BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED (2024) DOI:10.1163/23524588-00001218

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

RESEARCH ARTICLE

Dielectric drying of black soldier fly larvae (*Hermetia illucens*): impact on microbiological product quality, safety and stability

D. Vandeweyer[1]*, *D. Lachi*[1], *H. Geheniau*[2], *V. Goovaerts*[3], *P. van der Zweep*[2], *C. Groffils*[3], *M. Thijs*[2] *and M. Van Der Borght*[1]

[1]KU Leuven, Geel Campus, Department of Microbial and Molecular Systems (M²S), Research Group for Insect Production and Processing (IP&P), Kleinhoefstraat 4, 2440 Geel, Belgium; [2]Dymotec, Lammerdries-Oost 19, 2250 Olen, Belgium; [3]Microwave Energy Applications Management (MEAM), Centrum-Zuid 3060, 3530 Houthalen-Helchteren, Belgium; *dries.vandeweyer@kuleuven.be

Received 26 March 2024 | Accepted 9 October 2024 | Published online 29 October 2024

Abstract

Along with the increasing scale of industrial production of insects as food and feed, the need for processing methods applicable on large scale is rising within the insect sector. One important processing technology in this context is drying, which aims at stabilising the quality of the insects. This study investigated the impact of dielectric drying technologies, specifically microwave (μW) and radio frequency (RF) drying, on the microbiological quality, safety, and stability of black soldier fly (BSF) larvae in comparison to the traditional methods oven drying and freeze drying. Before, during and after subjecting a large batch of blanched BSF larvae to the four drying technologies, their water activity and moisture content as well as general microbial and pathogen counts were assessed. Dielectric drying effectively reduced water activity, thus enhancing product stability. Microbiological analyses revealed the efficacy of blanching in microbial reduction, with dielectric (especially RF) drying further contributing to these reductions. The results, however, do not conclusively support nonthermal microbial inactivation through dielectric treatment. Specific analyses targeting foodborne pathogens indicated the absence of most selected bacterial pathogens, except for (presumptive) *Bacillus cereus*. This pathogen was detected in a few samples but remained below safety thresholds and was effectively eliminated by dielectric drying. Finally, a shelf life stability evaluation showed minimal changes in dielectrically dried BSF larvae over six months, indicating a microbiologically stable product. In conclusion, dielectric drying technologies can retain or even improve the microbiological quality and stability of BSF larvae in the insect industry and can be considered as valuable and more efficient (time, energy consumption) alternatives to oven and freeze drying.

Keywords

insects – microwave drying – product quality – radio frequency drying – shelf life

1 Introduction

Since the global market introduction of insects as food and feed or source of biomolecules, the need for processing and stabilisation methods within the insect sector has risen. Over the past years, the farmed insect sector has evolved into a large-scale industry branch, with companies producing and processing tens of thou-

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-00001218
© D. VANDEWEYER *ET AL.*, 2024 | ISSN: 2352-4588 (online)
This is an open access article distributed under the terms of the CC BY 4.0 license.

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

sands of tons of insect products annually (IPIFF, 2020). Specifically for the black soldier fly (BSF, *Hermetia illucens*), which is among the most intensively reared insect species for food and feed, it is estimated that over 4.6 million tonnes of larvae will be reared by 2033 in Europe alone (Meticulous Research, 2024). In a perspective of insect production planning and logistics, this scale requires adequate large-scale processing methods that facilitate the conversion of insects into a diverse range of products and that, at the same time, ensure the stability of the insects and/or derived products during production and storage. A typical example of a stabilisation method applied in the insect sector is drying. While hot air (oven) drying and freeze drying are commonly used, they are often expensive and can be challenging to implement on a large scale and in a continuous set-up (Kusuma *et al.*, 2023; Stratta *et al.*, 2020). As a result, alternative technologies such as dielectric drying are gaining importance in the insect sector due to their efficiency and industrial-scale potential, although research regarding the application of this technology on insects is still limited (Bawa *et al.*, 2020; Hernández-Álvarez *et al.*, 2021; Lenaerts *et al.*, 2018; Parniakov *et al.*, 2022; Vandeweyer *et al.*, 2017).

Dielectric drying technology is generally based on either microwave (μW) or radio frequency (RF) heating. Within the electromagnetic spectrum, radio frequency waves exist at frequencies between 10 and 300 MHz, while microwaves correspond with frequencies between 300 and 30,000 MHz (Wang *et al.*, 2011). One substantial difference between μW and RF waves is their penetration depth. The lower frequency and thus longer wavelength of RF waves result in a larger penetration depth, leading to more uniform heating and a greater potential drying capacity compared to μW drying (Awuah *et al.*, 2005). μW drying, on the other hand, may allow more rapid heating and is particularly suitable for irregularly shaped products (Dubey *et al.*, 2016). Stabilisation through dielectric drying involves removing moisture from a biological matrix, thereby lowering its water activity (a_w). Reduced a_w, in turn, minimises (micro)biological, enzymatic and chemical deterioration processes and hence improves product stability and shelf life.

Apart from their role in stabilisation (through a decrease in a_w), μW and RF drying also impact the microbiological quality of insect products. Untreated (raw) insects may contain high amounts of microorganisms (up to a total viable count of 9.8 log cfu/g for BSF larvae (Wynants *et al.*, 2019)), potentially including spoilage or pathogenic species. Therefore, it is generally recommended to decontaminate the insects during post-harvest processing (Vandeweyer *et al.*, 2021). While no specific microbiological criteria exist for insect-based food and feed products, general microbiological limits that count for animal feeds of animal origin (e.g. EU Regulations 853/2004 and 142/2011: absence of *Salmonella* and presence of Enterobacteriaceae below 10 (lower limit) to 300 (upper limit) cfu/g) or ready-to-eat foodstuffs (e.g. EU Regulation 2073/2005: absence of *Listeria monocytogenes* in 25 g) should be complied to. Dielectric drying is a thermal technology and consequently, microorganisms in the drying matrix may be killed by the increase in temperature. While oven drying is a surface heating technology during which heat transfer occurs via conduction, convection and radiation, dielectric drying heats products in a volumetric way as a result of molecular friction and collision of ions in an alternating electromagnetic field (Wray and Ramaswamy, 2015). This means that, unlike oven drying, dielectric drying generates heat throughout the whole mass of the product, therefore improving heat transfer and decreasing drying times substantially. In a microbiological perspective, this phenomenon may be beneficial, given that a large number of microorganisms reside especially inside of the insect (Wynants *et al.*, 2017, 2019) and are thus directly subjected to volumetric heating as well. Freeze-drying, on the other hand, is a non-thermal drying method and is therefore not considered as decontaminating technology. Indeed, as reported by Saucier *et al.* (2022), freeze drying of BSF larvae could only reduce total viable counts by 1.5 log cfu/g, while hot-air drying resulted in a reduction of 3.5 log cfu/g. During post-harvest processing, blanching is typically applied as a method to kill insects, prior to drying. In that case, insects have already received a decontamination treatment. For example, a study by Larouche *et al.* (2019) found that blanching BSF larvae reduced the total viable count by 3.4 log cfu/g, resulting in the highest reduction among all killing methods (including oven drying alone). Blanching is, however, only a mild treatment, applied for a few seconds or minutes at a temperature of 100 °C or lower and thus only affecting (a part of) the vegetative cells present (Saucier *et al.*, 2022; Vandeweyer *et al.*, 2017). Dielectric drying may hence act as an additional decontamination step in insect processing. Indeed, during μW and RF drying, it is possible to reach a temperature higher than 100 °C which is necessary to impact bacterial endospores. Furthermore, the bactericidal and even sporicidal mechanisms of dielectric irradiation are claimed to be different than those of boiling (Kim *et al.*, 2009). At the same time, negative effects of dielectric treatment on nutritional quality

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This publication is licensed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

parameters are considered to be limited, due to the relatively short drying times necessary, as compared to oven drying. However, studies on the impact of microwave drying on the nutritional quality of BSF larvae have yielded varying results to date (Huang *et al.*, 2019; Lier *et al.*, 2024; Son *et al.*, 2023). Additionally, it is still under debate whether dielectric drying can also cause additional nonthermal microbial inactivation (Espitia *et al.*, 2023).

The aim of this study was to investigate the impact of two dielectric drying technologies, (hybrid) microwave and radio frequency drying, on the microbiological quality, safety and stability of larvae of the black soldier fly, a species with established industrial value within the insect sector (De Smet *et al.*, 2018). A comparison with two benchmark technologies, oven drying and freeze drying, was made with respect to the microbiological quality and safety of the BSF larvae.

2 Materials and methods

Experimental set-up

In order to compare two dielectric drying technologies, a large batch of black soldier fly larvae (*Hermetia illucens*) was subjected to both microwave (μW) and radio frequency (RF) drying on industrial scale. During preliminary experiments, both drying technologies were optimised in terms of processing parameters and these optimal parameters were applied during drying (see paragraph *Drying technologies*). Before and after drying, the microbiological quality and safety of the insects were assessed, as well as the intrinsic parameters water activity and moisture content. As a comparison, the same batch of BSF larvae was additionally dried via both oven drying and freeze drying (on lab scale), two benchmark technologies that are commonly applied in the insect industry. The same analyses (moisture content, water activity and microbiology) were performed for BSF larvae dried with these technologies. Finally, after dielectric drying, the BSF larvae were preserved in sealed plastic bags for six months at room temperature in order to evaluate their microbiological stability (shelf life).

Insects and pretreatments

A large batch of BSF larvae (50 kg) was reared by the Insect Research Centre of Inagro (Rumbeke-Beitem, Belgium). Larvae were produced on chicken feed (Farm 1 crumble, Hobbyfirst, Schoten, Belgium) supplemented with 2/3 tap water, following Inagro's standard protocol (27 °C, 60% relative humidity, 20,000 larvae/crate with 10 kg wet feed). The larvae were harvested at their final instar and transported alive to KU Leuven (Geel, Belgium) at ambient temperature in open crates. After taking samples for initial analyses, the larvae were killed by steam-blanching them for 5 min at 100 °C (Combi-Master Plus 61; Rational NV, Zwijndrecht, Belgium) in portions of 1 kg per perforated steam tray (30 mm layer thickness). After blanching, the larvae were allowed to cool down in open trays in a chamber refrigerator at 4 °C for 16 h. The largest part of blanched larvae were then distributed (ambient transport) to industrial drying facilities for μW and RF drying (30 and 10 min transport time, respectively), while the rest was used for analyses or to be subjected to benchmark drying technologies. Prior to freeze drying, BSF larvae were frozen in an ultra-low temperature freezer (REVCO UxF, Thermo Fisher Scientific, Merelbeke, Belgium) at –80 °C.

Drying technologies

A first part of BSF larvae was dried by application of hybrid microwave technology. Blanched larvae (12 kg) were dried in a 2,450 MHz MEAM Dry S20 system (Houthalen-Helchteren, Belgium), which is a hybrid second generation microwave drier with hot air heat recovery. In perforated baskets placed on a uni-MPB polypropylene belt, a layer of 15 mm larvae was dried for 65 min at 14.4 kW (eight μW units of 1.8 kW) set power. In total, this drying protocol for 12 kg BSF larvae using μW technology consumed 17.3 kWh of μW energy (excluding auxiliary equipment in the oven, e.g. fans and conveyor belt), as measured by the MEAM Dry S20 system. This corresponds with 1.44 kWh/kg (fresh) BSF larvae. The maximum temperature in the μW cavity was 85 °C, as measured using a built-in infrared sensor (Optris CT LT15, Berlin, Germany).

The second dielectric drying technology was based on radio frequency technology at a frequency of 27.12 MHz. This time, 10.6 kg of blanched BSF larvae were dried in a RF insect dryer (DI30, Dymotec, Olen, Belgium), specifically designed for drying insects. The BSF larvae were dried directly on a perforated belt, stacked in a layer of 45 mm in a Teflon frame. Using an auto-adjusted power between 6 and 9 kW, drying time was 90 min. To dry 10.6 kg of BSF larvae via this protocol, 15 kWh of total RF energy (excluding auxiliary equipment) was consumed, as measured by the RF insect dryer itself, which is 1.42 kWh of RF energy per kg (fresh) BSF larvae. Temperature of the insects was followed up during the drying process after inserting two fiber optic temperature sensors (OEM-PLUS, Weidmann

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

Technologies, Dresden, Germany) directly in a larva, at two random places in the drying tray. The maximum processing temperature was 130 °C.

As a comparison, the same batch of BSF larvae was also subjected to (lab scale) freeze and oven drying. For freeze drying, 400 g of frozen blanched BSF larvae were distributed in glass trays in a layer of 30 mm and dried for 72 h in a freeze dryer (Büchi Lyovapor L-200, Flawil, Switzerland) at –55 °C and a pressure of 0.200 mbar. For oven drying, on the other hand, 400 g blanched larvae were dried in a hot air oven (Memmert Universal Oven UF110, Schwabach, Germany). The larvae were placed on glass trays in a layer of 30 mm and dried for 21 h at a temperature of 60 °C, using full fan speed. During freeze and oven drying, energy consumption was measured using a Chacon (type 54355, Waver, Belgium) energy consumption meter. The freeze drying protocol consumed 145 kWh/kg (fresh) BSF larvae (excluding freezing of the larvae prior to the freeze drying process), while the oven drying consumed 9.5 kWh/kg (fresh) BSF larvae.

Preservation of dielectrically dried BSF larvae

After μW and RF drying, samples of 200 g dried insects were packed in plastic bags (VAC090, 20/70 PA/PE; Euralpack, Schoten, Belgium) and sealed (Multivac C200 packaging machine, Wolfertschwenden, Germany). The bags were characterised by a permeability of 50, 150 and 10 cm^3/m^2.day.bar for O_2, CO_2 and N_2, respectively. Next, they were stored at room temperature for 6 months. After 1, 3 and 6 months of storage, samples were analysed for moisture content, water activity and microbiological quality, in order to assess their shelf life stability

Moisture content and water activity

Before and after drying, as well as during preservation, moisture content and water activity (a_w) of the BSF larvae were analysed. Moisture content was determined after oven drying (Memmert Universal Oven UF110) of 5 g pulverised sample at 105 °C for 17 h and calculating the weight loss. Water activity was determined using an a_w meter (LabMaster a_w, Novasina, Lachen, Switzerland), after a_w and temperature (25 °C) were stable for 5 min. Both analyses were performed in threefold (technical replicates) per condition.

Microbiological analyses

The microbiological quality and food safety of the untreated and blanched insects as well as of the dried BSF larvae (by μW, RF, oven and freeze drying) were determined by analysing three samples (technical replicates) per condition for a selection of general microbial counts and pathogen analyses. Also during six months preservation, general microbiology was evaluated.

General microbiological quality was assessed by determining total viable aerobic (TVC) and anaerobic counts (ANTVC), aerobic (AES) and anaerobic (ANES) bacterial endospore counts and counts of fungi (yeasts and moulds), according to the ISO standards compiled by Dijk *et al.* (2015). In short, untreated, blanched and dried samples (whole larvae, three replicates) were pulverised with a flame-sterilised mixer (Bosch CNHR 25, Gerlingen, Germany). Next, 5 g of each sample was added to 45 g peptone physiological salt solution (0.85% (w/v) NaCl; 0.1% (w/v) peptone (Biokar Diagnostics, Beauvais, France)). This primary dilution was homogenised (Bagmixer 400W, Interscience, Saint Nom, France) for 1 min in a stomacher bag. Ten-fold dilutions were then plated as pour plates on Plate Count Agar (PCA, Biokar Diagnostics) and incubated for 72 h at 30 °C for the determination of TVC and ANTVC, and as spread plates on Dichloran Rose-Bengal Chloramphenicol agar (DRBC, Biokar Diagnostics) and incubated for 5 days at 25 °C to determine the amount of fungi. For the enumeration of AES and ANES, the primary 10^{-1} dilution was first given a heat treatment for 10 min at 80 °C before plating a dedicated ten-fold dilution onto PCA and incubation for 48 h at 37 °C. Anaerobic conditions were obtained by incubation in anaerobic jars (VWR, Leuven, Belgium) supplemented with gas generating sachets (Oxoid Anaerogen 2.5 L Sachet, Thermo Fisher Scientific, Merelbeke, Belgium) and anaerobic indicator strips (Oxoid Resazurin Anaerobic Indicator, Thermo Fisher Scientific) to evaluate anaerobic conditions. Microbial counts were calculated as log colony-forming units (cfu) per gram. To be able to compare results from dried insects with blanched insects, the microbial counts were expressed as log cfu/g dry matter for these samples.

Microbiological food safety was assessed for untreated, blanched and dried insects by monitoring the presence of selected bacterial pathogens. Three samples per condition (technical replicates) were provided to an external accredited laboratory (Micro-Smedt, Herentals, Belgium) and investigated for *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, (presumptive) *Bacillus cereus* and *Campylobacter* spp. counts. Additionally, all samples were evaluated for the presence of *Salmonella* spp. in 25 g.

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This publication is licensed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

Statistical analysis

In order to compare intrinsic parameters and microbial counts of the different BSF larvae samples, results were subjected to statistical analyses using JMP Pro 17.0.0 (JMP Statistical Discovery LLC). First, statistical assumptions normality and homogeneity of variance were checked using the Shapiro-Wilk and Levene's test, respectively. Next, differences in intrinsic parameters and in microbial counts of BSF larvae samples before, during and after processing (if applicable) were analysed via One-Way Analysis of Variance (ANOVA) followed by a Tukey HSD post hoc test. In case of unequal variances, a Welch's ANOVA with Games-Howell post hoc test was executed instead. When comparing only two groups (comparison of microbial counts between untreated and blanched BSF larvae samples), an Independent Samples t Test was used. For all statistical analyses, a significance level of 0.05 was adopted.

3 Results and discussion

Moisture content and water activity

Untreated, blanched, and dried BSF larvae were all analysed for their moisture content and water activity. Since a water activity below 0.60 is required for a microbiologically stable product (Madigan *et al.*, 2009), this value was aimed at when drying the larvae.

Figure 1 shows the mean water activity (Figure 1A) and moisture content (Figure 1B) of BSF larvae during the drying process towards μW-, RF-, oven- and freeze-dried products. The high water activity of untreated and blanched larvae (0.99 on average) dropped significantly after each drying method. Still, the target a_w of 0.60 was not always obtained. For example, while a moisture content of 8.66 ± 0.10% was obtained after μW drying (Figure 1B), the a_w of the μW-dried larvae did only decrease to a value of 0.73 ± 0.01. Consequently, these larvae were not dried sufficiently to warrant complete (microbiological) stability. RF-dried, oven-dried and freeze-dried BSF larvae were characterised by a water activity close to or below 0.60 and a moisture content that was even a few percentages lower than that of the μW-dried larvae. From this observation, it can be concluded that a moisture content below 6% was necessary to obtain an $a_w \leq 0.60$ and hence complete microbiological stability for this batch of BSF larvae. It should, however, be nuanced that a_w values below 0.75 do not support the growth of the majority of microorganisms, except for certain osmophilic yeasts and xerophilic moulds (Tapía *et al.*, 2020). Even the μW-dried BSF larvae may therefore be of acceptable quality, depending of the initial microbiological status of the larvae and of the intended application. Compared to oven drying (21 h, 9.5 kWh/kg BSF larvae) and freeze drying (72 h, 145 kWh/kg BSF larvae), it can be concluded that μW and RF drying are much faster (65 and 90 min, respectively), and therefore less energy consuming (1.44 and 1.42 kWh/kg BSF larvae, respectively (see paragraph *Drying technologies*)) processes to decrease the water activity and moisture content of BSF larvae to a microbiologically stable level. It should be nuanced that the energy consumption values reported are difficult to compare for the lab-scale benchmark drying technologies and the industrial-scale dielectric drying. Yet, while these values are rather indicative for this specific research context, the differences in order of magnitude clearly prove the highly increased efficiency of dielectric drying compared to freeze and oven drying.

Further, it should be noticed that the RF-dried BSF larvae had an a_w value of 0.33 ± 0.01, meaning that these insects were slightly overprocessed, as compared to the initial goal. This indicates that slightly more energy was consumed than essentially required to obtain an a_w of 0.60 and thus a microbiologically stable product. Yet, it has been observed that after a certain loss of water, water activity suddenly drops rapidly. As a consequence, only a minimal margin exists between the desired a_w of 0.60 and the risk of overprocessing. A practical implication for efficiently implementing RF drying in a large-scale industrial process is therefore to meticulously control the drying parameters, which requires proper and tailored optimisation. On the other hand, also other deteriorating processes such as lipid oxidation and browning reactions are function of the water activity in a product and can be minimised at specified a_w ranges. For example, lipid oxidation in certain low-moisture foods was reported to be the lowest at a_w values between 0.20 and 0.30 (Vu *et al.*, 2020). For certain applications, also for dried insects, it may therefore be desired to obtain a final a_w lower than 0.60 to avoid other unfavourable processes as well, at the cost of additional processing time.

Microbiological quality and safety

To gain a general understanding of the microbiological quality of the BSF larvae before, during and after processing them into dried insects, selected microbial counts were performed. Figure 2 illustrates aerobic (Figure 2A) and anaerobic (Figure 2B) total viable counts. Consistent with previous observations (Campbell *et al.*, 2020; Wynants *et al.*, 2019), untreated (raw) BSF larvae contained high amounts of microorganisms, with

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

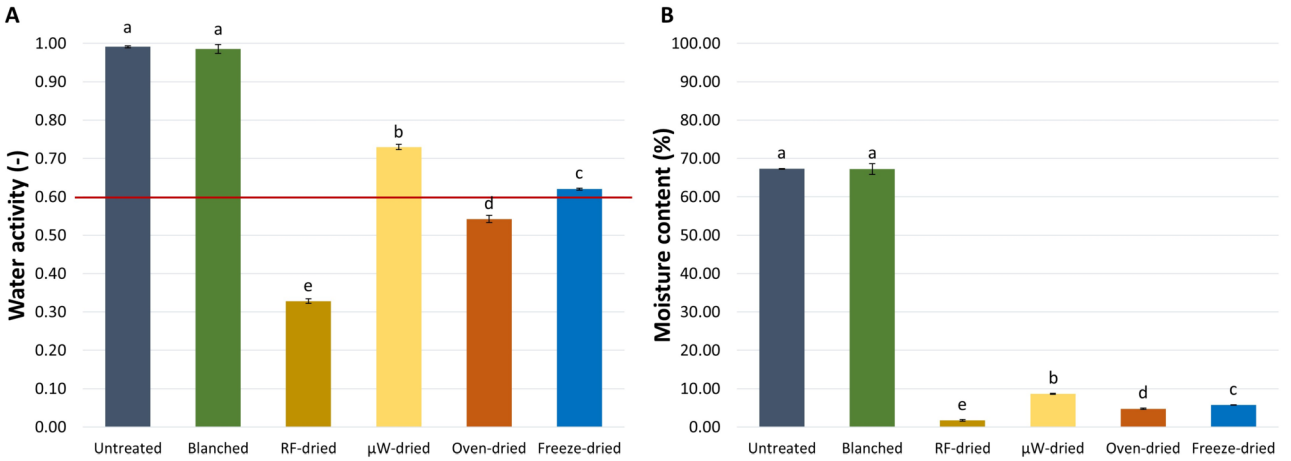

FIGURE 1 Water activity (A) and moisture content (B) of black soldier fly larvae before and after drying with different drying technologies. Results are mean values (n = 3) ± standard deviations. [abcde] For each figure, mean values (bars) with the same letter are not significantly different ($P > 0.05$). The red line indicates the target a_w value of 0.60 for the drying technologies.

mean TVC and ANTVC of 8.6 and 8.0 log cfu/g, respectively. Following the blanching step to kill the BSF larvae, a partial reduction of TVC and ANTVC to 6.0 and 4.1 log cfu/g, respectively, was noted. Again, such reduction was expected, as this was also observed in previous research (Larouche *et al.*, 2019). Since the main aim of drying the insects is stabilisation rather than decontamination (especially for nonthermal drying technologies such as freeze drying), this reduction in microbial counts, although limited, is beneficial for the general microbiological quality of the end products. Especially for the anaerobic counts, the limited reduction can be attributed to the survival of heat-resistant bacterial endospores. Indeed, as displayed in Figure 3, the aerobic (Figure 3A) and anaerobic (Figure 3B) bacterial endospore counts were not or only minimally impacted by the blanching treatment.

Due to a concentration effect of microorganisms after drying, a meaningful comparison between dried and non-dried larvae is only possible when comparing the microbial counts expressed per gram dry matter (DM). Accordingly, counts for blanched larvae were additionally presented as log cfu/g DM. Following the drying process, counts (per g DM) for the dried larvae remained relatively consistent, with a few noteworthy exceptions. For example, the TVC (Figure 2A) of RF-dried BSF larvae was further reduced to 5.3 ± 0.1 in comparison to blanched larvae (per g DM), while this was not observed for µW-dried, oven-dried and freeze-dried larvae. A similar additional microbial reduction was also noted for the AES after µW and RF drying (Figure 3A) and for ANES after RF drying. Those results suggest an additional decontaminating effect associated with the dielectric drying treatments, which also impacts bacterial endospores. Since this effect was particularly observed for RF drying, it was most probably correlated with the high temperature reached during RF drying (130 °C). In contrast, the other drying technologies, including µW drying, occurred at (much) lower temperatures (see paragraph *Drying technologies*) and did not result in such a prominent additional microbial inactivation. Given the variation in temperatures reached during drying, it remains inconclusive whether the additional microbial reductions observed following RF drying were predominantly caused by the high temperature or rather by potential (though disputed) nonthermal effects of dielectric drying technologies (Espitia *et al.*, 2023).

For ANTVC, instead of observing additional reduction compared to blanched larvae (per g DM), a slight increase was noted for µW- and particularly for oven-dried larvae. A plausible hypothesis is that during the storage period between blanching and (oven) drying, certain (facultative) anaerobic microorganisms had the opportunity to proliferate. Despite refrigerated storage of the BSF larvae, they passed the temperature zone which can facilitate microbial growth (5 to 60 °C, the so-called danger zone (Kapetanakou *et al.*, 2022)) at least twice: during the cooling phase after blanching and during the heating phase in the oven. Furthermore, it may be possible that psychrotrophic organisms were present, capable of growing at low temperatures, and/or that certain ((facultative) anaerobic) endospores germinated (e.g. illustrated in Figure 3B for RF-dried and freeze-dried larvae). Among the drying methods compared, passage through the microbiological danger zone was longest for larvae destined for oven-dried. Indeed, larvae for both dielectric drying methods experienced

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

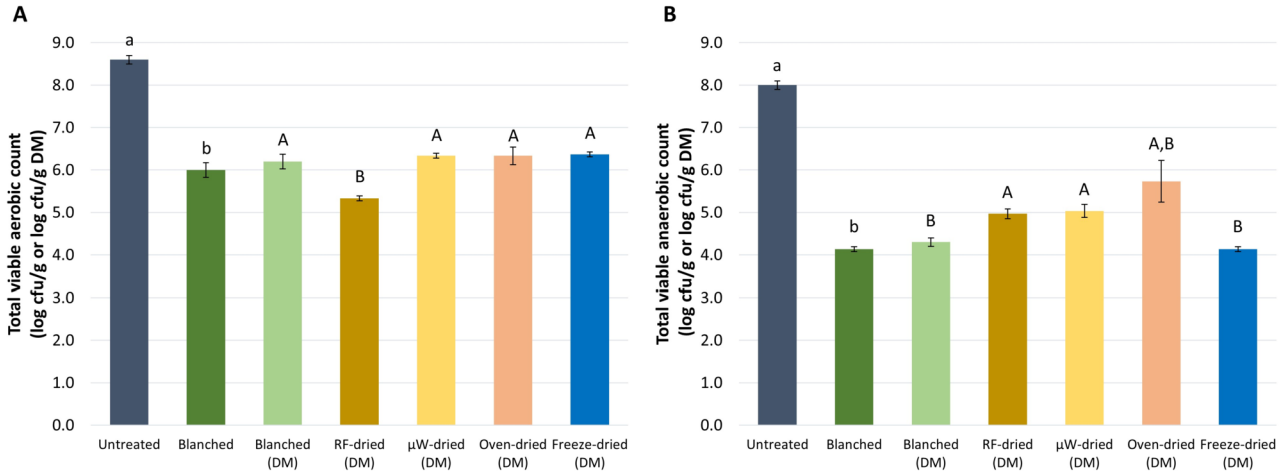

FIGURE 2 (A) Total viable aerobic (TVC) and (B) anaerobic (ANTVC) counts of black soldier larvae before and after drying with different drying technologies. Results are mean values (n = 3) ± standard deviations and expressed as log cfu/g for untreated and blanched BSF larvae and as log cfu/g dry matter (DM) for blanched and dried larvae. [ab]For each figure, mean values expressed on fresh weight with the same small letter are not significantly different ($P > 0.05$). [AB]For each figure, mean values expressed per g DM with the same capital letter are not significantly different ($P > 0.05$).

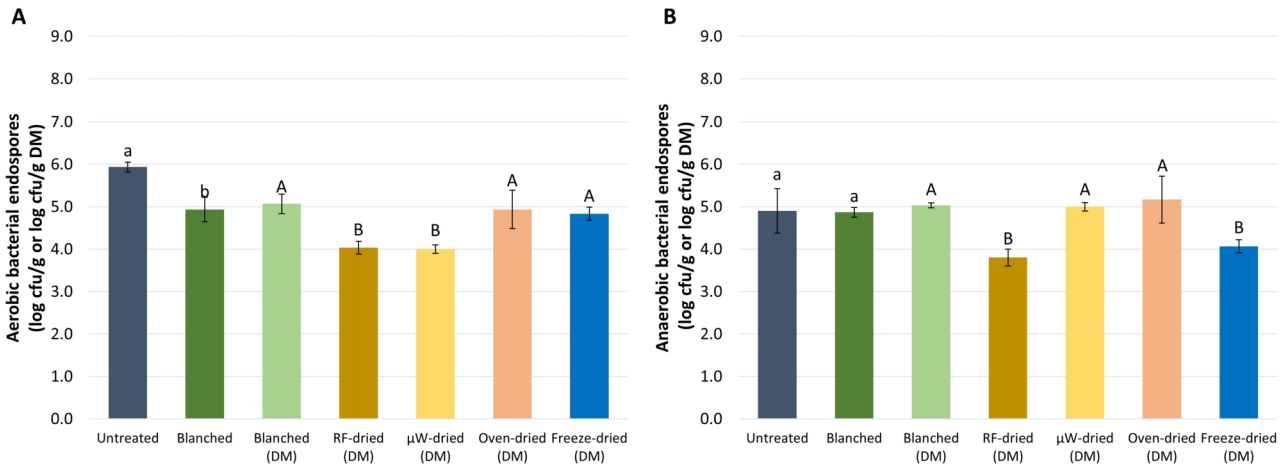

FIGURE 3 (A) Aerobic (AES) and (B) anaerobic (ANES) bacterial endospore counts of black soldier larvae before and after drying with different drying technologies. Results are mean values (n = 3) ± standard deviations and expressed as log cfu/g for untreated and blanched BSF larvae and as log cfu/g dry matter (DM) for blanched and dried larvae. [ab]For each figure, mean values expressed on fresh weight with the same small letter are not significantly different ($P > 0.05$). [AB]For each figure, mean values expressed per g DM with the same capital letter are not significantly different ($P > 0.05$).

only a brief heating time in the µW and RF ovens, while larvae prior to freeze drying were frozen immediately after cooling. Consequently, this hypothesis may explain the most substantial (but not significant) increase in ANTVC for oven-dried BSF larvae. To mitigate the risk of outgrowth of microorganisms between decontamination and drying, it is advised to minimise storage time between blanching and drying. Ideally, insects can be dried immediately after the blanching treatment. Further, it is crucial to consider that the slower heating (from the surface) in a hot air oven, compared to quick volumetric heating during dielectric drying, may pose a challenge in maintaining the microbiological quality after decontamination via blanching. Finally, the large number of fungi present in untreated BSF larvae (6.2 ± 0.2 log cfu/g) were significantly reduced to below the detection limit (2.0 log cfu/g) through blanching. After drying into all end products, fungal counts remained below this detection limit.

While microbial counts offer insights into the general microbiological quality and dynamics of insects during the drying process, they do not directly inform about potential food safety risks. To address this, untreated, blanched and dried BSF larvae were subjected to analyses for a selection of relevant foodborne pathogens. For all samples of untreated, blanched, µW-dried, RF-dried, oven-dried and freeze-dried BSF larvae, *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocy-*

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms
of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

togenes, *Campylobacter* spp. and *Salmonella* spp. were not detected. (Presumptive) *Bacillus cereus*, however, were encountered in blanched, oven-dried and freeze-dried samples. However, all values detected were at most 2.8 log cfu/g (in a sample of oven-dried BSF larvae). *B. cereus* was already associated with insects for food and feed in previous research, both in vegetative and endospore form (Fasolato *et al.*, 2018; Vandeweyer *et al.*, 2020, 2021). Notably in this study, *B. cereus* was not detected in any of the dielectrically dried samples anymore, which suggests an effective elimination of the pathogen when present in low amounts. While the presence of this pathogen requires caution regarding food safety of the insects, observed values were below the generally accepted food safety risk threshold of 5.0 log cfu/g (3.0 log cfu/g in some cases) for *B. cereus* (EFSA Panel on Biological Hazards, 2016). Strict monitoring and proper storage of the insects after drying should therefore be sufficient to warrant microbiological safety of the dried BSF larvae in this case. Since *B. cereus* may remain a concern for insects as food and feed, it would be interesting to develop fast diagnostic tools (e.g. RNA-based qPCR) to evaluate the survival of this pathogen in processed insect products.

Shelf life stability

Following the drying process, μW- and RF-dried BSF larvae were packed and preserved for six months at ambient temperature. Throughout the storage period (at one, three and six months), the stability of the dielectrically dried larvae was monitored by investigating changes in water activity, moisture content and (general) microbial counts.

Table 1 shows the water activity and moisture content of the μW- and RF-dried BSF larvae during storage. For RF-dried BSF larvae, the a_w rose significantly during storage, but in absolute terms only marginally. For μW-dried BSF larvae, the a_w even showed a significant decrease. At the same time, a significant increase in moisture content (Table 1) was observed for dried BSF larvae from both technologies, although this increase did not continue over the complete six month period. The slight changes in moisture content and a_w can be the result of migration of water vapour from the storage environment through the packaging material and vice versa and the achievement of a dynamic equilibrium due to redistribution of moisture in the package and the product. For this study, however, the limited uptake of water was not sufficient to considerably change the a_w and thus the (microbiological) stability of the dielectrically dried larvae. As mentioned earlier, the a_w values of the dielectrically dried BSF larvae do not allow substantial microbial proliferation. A water activity below 0.60 for RF-dried insects correlates with a complete microbiologically stable product, while an a_w below 0.75 for μW-dried insects only allows specific extreme (osmophilic or xerophilic) microorganisms to grow (Tapía *et al.*, 2020).

The stability of dried BSF larvae as determined by physiochemical parameters corresponds in part with their microbial counts, shown in Table 1. For RF-dried BSF larvae, the TVC, AES and amount of fungi did not change significantly during the six months of storage. However, the two anaerobic counts differed to a larger extent over time and showed two major declines (after one month for ANTVC and at three months for ANES). This may be explained by an inhomogeneous distribution of the anaerobic microorganisms in the samples. For the μW-dried larvae, TVC, ANTVC and AES remained (relatively) stable during the 6 months of storage. ANES, on the other hand decreased significantly after one and three months of storage and increased again after six months. Again, an inhomogeneous distribution of the anaerobic endospores may have caused this aberration. The amount of fungi was observed to slightly increase after 6 months of storage, potentially caused by proliferation of certain osmophilic or xerophilic fungi. However, the increase is not significant, as a result of the large standard deviation. A similar experiment was executed by Kamau *et al.* (2020) regarding preservation of BSF prepupae meal. After a six-month storage period at ambient temperature, they observed increases of approximately 1.5 log cfu/g for TVC and even 3.0 log cfu/g for fungi. Additionally, they concluded that the packaging concept and storage conditions greatly influenced the microbiological quality during storage. For our study, comparing the microbial counts immediately after drying with those after six months of storage, it can be concluded that the general microbiological quality remained consistent. From a microbiological point of view, a shelf life of six months can therefore be adopted for the μW- and RF-dried BSF larvae, provided that they are properly packed (in this case in sealed plastic bags). It is, however, advised to further monitor the dynamics of the different microbial counts in further research. Ideally, the μW-dried insects should be dried to an a_w below 0.60 to completely exclude microbiological changes.

Downloaded from Brill.com 10/31/2024 05:07:22PM
Wageningen University & Research Library
This work is published and licensed under the terms of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

TABLE 1 Moisture content, water activity and microbial counts of dielectrically dried black soldier fly larvae during six-month storage. Data are the mean of three measurements (n = 3) ± standard deviation

Drying method	Storage time (months)	Water activity (–)	Moisture content (%)	Microbial counts (log cfu/g DM)[1,2]				
				Total viable count	Anaerobic total viable count	Aerobic bacterial endospores	Anaerobic bacterial endospores	Fungi
Radio frequency	0	0.33 ± 0.01^{c}	1.77 ± 0.22^{c}	5.3 ± 0.1^{a}	5.0 ± 0.1^{a}	4.0 ± 0.2^{a}	3.8 ± 0.2^{a}	< 2.1 ± 0.1^{a}
	1	0.34 ± 0.00^{bc}	3.39 ± 0.03^{b}	5.1 ± 0.3^{a}	3.3 ± 0.1^{c}	3.7 ± 0.1^{a}	3.2 ± 0.1^{b}	< 2.0 ± 0.0^{a}
	3	0.34 ± 0.00^{b}	3.75 ± 0.02^{a}	5.2 ± 0.1^{a}	4.6 ± 0.1^{b}	3.7 ± 0.1^{a}	2.1 ± 0.2^{c}	< 2.0 ± 0.1^{a}
	6	0.38 ± 0.00^{a}	3.52 ± 0.05^{b}	5.1 ± 0.2^{a}	4.6 ± 0.1^{b}	3.9 ± 0.2^{a}	4.2 ± 0.4^{a}	< 2.0 ± 0.1^{a}
Microwave	0	0.73 ± 0.01^{a}	8.66 ± 0.10^{c}	6.3 ± 0.1^{a}	5.0 ± 0.2^{a}	4.0 ± 0.1^{c}	5.0 ± 0.1^{a}	< 2.3 ± 0.2^{a}
	1	0.70 ± 0.00^{b}	9.72 ± 0.04^{a}	5.1 ± 0.1^{b}	3.9 ± 0.6^{ab}	5.1 ± 0.1^{a}	3.4 ± 0.2^{c}	< 2.1 ± 0.2^{a}
	3	0.68 ± 0.00^{c}	9.21 ± 0.39^{ab}	6.2 ± 0.2^{a}	4.2 ± 0.9^{ab}	4.4 ± 0.1^{b}	< 2.0 ± 0.3^{d}	< 2.0 ± 0.1^{a}
	6	0.68 ± 0.00^{c}	8.94 ± 0.05^{bc}	6.2 ± 0.1^{a}	3.8 ± 0.1^{bc}	4.3 ± 0.1^{b}	4.0 ± 0.1^{b}	< 3.1 ± 1.0^{a}

1 Mean microbial counts are expressed as log colony-forming units per gram dry matter, in order to correct for differences in drying efficiency.

2 For mean values with '<', at least one of the three measurements was below the detection limit.

^{abcd} Mean values per drying method and per column with the same superscript are not significantly different ($P > 0.05$).

4 Conclusions

This study explored the efficacy of two dielectric drying technologies, microwave and radio frequency drying, in processing black soldier fly larvae for the insect industry. A comparison with conventional methods, oven drying and freeze drying, was included. From moisture content and water activity analyses, it was confirmed that dielectric drying can effectively reduce a_w and moisture content to values that indicate microbiological stability, but in a faster, less energy consuming and thus more efficient way compared to the benchmarks. Microbiological assessments confirmed the effectiveness of blanching in reducing (vegetative) microbial counts, but additionally revealed microbial reductions after dielectric drying. Especially for RF-dried BSF larvae, even pathogens like *Bacillus cereus* and bacterial endospores were affected. It should, however, be mentioned that outgrowth of microorganisms between decontamination and drying should be maximally avoided by reducing the storage time between processing steps. Finally, a shelf life stability evaluation demonstrated minimal changes in moisture content, a_w and microbiological parameters of dielectrically dried BSF larvae over six months, confirming the stability of the product. In this view, both μW and RF drying technologies, although different in application, are well-suited to be implemented in the insect industry and are valuable alternative drying methods compared to oven and freeze drying. A well-controlled drying process is, however, crucial to obtain the desired end product. Further research can contribute to the optimisation of these methods in specific industrial contexts as well as for different insect species.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 861976 (project SUSINCHAIN). DV is financed by the SUSINCHAIN project as well as by the Research Foundation Flanders (FWO) via the SBO project ENTOBIOTA (S008519N). DL is funded by the SUSINCHAIN project. We would like to thank the Insect Research Centre of Inagro (Rumbeke-Beitem, Belgium) for providing the black soldier fly larvae.

Conflict of interest

The authors have no conflicts of interest to declare.

References

Awuah, G.B., Ramaswamy, H.S., Economides, A. and Mallikarjunan, K., 2005. Inactivation of *Escherichia coli* K-12 and *Listeria innocua* in milk using radio frequency (RF) heat-

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

ing. Innovative Food Science & Emerging Technologies 6: 396-402. https://doi.org/10.1016/J.IFSET.2005.06.002

Bawa, M., Songsermpong, S., Kaewtapee, C. and Chanput, W., 2020. Effects of microwave and hot air oven drying on the nutritional, microbiological load, and color parameters of the house crickets (*Acheta domesticus*). Journal of Food Processing and Preservation 44: e14407. https://doi.org/10.1111/JFPP.14407

Campbell, M., Ortuño, J., Stratakos, A.C., Linton, M., Corcionivoschi, N., Elliot, 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. https://doi.org/10.3390/ANI10040682

De Smet, J., Wynants, E., Cos, P. and Van Campenhout, L., 2018. Microbial community dynamics during rearing of black soldier fly larvae (*Hermetia illucens*) and impact on exploitation potential. Applied and Environmental Microbiology 84: e02722-17. https://doi.org/10.1128/AEM.02722-17

Dijk, R., van den Berg, D., Beumer, R., de Boer, E., Dijkstra, A., Mout, L., Stegeman, H., Uyttendaele, M. and in 't Veld, S., 2015. Microbiologie van voedingsmiddelen: Methoden, principes en criteria. 5th ed. MYbusinessmedia, Capelle aan den IJssel, The Netherlands.

Dubey, M.K., Janowiak, J., Mack, R., Elder, P. and Hoover, K., 2016. Comparative study of radio-frequency and microwave heating for phytosanitary treatment of wood. European Journal of Wood and Wood Products 74: 491-500. https://doi.org/10.1007/s00107-016-1025-2

EFSA Panel on Biological Hazards, 2016. Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal 14: 93. https://doi.org/10.2903/j.efsa.2016.4524

Espitia, J., Verheyen, D., Kozak, D.S. and Van Impe, J.F.M., 2023. Influence of microbial cell morphology and composition on radio frequency heating of simple media at different frequencies. Scientific Reports 13: 1-10. https://doi.org/10.1038/s41598-023-35705-4

Fasolato, L., Cardazzo, B., Carraro, L., Fontana, F., Novelli, E. and Balzan, S., 2018. Edible processed insects from e-commerce: food safety with a focus on the *Bacillus cereus* group. Food Microbiology 76: 296-303. https://doi.org/10.1016/j.fm.2018.06.008

Hernández-Álvarez, A.J., Mondor, M., Piña-Domínguez, I.A., Sánchez-Velázquez, O.A. and Melgar Lalanne, G., 2021. Drying technologies for edible insects and their derived ingredients. Drying Technology 39: 1991-2009. https://doi.org/10.1080/07373937.2021.1915796

Huang, C., Feng, W., Xiong, J., Wang, T., Wang, W., Wang, C. and Yang, F., 2019. Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (*Hermetia illucens* L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties. European Food Research and Technology 245: 11-21. https://doi.org/10.1007/s00217-018-3136-y

IPIFF, 2020. Edible insects on the European market. Available at: https://ipiff.org/10-06-2020-ipiff-edible-insects-market-factsheet/

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Ekesi, S., Ekesi, S., Nakimbugwe, D. and Fiaboe, K.K.M., 2020. Changes in chemical and microbiological quality of semi-processed black soldier fly (*Hermetia illucens* L.) larval meal during storage. Journal of Insects as Food and Feed 6: 417-428. https://doi.org/10.3920/JIFF2019.0043

Kapetanakou, A.E., Athanaseli, K.G., Kolostoumpi, M., Passiou, K. and Skandamis, P.N., 2022. Raw vs frozen pork "gyros": predicting simultaneous growth of pathogenic and spoilage microorganisms under commercially occurring roasting scenarios. Food Control 137: 108900. https://doi.org/10.1016/J.FOODCONT.2022.108900

Kim, S.Y., Shin, S.J., Song, C.H., Jo, E.K., Kim, H.J. and Park, J.K., 2009. Destruction of Bacillus licheniformis spores by microwave irradiation. Journal of Applied Microbiology 106: 877-885. https://doi.org/10.1111/J.1365-2672.2008.04056.X

Kusuma, H.S., Izzah, D.N. and Linggajati, I.W.L., 2023. Microwave-assisted drying of *Ocimum sanctum* leaves: analysis of moisture content, drying kinetic model, and techno-economics. Applied Food Research 3: 100337. https://doi.org/10.1016/J.AFRES.2023.100337

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. https://doi.org/10.3390/ANI9040182

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. https://doi.org/10.1016/j.foodchem.2018.02.006

Lier, I.S., Ong, M.K., Leong, S.Y. and Teoh, C.Y., 2024. Comparison of microwave and vacuum oven drying on the amino acid composition and antioxidant properties of black soldier fly (*Hermetia illucens*) prepupae. LWT 196: 115870. https://doi.org/10.1016/J.LWT.2024.115870

Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P., 2009. Brock biology of microorganisms. 12th ed. Pearson/Benjamin Cummings, San Francisco, CA, USA.

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Wageningen University & Research
This is an open access article distributed under the terms of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/

Meticulous Research, 2024. Europe black soldier fly market by product (protein meals, whole dried larvae, biofertilizers {frass}, larvae oil, others {cocoons, pupa}), by application (animal feed, agriculture, pet food, others), and country – forecast to 2033. Available at: https://www.meticulousresearch.com/product/europe-black-soldier-fly-market-5562

Parniakov, O., Mikhrovska, M., Wiktor, A., Alles, M., Ristic, D., Bogusz, R., Nowacka, M., Devahastin, S., Mujumdar, A., Heinz, V. and Smetana, S., 2022. Insect processing for food and feed: a review of drying methods. Drying Technology 40: 1500-1513. https://doi.org/10.1080/07373937.2021.1962905

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. https://doi.org/10.3920/JIFF2021.0002

Son, J., Park, S.H., Jung, H.J., You, S.J. and Kim, B.G., 2023. Effects of drying methods and blanching on nutrient utilization in black soldier fly larva meals based on in vitro assays for pigs. Animals 13: 858. https://doi.org/10.3390/ANI13050858

Stratta, L., Capozzi, L.C., Franzino, S. and Pisano, R., 2020. Economic analysis of a fFreeze-drying cycle. Processes 8: 1399. https://doi.org/10.3390/PR8111399

Tapía, M.S., Alzamora, S.M. and Chirife, J., 2020. Effects of water activity (a_w) on microbial stability as a hurdle in food preservation. In: Barbosa-Cánovas, G.V., Fontana Jr., A.J., Schmidt, S.J. and Labuza, T.P. (eds.) Water activity in foods: fundamentals and applications. John Wiley & Sons, Ltd., pp. 323-355. https://doi.org/10.1002/9781118765982.CH14

Vandeweyer, D., De Smet, J., Van Looveren, N. and Van Campenhout, L., 2021. Biological contaminants in insects as food and feed. Journal of Insects as Food and Feed 7: 807-822. https://doi.org/10.3920/JIFF2020.0060

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. https://doi.org/10.1016/j.foodcont.2016.07.011

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. https://doi.org/10.1038/s43016-020-0120-z

Vu, T.P., He, L., McClements, D.J. and Decker, E.A., 2020. Effects of water activity, sugars, and proteins on lipid oxidative stability of low moisture model crackers. Food Research International 130: 108844. https://doi.org/10.1016/J.FOODRES.2019.108844

Wang, Y., Li, Y., Wang, S., Zhang, L., Gao, M. and Tang, J., 2011. Review of dielectric drying of foods and agricultural products. International Journal of Agricultural and Biological Engineering 4: 1-19. https://doi.org/10.3965/J.ISSN.1934-6344.20

Wray, D. and Ramaswamy, H.S., 2015. Novel concepts in microwave drying of foods. Drying Technology 33: 769-783. https://doi.org/10.1080/07373937.2014.985793

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. https://doi.org/10.1016/j.ifset.2017.06.004

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. https://doi.org/10.1007/S00248-018-1286-X

Downloaded from Brill.com 10/31/2024 05:07:22PM
via Open Access. This is an open access article distributed under the terms of the CC BY 4.0 license.
https://creativecommons.org/licenses/by/4.0/