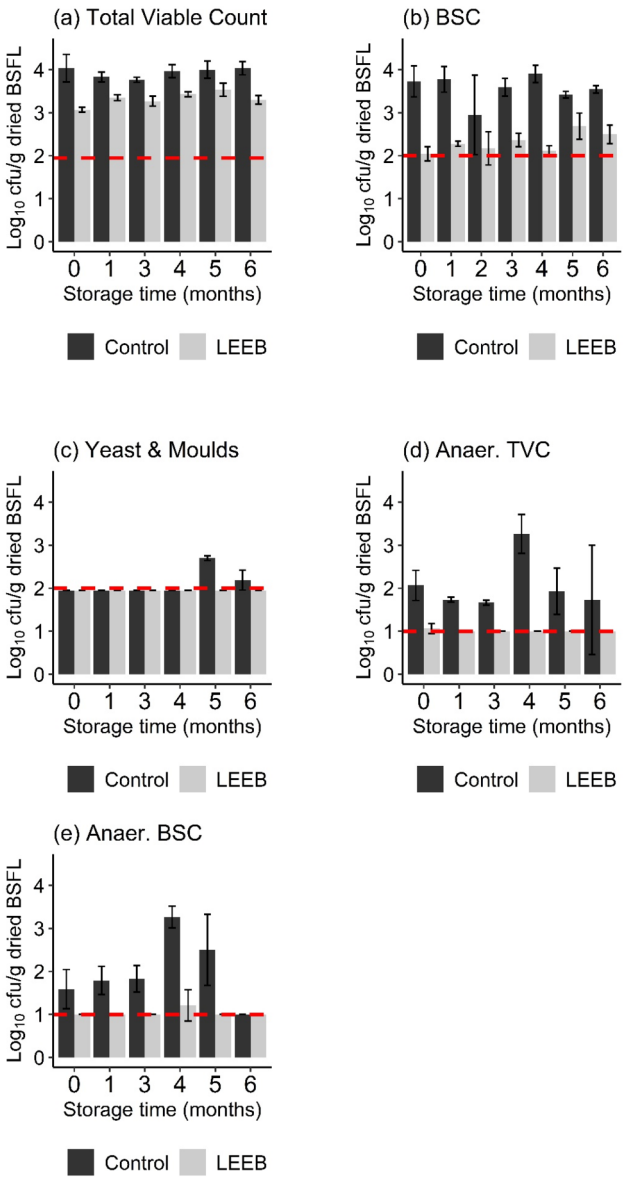

FIGURE 2 Microbial counts of control (i.e. oven-dried) and LEEB-treated BSFL samples over 6 months storage time. Control is blanched followed by drying without LEEB treatment. BSC: bacterial spore count, Anaer. TVC: anaerobic total viable count, and Anaer. BSC: anaerobic bacterial spore count. Data displayed are mean values and standard deviations shown as error bars (n = 3). Samples where colonies were not observed are graphed as the detection limit (shown as red dashed line). For aerobic microorganisms, detection limit was 2-$\log_{10}$ cfu/g dried insect and for anaerobic was 1-$\log_{10}$ cfu/g dried insects. Results in month two are not displayed due to sampling error.

observed in experiment 2 (e.g. 4 $\log_{10}$ cfu/g dried YMW, Table 2). However, TVCs were slightly lower than what is typically observed in fresh YMWs at harvest (8-9 $\log_{10}$ cfu/g fresh YMW) (Caparros Megido *et al.*, 2017; Stoops *et al.*, 2016; Wynants *et al.*, 2017). Anaerobic TVC were in a similar range to aerobic TVC, indicating facultative

TABLE 3 Primary (peroxide value) and secondary (p-anisidine value) lipid oxidation were monitored during the oven-dried BSFL shelf-life at four different time points. Data displayed are mean values and standard deviation (n = 3)

Storage Time (months)	Treatment	Peroxide value (meq./kg fat)	p-Anisidine value (–)
0	Control	2.2 ± 0.5	1.5 ± 0.1
	LEEB	3.9 ± 1.0	2.3 ± 0.7
1	Control	6.0 ± 2.5	1.9 ± 0.1
	LEEB	3.5 ± 1.3	2.1 ± 1.2
3	Control	12.0 ± 0.0	3.8 ± 1.0
	LEEB	11.7 ± 0.6	4.2 ± 0.2
6	Control	15.0 ± 0.0	6.8 ± 1.0
	LEEB	14.3 ± 0.6	6.6 ± 0.6

TABLE 4 Comparison of microwave average (experiment 2, n = 2) and oven dried average (experiment 3, n = 3) $\log_{10}$ reduction of initial microbial load after LEEB treatment for both BSFL and YMW

Experiment	BSFL		YMW	
	2	3	2	3
TVC	2.0	0.96	1.7	4.1
Bacterial spore count	1.6	1.7	0.0	0.82
Yeast & moulds	0.0	0.0	0.0	0.0
Anaerobic TVC	0.0	1.0	1.7	3.9
Anaerobic bacterial spore count	0.0	0.6	0.0	0.86

anaerobes as discussed in experiment 2 (Table 2). Aerobic bacterial spore counts were around 3-$\log_{10}$ cfu/g dried YMW (Figure 3).

Similar to experiment 2 with microwave dried YMW, LEEB treatment decreased all microbiological parameters to at or below the detection limit, except anaerobic TVC which was reduced to 2-$\log_{10}$ cfu/g dried YMW (Table 4, Figure 3). The largest decrease was observed for aerobic and anaerobic TVC, both resulting in a 4-$\log_{10}$ reduction (Table 4). Because of the naturally higher initial microbial load in experiment 3, LEEB was overall more effective in reducing microbial numbers in the oven dried YMW compared to microwave dried YMW used in experiment 2 (Table 4). Contrary to BSFL, drying method does not seem to heavily impact the surface morphology YMW, which could be why LEEB treatment was similar within experiment 2 and 3. When intended for human consumption, IPIFF (2019) recommends less than 3-$\log_{10}$ cfu/g of aerobic TVC. Therefore, LEEB was

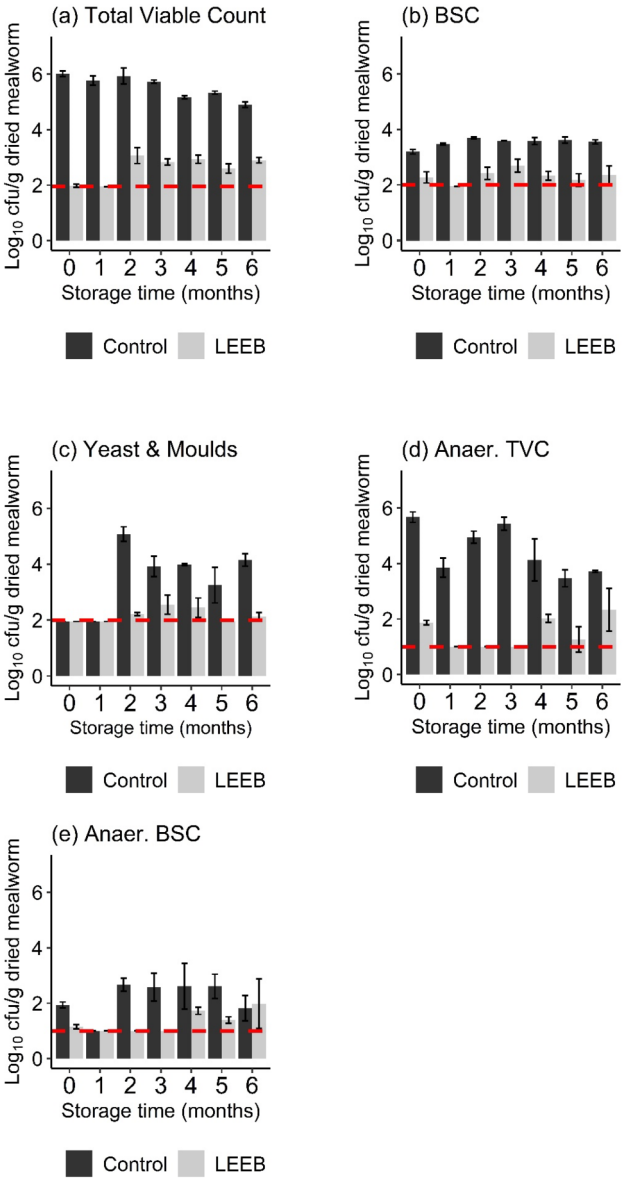

FIGURE 3 Microbial counts of control (i.e. oven dried) and LEEB-treated YMW samples over 6 months storage time. Control is blanched followed by drying without LEEB treatment. BSC: bacterial spore count, Anaer. TVC: anaerobic total viable count, and Anaer. BSC: anaerobic bacterial spore count. Data are displayed with mean values and standard deviations shown as error bars (n = 3). Samples where colonies were not observed are graphed as the detection limit (shown as red dashed line). For aerobic microorganisms, detection limit was 2-$\log_{10}$ cfu/g dried insect and for anaerobic was 1-$\log_{10}$ cfu/g dried insects.

effective in both experiments in reducing aerobic TVC to below the acceptable limit.

Throughout the six-months shelf-life study, LEEB treated YMW had lower counts in all microbiological parameters than the untreated control. Within the first two months of storage, aerobic TVC and bacterial spore counts for LEEB-treated YMWs remained at or below the detection limit, with anaerobic bacterial spore counts remaining at or below for the first three months. After two months of product storage, higher numbers were observed for aerobic TVC for LEEB-treated samples and remained around 3-$\log_{10}$ cfu/g dried YMW for the entire shelf life. This could be explained by a potential slight recovery of survived microbial cells, which were not culturable shortly after LEEB treatment. Further studies on such cell fractions are needed to verify this hypothesis. All microbial counts of the control remained stable throughout the 6-months shelf-life study with aerobic and anaerobic TVC ranging from 4–6 $\log_{10}$ cfu/g dried YMW. Yeast and mould counts was detected at 5-$\log_{10}$ cfu/g dried YMW in the control after two months of storage indicating some spoilage, as its close the limit set for animal feed of 6-$\log_{10}$ cfu/g (GMP + International, 2020). In contrast, LEEB-treated yeast and moulds remained at or below the detection limit throughout the shelf-life study.

4 Conclusion

LEEB treatment was effective in reducing microbial numbers in BSFL and YMW following thermal treatment, leading to an improved shelf-life. Further research should investigate the effect of LEEB treatment on BSFL with a higher initial microbial load. Despite a slight increase in lipid oxidation after LEEB treatment, the initial peroxide value for BSFL and YMW was still considered relatively low, meeting the target suitable for animal-based fat oils. Nevertheless, further research is necessary to investigate the shelf-life of microwave dried insect products, particularly in terms of lipid oxidation as this has yet to be conducted. Overall, these findings suggest that LEEB could be a complementary post-processing step to a gentler and more efficient thermal step, especially among regions of the world where irradiation is gaining increased interest.

Supplementary Material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.24032067

Acknowledgements

The authors would like to thank Lucio Bianchetti and Lorenz Fanger from the Sustainable Food Processing

Laboratory at ETH Zurich for their support with the laboratory work. The authors would also like to thank TingTing Wu at Empa for her assistance with measuring density of the dried insects, Bühler for providing access to the industrial LEEB unit and SUSINCHAIN WP3 partner Dr. Vincent Goovaerts from MEAM and WP2 partner Insect Research Centre of Inagro for supply of microwave dried BSFL and yellow mealworm.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

All original data supporting the findings of this study are publicly available. Raw data and custom R scripts developed for the analyses and visualizations can be found at: https://github.com/dapeguero/Leeb.

Funding

This work was supported by the European Union's Horizon 2020 research and innovation program under grant agreement no. 861976 project SUStainable INsect CHAIN (SUSINCHAIN).

References

Aisala, H., Nygren, H., Seppänen-Laakso, T., Heiniö, R.L., Kiessling, M., Aganovic, K., Waser, A., Kotilainen, H. and Ritala, A., 2021. Comparison of low energy and high energy electron beam treatments on sensory and chemical properties of seeds. Food Research International 148: 110575.

Alp, D. and Bulantekin, Ö., 2021. The microbiological quality of various foods dried by applying different drying methods: a review. European Food Research and Technology 247: 1333-1343.

American Oil Chemist Society (AOCS), 2009. Peroxide value Acetic Acid-Isooctane Method. Official Methods and Recommended Practices of the AOCS, Official Method Cd. 8b-90. AOCS Press.

Barden, L. and Decker, E.A., 2016. Lipid oxidation in low-moisture food: a review. Critical Reviews in Food Science and Nutrition 56: 2467-2482.

Barkai-Golan, R. and Follett, P.A., 2017. Ionizing radiation for shelf life extension. In: Irradiation for quality improvement, microbial safety and phytosanitation of fresh produce. Academic Press, Cambridge, MA, USA, pp. 7-28. https://doi.org/10.1016/B978-0-12-811025-6.00002-1

Berk, Z., 2013. Ionizing irradiation and other non-thermal preservation processes. Food process engineering and technology. 3rd ed. Academic Press, Cambridge, MA, USA.

Bessa, L.W., Marais, J. and Hoffman, L.C., 2020. Why for feed and not for human consumption? The black soldier fly larvae. Comprehensive Reviews in Food Science and Food Safety 19: 2747-2763.

Black, J.L. and Jaczynski, J., 2008. Effect of water activity on the inactivation kinetics of *Escherichia coli* O157:H7 by electron beam in ground beef, chicken breast meat, and trout fillets. International Journal of Food Sciences and Technology 43: 579-586.

Blank, G. and Corrigan, D., 1995. Comparison of resistance of fungal spores to gamma and electron beam radiation. International Journal of Food Microbiology 26: 269-277.

Bonazzi, C. and Dumoulin, E., 2011. Quality changes in food materials as influenced by drying processes. In: Tsotsas, E. and Mujumdar, A.S. (eds.) Modern drying technology: volume 3 – product quality and formulation. Wiley-VCH Verlag, Rastede, Germany. https://doi.org/10.1002/9783527631667.ch1

Bosch, G. and Swanson, K.S., 2021. Effect of using insects as feed on animals: pet dogs and cats. Insects as Food and Feed 7: 795-805.

Bruno, D., Bonelli, M., De Filippis, F., Casartelli, M. and Ercolini, D., 2019. The intestinal microbiota of *Hermetia illucens* larvae is affected by diet and shows a diverse composition in the different midgut regions. Applied and Environmental Microbiology 85: e01864-18.

Campbell, M., Ortuño, J., Stratakos, A.C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A. and Theodoridou, K., 2020. Impact of thermal and high-pressure treatments on the microbiological quality and in vitro digestibility of black soldier fly (*Hermetia illucens*) larvae. Animals 10: 682.

Caparros Megido, R., Desmedt, S., Blecker, C., Béra, F., Haubruge, É., Alabi, T. and Francis, F., 2017. Microbiological load of edible insects found in Belgium. Insects 8: 12.

CFR, 2023. Cod of federal regulations 21CFR179.26.

Clemmons, H.E., Clemmons, E.J. and Brown, E.J., 2015. Electron beam processing technology for food processing. In: Pillai, S.D. and Shayanfar, S. (eds.) Electron beam pasteurization and complementary food processing technolo-

gies. Woodhead Publishing, Cambridge, UK, pp. 11-25. https://doi.org/10.1533/9781782421085.1.11

De Lara, J., Fernandez, P.S., Periago, P.M. and Palop, A., 2002. Irradiation of spores of *Bacillus cereus* and *Bacillus subtilis* with electron beams. Innovative Food Science and Emerging Technologies 3: 379-384.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. and Schiavone, A., 2015. Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Animal Feed Science and Technology 209: 211-218.

Delbrück, A.I., Zhang, Y., Heydenreich, R. and Mathys, A., 2021. Bacillus spore germination at moderate high pressure: a review on underlying mechanisms, influencing factors, and its comparison with nutrient germination. Comprehensive Reviews in Food Science and Food Safety 20: 4159-4181.

Dreassi, E., Cito, A., Zanfini, A. and Materozzi, L., 2017. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). Lipids 52: 285-294.

EFSA, 2005. Opinion of the scientific panel on biological hazards (BIOHAZ) on *Bacillus cereus* and other *Bacillus* spp in foodstuffs. EFSA Journal 3: 175.

European Commission (EC), 2005. Commission Regulation (EU) 2073/2005 of 15 November on microbiological criteria for foodstuffs. Official Journal of the European Union, 1-26.

European Commission (EC), 2011. Commission Regulation (EU) 142/2011 implementing Regulation (EC) No. 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Co. Official Journal of the European Union, 1-254.

European Commission (EC), 2017. Commission regulation (EU) 2017/893: amending regulation No 999/2001 and No 142/2011. Official Journal of the European Union.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. and Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – possibilities and limitations for modification through diet. Waste Management 102: 40-47.

Fan, X., Sokorai, K., Weidauer, A., Gotzmann, G., Rögner, F. and Koch, E., 2017. Comparison of gamma and electron beam irradiation in reducing populations of *E. coli* artificially inoculated on mung bean, clover and fenugreek seeds, and affecting germination and growth of seeds. Radiation Physics and Chemistry 130: 306-315.

FAO and WHO, 2001. Codex Alimentarius. Fats, oils and related products. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission.

FAO and WHO, 2017. Codex Alimentarius Comission: Standard for fish oils CODEX STAN 329-2017.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21: 269285.

Gasco, L., Biasato, I., Enes, P. and Gai, F., 2023. Potential and challenges for the use of insects as feed for aquaculture. In: Morales-Ramos, J.A., Rojas, M.G. and Shapiro-Ilan, D.I. (eds.) Mass production of beneficial organisms. 2nd ed. Academic Press, Cambridge, MA, USA, pp. 465-492. https://doi.org/10.1016/B978-0-12-822106-8.00009-9

Ghomi, H., Rahman, S.R., Chalise, P.R., Hayashi, Y., Watanabe, M., Okino, A., Ano, T., Shoda, M. and Hotta, E., 2005. Experimental investigation of effect of low-energy pulsed atmospheric electron beam on bacterial cells. Japanese Journal of Applied Physics 44: 8698-8701.

GMP + International, 2020. TS 1.5 Specific feed safety limits. GMP+ International, Rijswijk, the Netherlands, 1-85 pp.

Gold, M., Tomberlin, J.K., Diener, S., Zurbrügg, C. and Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. Waste Management 82: 302-318.

Grabowski, N.T. and Klein, G., 2017. Microbiology of processed edible insect products – results of a preliminary survey. International Journal of Food Microbiology 243: 103-107.

Grasso, E.M., Uribe-Rendon, R.M. and Lee, K., 2011. Inactivation of Escherichia coli inoculated onto fresh-cut chopped cabbage using electron-beam processing. Journal of Food Protection 74: 115-118.

Grutsch, A.A., Nimmer, P.S., Pittsley, R.H., Kornilow, K.G. and McKillip, J.L., 2018. Molecular pathogenesis of *Bacillus* spp., with emphasis on the dairy industry. Fine Focus 4: 203-222.

Gryczka, U., Madureira, J., Cabo, S. and Migda, W., 2021. Determination of pepper microbial contamination for low energy e-beam irradiation. Food Microbiology 98: 1-5.

Helt-Hansen, J., Miller, A., Sharpe, P., Laurell, B., Weiss, D. and Pageau, G., 2010. Dμ – a new concept in industrial low-energy electron dosimetry. Radiation Physics and Chemistry 79: 66-74.

Heredia, N. and García, S., 2018. Animals as sources of foodborne pathogens: a review. Animal Nutrition 4: 250-255.

Hertwig, C., Meneses, N. and Mathys, A., 2018. Cold atmospheric pressure plasma and low energy electron beam as alternative nonthermal decontamination technologies for

dry food surfaces: a review. Trends in Food Science and Technology 77: 131-142.

Hong, J., Han, T. and Kim, Y.Y., 2020. Mealworm (*Tenebrio molitor* larvae) as an alternative protein source for monogastric animal: a review. Animals 10: 2068.

Hurtado-Ribeira, R., Hernández, M.D., Villanueva-Bermejo, D., Garcia-Risco, M.R., Hernández, M.D., Vázquez, L., Fornari, T. and Martin, D., 2023. The interaction of slaughtering, drying, and defatting methods differently affects oxidative quality of the fat from black soldier fly (*Hermetia illucens*) larvae. Insects 14: 368.

IPIFF, 2019. International platform of insects for food and feed (IPIFF) guide on good hygiene practices for European Union (EU) producers of insects as food and feed. OPIFF, Brussels, Belgium, 6-100 pp.

ISO, 2013. ISO 4833-1-Microbiology of the food chain – horizontal method for the enumeration of microorganisms.

ISO, 2020. ISO/ASTM 51818:2020 Practice for dosimetry in an electron beam facility for radiation processing at energies between 80 and 300 keV.

Jeon, Y., Son, Y., Kim, S., Yun, E., Kang, H. and Hwang, I., 2016. Physicochemical properties and oxidative stabilities of mealworm (*Tenebrio molitor*) oils under different roasting conditions. Food Science and Biotechnology 25: 105-110.

Johnson, D.R. and Decker, E.A., 2015. The role of oxygen in lipid oxidation reactions: a review. Annual Review of Food Science and Technology 6: 171-190.

Kashiri, M., Marin, C., Garzón, R., Rosell, C.M., Rodrigo, D. and Martínez, A., 2018. Use of high hydrostatic pressure to inactivate natural contaminating microorganisms and inoculated *E. coli* O157:H7 on *Hermetia illucens* larvae. PLoS ONE 13: e0194477.

Kathumbi, L.K., Home, P.G., Raude, J.M., Gathitu, B.B., Gachanja, A.N., Wamalwa, A. and Mibei, G., 2022. Influence of transesterification catalysts synthesized with citric acid on the quality and oxidative stability of biodiesel from black soldier fly larvae. Fuels 3: 533-554.

Klunder, H.C., Wolkers-Rooijackers, J., Korpela, J.M. and Nout, M.J.R., 2012. Microbiological aspects of processing and storage of edible insects. Food Control 26: 628-631.

Kong, Q., Wu, A., Qi, W., Qi, R., Carter, J.M., Rasooly, R. and He, X., 2014. Effects of electron-beam irradiation on blueberries inoculated with *Escherichia coli* and their nutritional quality and shelf life. Postharvest Biology and Technology 95: 28-35. https://doi.org/10.1016/j.postharvbio.2014.04.004

Kroeckel, S., Harjes, A.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. and Schulz, C., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute – growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364: 345-352.

Kume, T., Furuta, M., Todoriki, S., Uenoyama, N. and Kobayashi, Y., 2009. Status of food irradiation in the world. Radiation Physics and Chemistry 78: 222-226.

Lang, E., Zoz, F., Iaconelli, C., Guyot, S., Alvarez-Martin, P., Beney, L., Perrier-Cornet, J.M. and Gervais, P., 2016. Recovery estimation of dried foodborne pathogens is directly related to rehydration kinetics. PLOS ONE 11: e0160844.

Larouche, J., Deschamps, M.-H., Saucier, L., Lebeuf, Y., Doyen, A. and Vandenberg, G.W., 2019. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. Animals 9: 182.

Lenaerts, S., Van Der Borght, M., Callens, A. and Van Campenhout, L., 2018. Suitability of microwave drying for mealworms (*Tenebrio molitor*) as alternative to freeze drying: impact on nutritional quality and colour. Food Chemistry 254: 129-136.

Mancini, S., Fratini, F., Tuccinardi, T., Turchi, B., Nuvoloni, R. and Paci, G., 2019. Effects of different blanching treatments on microbiological profile and quality of the mealworm (*Tenebrio molitor*). Insects as Food and Feed 5: 225-234.

Moeller, R., Setlow, P., Horneck, G., Berger, T., Reitz, G., Rettberg, P., Doherty, A.J., Okayasu, R. and Nicholson, W.L., 2008. Roles of the major, small, acid-soluble spore proteins and spore-specific and universal DNA repair mechanisms in resistance of *Bacillus subtilis* spores to ionizing radiation from X rays and high-energy charged-particle bombardment. Journal of Bacteriology 190: 1134-1140.

Murdoch, M., Waser, A., Morantes, G., Dubovcova, B., Akepsimaidis, G., Currie, A. and Pillai, S.D., 2022. A new proposed validation method for low energy electron beam processing of dry spices. Innovative Food Science and Emerging Technologies 81: 103141.

Raimondi, S., Spampinato, G., Macavei, L.I., Lugli, L., Candeliere, F., Rossi, M., Maistrello, L. and Amaretti, A., 2020. Effect of rearing temperature on growth and microbiota composition of *Hermetia illucens*. Microorganisms 8: 902.

Ratti, C., 2001. Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering 49: 311-319.

Ravzanaadii, N., Kim, S., Choi, W.H., Hong, S. and Kim, N.J., 2012. Nutritional value of mealworm, *Tenebrio molitor* as food source. International Journal of Industrial Entomology 25: 93-98.

Rodriguez, O., Castell-Perez, M.E., Ekpanyaskun, N., Moreira, R.G. and Castillo, A., 2006. Surrogates for validation of electron beam irradiation of foods. International Journal of Food Microbiology 110: 117-122.

Rumpold, B.A., Fröhling, A., Reineke, K., Knorr, D., Boguslawski, S., Ehlbeck, J. and Schlüter, O., 2014. Comparison of volumetric and surface decontamination techniques for

innovative processing of mealworm larvae (*Tenebrio molitor*). Innovative Food Science and Emerging Technologies 26: 232-241.

Saucier, L., M'ballou, C., Ratti, C., Deschamps, M.H., Lebeuf, Y. and Vandenberg, G.W., 2022. Comparison of black soldier fly larvae pre-treatments and drying techniques on the microbial load and physico-chemical characteristics. Journal of Insects as Food and Feed 8: 45-64.

Schopf, S., Gotzmann, G., Dietze, M., Gerschke, S., Kenner, L. and König, U., 2022. Investigations into the suitability of bacterial suspensions as biological indicators for low-energy electron irradiation. Frontiers in Immunology 13: 814767.

Siavashani, A.Z., Mohammadi, J., Maniura-weber, K., Senturk, B., Nourmohammadi, J., Sadeghi, B., Huber, L. and Rottmar, M., 2020. Silk based scaffolds with immunomodulatory capacity: anti-inflammatory effects of nicotinic acid. Biomaterials Science Royal Society of Chemistry 8: 148162.

Smetana, S., Schmitt, E. and Mathys, A., 2019. Sustainable use of *Hermetia illucens* insect biomass for feed and food: attributional and consequential life cycle assessment. Resources, Conservation and Recycling 144: 285-296.

Son, Y., Choi, S.Y., Hwang, I., Nho, C.W. and Kim, S.H., 2020. Could defatted mealworm (*Tenebrio molitor*) and mealworm oil be used as food ingredients? Foods 9: 40.

Sperber, W.H. and Doyle, M.P., 2009. Compendium of the microbiological spoilage of foods and beverages. Springer-Verlag, New York, NY, USA. https://doi.org/10.1007/978-1-4419-0826-1

Spykman, R., Hossaini, M.S., Peguero, D.A., Green, A., Heinz, V. and Smetana, S., 2021. A modular environmental and economic assessment applied to the production of *Hermetia illucens* larvae as a protein source for food and feed. The International Journal of Life Cycle Assessment 26: 1959-1976.

Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B. and Van Campenhout, L., 2016. Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. Food Microbiology 53: 122-127.

Tahergorabi, R., Matak, K.E. and Jaczynski, J., 2012. Application of electron beam to inactivate *Salmonella* in food: recent developments. Food Research International 45: 685-694.

Tome, N.M., Boogaard, T., Serteyn, D., Schmitt, E. and Paul, A., 2021. Evaluation of the fat oxidation quality of commercial *Hermetia illucens* meal. Journal of Insects as Food and Feed 7: 965-974.

Trinetta, V., Vaidya, N., Linton, R. and Morgan, M., 2011. A comparative study on the effectiveness of chlorine dioxide gas ozone gas and e-beam irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds. International Journal of Food Microbiology 146: 203-206. https://doi.org/10.1016/j.ijfoodmicro.2011.02.014

Vandeweyer, D., Lenaerts, S., Callens, A. and Van Campenhout, L., 2017. Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). Food Control 71: 311-314.

Vandeweyer, D., Lievens, B. and Van Campenhout, L., 2020. Identification of bacterial endospores and targeted detection of foodborne viruses in industrially reared insects for food. Nature Food 1: 511-516.

Vandeweyer, D., De Smet, J., Van Looveren, N. and Van Campenhout, L., 2021. Biological contaminants in insects as food and feed. Insects as Food and Feed 7: 807-822.

Wang, Y.-S. and Shelomi, M., 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 6: 91.

Woo, I.S., Rhee, I.K. and Park, H.D., 2000. Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Applied and Environmental Microbiology 66: 2243-2247.

Wynants, E., Crauwels, S., Lievens, B., Luca, S., Claes, J., Borremans, A., Bruyninckx, L. and Van Campenhout, L., 2017. Effect of post-harvest starvation and rinsing on the microbial numbers and the bacterial community composition of mealworm larvae (*Tenebrio molitor*). Innovative Food Science and Emerging Technologies 42: 8-15.

Wynants, E., Frooninckx, L., Crauwels, S., Verreth, C., De Smet, J., Sandrock, C., Wohlfahrt, J., Van Schelt, J., Depraetere, S., Lievens, B., Van Miert, S., Claes, J. and Van Campenhout, L., 2019. Assessing the microbiota of black soldier fly larvae (*Hermetia illucens*) reared on organic waste streams on four different locations at laboratory and large scale. Microbial Ecology 77: 913-930.

Yun, J. and Surh, J., 2012. Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress. Preventive Nutrition and Food Science 17: 158-165.

Zhang, Y., Moeller, R., Tran, S., Dubovcova, B., Akepsimaidis, G., Meneses, N., Drissner, D. and Mathys, A., 2018. *Geobacillus* and *Bacillus* spore inactivation by low energy electron beam technology: resistance and influencing factors. Frontiers in Microbiology 9: 2720.

Zhao, X., Vázquez-Gutiérrez, J.L., Johansson, D.P., Landberg, R. and Langton, M., 2016. Yellow mealworm protein for food purposes – extraction and functional properties. PLoS ONE 11: e0147791.

Zhen, Y., Chundang, P., Zhang, Y., Wang, M., Vongsangnak, W., Pruksakorn, C. and Kovitvadhi, A., 2020. Impacts of killing

process on the nutrient content, product stability and *in vitro* digestibility of black soldier fly (*Hermetia illucens*) larvae meals. Applied Sciences 10: 6099.