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MICROBIOLOGICAL AND NUTRITIONAL ASPECTS OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE AFTER BIOCONVERSION OF DIFFERENT FOOD WASTES

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ABSTRACT

Black soldier fly larvae (BSFL), *Hermetia illucens*, have shown remarkable potential for food waste bioconversion, contributing to sustainability and waste management efforts. This study evaluated the effects of different food waste compositions (three types of diets: egg pasta in milk, rice with peas, and couscous mixture with egg, spinach, and carrot peels) and their microbial quality (fresh vs. spoiled) on BSFL bioconversion efficiency, microbiology, and nutritional value. Microbiological analyzes of the larvae were performed using the plate dilution method and MALDI-TOF mass spectrometer. All test variants spotted successful bioconversion. However, bioconversion efficiency (BE) and waste reduction rate (WR) were highest for couscous-based diets, with fresh couscous yielding a highest BE of 27.41% and WR of 81.34%. Nutritionally, larvae reared on mixture of couscous, egg, spinach, and carrot peels displayed the highest protein content (up to 35.5%). Microbial analysis indicated increased microbial counts, particularly Enterobacteriaceae and spore-forming bacteria, in larvae fed spoiled diets. Potentially pathogenic microorganisms such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Morganella morganii* were detected, underscoring the need for microbial safety measures in BSFL-based applications. This study highlights the critical role of feed composition and quality in determining the efficiency and safety of BSFL bioconversion, providing valuable insights for optimizing their use in sustainable food waste management and as a resource in feed or food production.

Keywords: Biodegradation, BSFL, Food waste, Food safety, Microbial counts, Protein content, Fat content

INTRODUCTION

Food waste (FW) is a substantial component of organic waste generated at a growing rate around the world. The rise in living standards and the human population has led to a growing demand for food. As a result, FW is increasing at an alarming rate, raising several socioeconomic concerns **(Kim *et al.*, 2021; Papargyropoulou *et al.*, 2014)**. The growing global issue of FW has a significant influence on environmental pollution and necessitates the application of effective management techniques **(Mishra *et al.*, 2024)**. According to estimates from the Food and Agriculture Organization of the United Nations (FAO), 1.3 billion metric tons, or one-third, of the world's food supply is wasted annually **(Flanagan *et al.*, 2019)**. In the high-income countries, household FW accounts for over half of total FW, making a major contribution to the problem **(Stancu *et al.*, 2016)**.

Food disposal includes waste management activities such as composting and anaerobic digestion, co-generation, incineration, and landfilling, as well as alternative pathways such as bio-energy production and disposal via sewage, plough-in/not harvested, and any discards **(Scherhaufer *et al.*, 2018)**. Moreover, large amounts of FW are frequently discarded with other municipal solid waste in landfills, which provide a substantial challenge.

due to their putrescent properties and the production of greenhouse gas emissions **(Zhang, 2024)**. This problem concerns practically all countries in the world. However, most of the generated food waste may be managed with benefits **(Moustakas *et al.*, 2019)**. The search for new solutions to their valorization is a viable and potentially advantageous alternative in a circular economy context **(Salomone *et al.*, 2017)**, in which waste does not exist and organic waste should not be reduced because it is still a source of many other potential uses. One of the attractive FW treatment options is the bioconversion method **(Kiran *et al.*, 2014)**. Based on bioconversion principles, waste can be treated by utilizing live organisms such as insects. The use of insect larvae for waste processing is one example of an alternative approach that enables waste management, with additional benefits **(Čičková *et al.*, 2015)**. Insect bioconversion of organic waste streams effectively recycles nutrients and energy that would otherwise be misused. It additionally promotes the use of a more sustainable protein source, which can help in food security **(Huis *et al.*, 2013)**.

An example of a useful insect is the larvae of *Hermetia illucens* (black soldier fly larvae, BSFL), a native of America, which can today be found in many countries with year-round warm, tropical or sub-tropical climates **(Dortmans *et al.*, 2017)**. The main advantage of BSF, compared to house flies, for example, is their behavior. The BSF avoids approaching people or animals and prefers to rest on plants **(Sheppard *et al.*, 1994)**. Adult flies do not bite or sting humans, they merely consume water, and do not transmit any specific diseases **(Čičková *et al.*, 2015)**. BSFL are distinguished by rapid biomass growth, the capacity to convert a wide range of organic substrates into high-quality protein and fat biomass **(Gligorescu *et al.*, 2019)** and leaving behind a compost-like residue with properties comparable to immature compost **(Zurbrűgg *et al.*, 2018)**, which becomes useful fertilizer **(Moustakas *et al.*, 2019)**. Valuable proteins and lipids make them suitable for use as feedstock in the swine, poultry, and aquaculture industries **(Magalhães *et al.*, 2017)**. They are known as workhorse when it comes to valorizing biodegradable organic waste **(Clariza Samayoa *et al.*, 2016)**. Moreover, they provide potential chemical precursors for biodiesel production **(Barragan-Fonseca *et al.*, 2017)**. For the above reasons, BSFL are undoubtedly highly suitable for waste management **(Lalander *et al.*, 2015)**. Their inclusion into organic food waste recycling systems has proven to be a cost-effective and sustainable process **(Rehman *et al.*, 2023)**.

Characteristics such as rapid growth, broad degrading capabilities, and lack of ability to spread human diseases make it perfect for industrial application **(Wang & Shelomi, 2017)**. For commercial use in human foods, larvae could potentially be milled and converted into a textured protein with a strong flavor. They are of far greater value than other insects because of their ability to convert waste into food, completing nutrient cycles, and reducing pollution. But this all-encompassing advantage is also their greatest disadvantage because, in addition to the existing taboos around insect intake, there are societal stigmas and legal restrictions against consuming organisms that consume trash **(Wang & Shelomi, 2017)**. Even though the potential role of BSFL as a sustainable protein source for the rapidly growing world population has been debated increasingly **(Kouřimská *et al.*, 2016)**, *H. illucens* has received less attention as a potential food source for humans. Moreover, there is the unanswered question of their safety when consumed as food. Although there are not many insects that seem to be dangerous to consume **(Blum, 1994)**, allergies excluding **(Barre *et al.*, 2014)**, one should not disregard problems like insect microbiological contamination **(EFSA, 2015)**. The compost of kitchen waste can lead to an increase in relative abundance of various potentially pathogenic or mycotoxigenic microorganisms **(Shi *et al.*, 2024)**. If the larvae are kept on contaminated substrate for an extended period, they may become contaminated with the bacteria themselves **(Erickson *et al.*, 2004; Gold *et al.*, 2018)**. As a result, one of the most serious biological risks linked with edible insects is the presence of pathogenic and toxigenic microorganisms **(Garofalo *et al.*, 2019)**. Therefore, it is highly important to study microbiomes of BSFL raised on a specific diet under specific conditions **(Wu *et al.*, 2020)**.

The aim of this study was to evaluate the influence of feed composition and microbiological quality on the bioconversion performance, microbiology, and nutritional characteristics of *H. illucens* larvae reared on different types of food waste. By comparing fresh and spoiled diets, the research seeks to identify key factors affecting the safety and nutritional value of the resulting larval biomass, with implications for optimizing their use in sustainable food waste management and as a potential resource in feed or food production.

MATERIAL AND METHODS

The experiment consisted of rearing BSFL on different types of kitchen waste with the aim of organic food waste bioconversion into valuable and potentially usable biomass and in subsequent analysis of the feed composition effect on the overall larval microbiology and selected nutritional characteristics. Approximately 10-day-old BSFLs obtained from ecol Trade s.r.o. (Nitra, Slovakia) were utilized. Prior to our experiment, they were reared under standard conditions and fed standard chicken feed (a mixture of cereals, proteins, and fats).

Experimental rearing conditions and sample preparation

Larvae breeding took place in rooms without access to direct light, at a temperature of 27 ± 1 °C and a relative humidity of 55±10%. Larvae were kept in plastic closable containers with a built-in metal insect net, which was used for ventilation. The air flow of the room was provided by stand fans.

BSFL were fed with six different diet variants (**Table 1**), each of four replicates. Every replicate used 100 g of BSFL at the start of the experiment (totally 400 g of BSFL per variant). Each diet was prepared in two versions: fresh and spoiled, to evaluate differences between larvae fed diets of varying microbiological quality. At the start of the experiment, 1140 g of feed was distributed to each experimental container. The breeding period lasted 8 days, with the larvae starving for the final 1-2 days. On the eighth day, BSFL were separated from the residue (frass) and frozen at -80 °C.

Table 1 Variants and feeding design of the experiment

Variant		**Diet formulation (proportion of feed components)**
P-F	pasta-fresh	egg pasta cooked in whole milk (1:2)
P-S	pasta-spoiled	
C-F	couscous-fresh	hydrated couscous with boiled egg, fresh spinach, and carrot peels (30:5:1:2)
C-S	couscous-spoiled	
R-F	rice-fresh	boiled rice with thawed peas (7:1)
R-S	rice-spoiled	

Moisture content and water activity of feed

Moisture content (MC) and water activity (a_w) were assessed in each feed variant. The weight difference of 5 g of the initial sample before and after 4 hours of drying in an oven at 105 °C to a constant weight was used to calculate MC. The LabMaster-aw device (Novasina, Lachen, Switzerland) was used to determine a_w.

Biometric and nutritional evaluations

Weigh

To measure larval weight, 100 individuals of representative size from each BSFL group (larvae before and larvae after experimental feeding) were selected by tweezers. Weighing was performed using laboratory scales (PCB 3000-2, Kern, Frankfurt, Germany) and mean values for each variant were calculated from the values obtained across the 4 experimental replicates.

Dry matter

Gravimetric analysis of the dry matter (DM) of diet samples, residues (frass), input larvae, and output larvae was performed in accordance with **Ruiz (2001)**. In an oven set at 105 ± 5 °C, samples were dried to a constant mass. The dry matter was calculated by comparing the mass of the samples before and after the drying procedure.

Protein content

Totally, 100 g of each BSFL sample were lyophilized (Laboratory freeze-dryer Christ LyoCube Alpha 1-4 LSC plus, Osterode am Harz, Germany). Subsequently, 10 g of the larvae were homogenized using a blender. To 1 g of homogenate, 10 mL of demineralized water was added. Protein extraction was performed under constant stirring (Rocker shaker BioSan MR-12, Riga, Latvia) for 60 min at 20-22 °C. The extract was filtered through filter paper no. 390 (Munktell and Filtrak Gmbh, Bärenstein, Germany) and used to determine protein content according to the **Bradford (1976)**. The protein concentration was calculated using a calibration curve. The standard protein solution was prepared using bovine serum albumin (SERVA Electrophoresis GmbH, Heidelberg, Germany) at a concentration of 1 mg/mL in deionized water. The calibration curve expresses the dependence of the absorbance at 595 nm on the protein concentration.

Fat content

An Ancom XT15 Fat Extractor (Ancom, Macedon, NY, USA) was used to detect the amount of fat in the larval samples. Following the manufacturer's instructions, a total of 1.5 g of sample was weighed (W1) into a special filter bag XT4 (Ancom, Macedon, NY, USA) and dried for 3 h at 105 °C to remove moisture before extraction. The samples were then placed in a desiccant bag and left for 15 minutes, reweighed (W2) and then extracted with petroleum ether for 60 minutes at 90 °C. After this procedure, the samples were removed, oven-dried for 30 minutes at 105 °C, placed in a desiccant bag and weighed again (W3). The fat content (FC) was calculated using the formula:

$$FC\ (\%) = \frac{W2 - W3}{W1} \times 100$$

Bioconversion performance calculation

Estimates of how effectively the larvae reduced or converted the experimental feeds into larval biomass was obtained by correlating dry matter analyses with the weights of the feeds, residue, and total larval biomass (fresh larval weight). Bioconversion efficiency (BE) was estimated with the equation:

$$BE\ (\%) = \frac{Dry\ matter\ of\ the\ output\ larvae - Dry\ matter\ of\ the\ input\ larvae}{Dry\ matter\ of\ the\ feed} \times 100$$

The residue at the end of the experiment, consisting of diet, exuvia, and excreta, was quantified (in g of dry matter) and used to correct the amount of diet provided based on the following formula expressing bioconversion efficiency corrected for residue (BER):

$$BER\ (\%) = \frac{Dry\ matter\ of\ the\ output\ larvae - Dry\ matter\ of\ the\ input\ larvae}{Dry\ matter\ of\ the\ feed - Dry\ matter\ of\ the\ residues} \times 100$$

This corrected formula provides better insight in the relation between consumed substrate and biomass production. The formulas used were designed by **Bosch *et al.* (2020)**. Waste reduction rate (WR) was estimated, according to **Rehman *et al.* (2017)**, based on the amount of diet provided during the experiment and the residue obtained at the end of the experiment, using formula:

$$WR\ (\%) = \frac{Dry\ matter\ of\ the\ feed - Dry\ matter\ of\ the\ residues}{Dry\ matter\ of\ the\ feed} \times 100$$

Microbiological analyses

Dead larvae samples, collected before and after the feeding process, were subjected to microbiological analyses aimed at the quantitative detection of the following parameters: total microbial count (TMC), Enterobacteriaceae (ENT), sporulating bacteria (SB), yeasts (Y), and microscopic filamentous fungi (FFM). In all cases, the plate dilution method was used, always in duplicate. Analyses were performed according to the relevant ISO standards (ISO 4833-1; ISO 21528-1; ISO 21527-1). TMC was determined using Plate Count Agar (PCA, Biokar Diagnostics, Beauvais, France) and incubation at 30 °C for 72 h. Enterobacteriaceae were determined on Violet Red Bile Glucose agar (VRBG, Biokar Diagnostics, Beauvais, France) after incubation at 37 °C for 24 h. SB detection was performed on Plate Count Agar at 37 °C for 48 hours after heat shock (80 °C for 10 min followed by rapid cooling) to eliminate vegetative microorganisms in the basic dilutions. Fungi (FFM and Y) were determined on Dichloran Rose Bengal Chloramphenicol agar (DRBC, Biokar Diagnostics, Beauvais, France) and incubated at 25 °C for 6 days, while the calculation was carried out separately for each group of microscopic fungi.

The microbiological results obtained were expressed in log CFU.g^{-1}. To monitor changes after bioconversion, microbiological analyses were also done before the experiment.

Enterobacteriaceae and other bacteria identification

Grown colonies of bacteria were subjected to analysis carried out by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) based on protein fingerprints. Single bacterial colonies of fresh overnight cultures were used for the ethanol-formic acid extraction, followed in accordance with the protocol recommended by the manufacturer. A previously published article **(Hleba *et al.*, 2020)** contains comprehensive details regarding the precise procedures involved in protein extraction, sample preparation, and the identification. Raw data of protein spectrum of each isolate was imported into the Biotyper software, version 3.0 (Bruker Daltonics, Bremen, Germany) and analyzed. The result of the MALDI Biotyper was a log score value between 0 and 3.0, which indicated the probability that the bacteria isolate was correctly identified. A score of 2.300 to 3.000 suggested a high probability of species identification; a score of 2.000 to 2.299 indicated a secure genus with possible species identification; a score of 1.700 to 1.999 showed genus level identification; and a score of less than 1.700 indicated unreliable identification.

Statistical analysis

Since all experiments and measurements were performed in repetitions, all obtained and measured values were averaged, and the standard deviation was expressed.

RESULTS AND DISCUSSION

Feed properties and bioconversion performance

The moisture content and water activity (**Table 2**) of the feed indicate the variability among diet types and conditions (fresh vs. spoiled). Feeds of all spoiled variants displayed observable sensory alterations, which were indicative of microbial activity. Feeds displayed a musty smell, discolorations, and a notable proliferation of microscopic filamentous fungi. According to **Brulé *et al.* (2024)**, microorganisms that proliferate during storage prior to bioconversion are likely to be even more abundant in the final larval microbiome, including potentially hazardous genera. This fact shaped the experimental design of our study, where we intentionally included both fresh and spoiled feed variants to observe how the properties of the feed substrate affect the microbiological and nutritional quality of the resulting larvae.

In the study, spoiled diets exhibited higher moisture levels compared to their fresh counterparts, which is consistent with fermentation-induced breakdown processes releasing water. The water activity values, however, remained relatively similar across variants.

Table 2 Moisture content and water activity of feed (mean value ± standard deviation, *n*=3).

Variant	Moisture [%]	a_w
P-F	63.45 ± 1.13	0.820 ± 0.003
P-S	65.14 ± 1.43	0.817 ± 0.002
C-F	70.07 ± 1.18	0.818 ± 0.005
C-S	73.69 ± 1.02	0.811 ± 0.005
R-F	67.62 ± 1.64	0.831 ± 0.002
R-S	72.88 ± 0.55	0.818 ± 0.006

Legend: P-* – variant fed by egg pasta with milk; C-* – variant fed by mixture of hydrated couscous, boiled egg, fresh spinach, and carrot peels; R-* – variant fed by boiled rice with thawed peas; *-F – freshly prepared diet; *-S – spoiled diet

The results show that bioconversion using BSFL can effectively break down food wastes (**Table 3**) no matter what the designed diet is. However, the substrate conditions affect the quality and safety of the larval biomass that is produced. Bioconversion efficiency (BE) and efficiency corrected for residue (BER) were highest for fresh couscous (27.41% and 33.63%), followed by spoiled couscous (23.51% and 30.05%), indicating that this substrate provides the most suitable nutrient profile for larval growth and compatibility with BSFL digestion. In contrast, other spoiled diets exhibited reduced efficiency, which may be attributed to nutrient loss during spoilage or the presence of inhibitory microbial byproducts. The lowest bioconversion efficiency was recorded in the case of spoiled pasta (19.40%), which is a comparable result of 20.7% previously found for BSFL reared on industrial food waste **(Broeckx *et al.*, 2021)**. In the study of **Van Looveren *et al.* (2023)**, differences between untreated and heat-treated food waste substrates were analysed, and BE values for both variants were similar, 21.3% and 22.3% respectively. The BER reached 30.9% for untreated and 30.4% for heat-treated FW.

The waste reduction rate (WR) was highest for fresh couscous (81.34%) and lowest for spoiled rice (76.74%), highlighting the superior bioconversion capacity of certain fresh diets and the challenges posed by spoilage. The higher waste reduction rates (WR) in fresh diets further demonstrate their suitability for maximizing resource utilization. Compared to other studies, our WR rates showed higher values. **Van Looveren *et al.* (2023)** state results around 70% for untreated and treated FW; studies **Broeckx *et al.* (2021)**, **Diener *et al.* (2011)**, **Lalander *et al.* (2015, 2019)** reported WR ranging from 45% to 70%. The leftover residue can be applied to soil or utilized as plant fertilizer **(Van Looveren *et al.*, 2021)**.

Table 3 Efficiency of bioconversion of food wastes with different composition and quality performed by *Hermetia illucens* larvae (mean value ± standard deviation, *n*=4).

Variant	BE [%]	BER [%]	WR [%]
P-F	23.04 ± 2.04	28.47 ± 2.27	80.89 ± 1.01
P-S	19.40 ± 2.41	24.50 ± 3.16	79.21 ± 0.83
C-F	27.41 ± 2.80	33.63 ± 1.77	81.34 ± 4.37
C-S	23.51 ± 4.98	30.05 ± 3.35	77.62 ± 7.50
R-F	23.55 ± 0.32	29.79 ± 1.01	79.12 ± 2.80
R-S	23.93 ± 1.08	31.32 ± 2.09	76.74 ± 7.06

Legend: BE – bioconversion efficiency; BER – bioconversion efficiency corrected for residue; WR – waste reduction rate; P-* – variant fed by egg pasta with milk; C-* – variant fed by mixture of hydrated couscous, boiled egg, fresh spinach, and carrot peels; R-* – variant fed by boiled rice with thawed peas; *-F – freshly prepared diet; *-S – spoiled diet

Nutritional quality of larval biomass

The effects of different composition and quality of food wastes on biometric and nutritional parameters of larvae are shown in **Table 4**. Larval weight and nutritional composition were significantly influenced by diet type and condition. Fresh diets generally yielded higher weights and dry matter content, while spoiled diets led to slightly lower values. During feeding, the larval weight increased 2.8 (in R-S variant) – 3.5 (in P-F variant) times. Evaluated dry matter (%) of BSFL was significantly highest in case of feeding them rice-based feed, while the lowest mean value (after the bioconversion process) was revealed in larvae fed by spoiled couscous.

The two most significant nutritional elements of edible insects are their protein and fat content. The growth and development of black soldier fly larvae are thought to be significantly impacted by both nutritional, and microbiological quality of the substrate or waste **(Nguyen *et al.*, 2015)**, larval growing conditions, processing methods, and life stage at harvest (**Surendra *et al.*, 2020**). **Oonincx *et al.* (2015)** stated, that the composition of the substrates may have a major impact on BSF development, survival, nutritional composition, and the substrate bioconversion rate. For example, depending on the substrate type, protein contents can range from 10 to 40% of body weight. The same variability also occurs with both fat content and composition of resulting BSF larvae reared on different substrates **(Surendra *et al.*, 2020)**.

From the perspective of the diet's composition, we clearly recorded the highest protein content after couscous-based feeding, regardless of whether it was a fresh (35.5%) or spoiled (34.7%) variant. When comparing fresh and spoiled diets, it was found that the proportion of protein per dry weight was higher in the spoiled pasta- and rice-based diets. However, this effect was not consistent, as seen in the couscous-based diet, where fresh feed yielded better protein content. The opposing trends in protein and fat content further highlight the complex interactions between feed quality and larval metabolism. The higher protein content in larvae fed spoiled diet could indicate the role of microbial fermentation in releasing bioavailable nutrients. By starting the breakdown of complex organic substances, microbial activity can make the substrate more appetizing and accessible to the larvae **(Lalander *et al*., 2015)**. However, excessive microbial degradation can result in the loss of nutrients or the formation of metabolites like ethanol or organic acids, which may inhibit the growth of larvae or the efficiency of feed conversion **(Gold *et al*., 2018; Jalil *et al*., 2021)**.

Table 4 Biometric and nutritional values of *Hermetia illucens* larvae before and after bioconversion of kitchen waste with different composition and quality (mean value ± standard deviation, *n*=4)

Variant	Weigh of larvae [g]*	Larvae dry matter [%]	Proteins [%]	Fats [%]
P-I	3.9 ± 0.27	20.05 ± 0.18	34.3 ± 0.83	25.5 ± 0.24
P-F	13.7 ± 1.15	33.18 ± 1.07	30.9 ± 3.06	35.7 ± 0.66
P-S	11.7 ± 1.10	32.36 ± 0.44	34.9 ± 0.62	27.3 ± 0.84
C-I	3.1 ± 0.17	18.94 ± 0.31	37.5 ± 0.19	25.1 ± 0.13
C-F	10.0 ± 0.81	35.10 ± 0.29	35.5 ± 0.68	25.8 ± 0.18
C-S	9.0 ± 1.25	31.14 ± 0.84	34.7 ± 0.43	34.0 ± 0.12
R-I	3.2 ± 0.68	19.46 ± 0.61	28.1 ± 0.33	27.4 ± 0.19
R-F	9.7 ± 0.10	35.29 ± 0.59	29.6 ± 1.07	32.7 ± 0.73
R-S	8.9 ± 0.41	33.83 ± 1.21	34.6 ± 0.15	27.7 ± 0.21

Legend: *weigh of 100 representative individuals; P-* – variant fed by egg pasta with milk; C-* – variant fed by mixture of hydrated couscous, boiled egg, fresh spinach, and carrot peels; R-* – variant fed by boiled rice with thawed peas; *-I – larvae before the experiment; *-F – freshly prepared diet; *-S – spoiled diet

Fat content was notably highest in larvae fed fresh pasta (35.7%). The amount of fat is, similarly, very variable and depends on the type of diet. For example,

Newton *et al.* (1977) reported 35% on cattle manure, **Newton *et al*. (2005)** 28% on swine manure, and **Barry (2004)** 42–49% on oil-rich food waste.

Microbial counts

To determine the presence and amount of significant microbial groups, a microbial examination of the *H. illucens* larvae used in the bioconversion process was carried out. **Table 5** offers information about the samples' microbiological load. As we hypothesized, larvae reared on spoiled diets generally exhibited higher microbial loads, particularly for sporulating bacteria in all diet types (ranging from 5.60 to 6.12 log CFU.g^{-1}), and for all monitored groups of microorganisms, except yeasts, in couscous-based diet. These findings underscore the impact of feed microbial quality on the resulting microbiome of the larvae, with potential safety implications for their downstream applications. While some microbial activity may positively impact nutrient release, the elevated levels of potentially pathogenic microorganisms, such as Enterobacteriaceae or spore-forming bacteria, pose safety concerns. And this despite the fact that they can positively influence the degradation process when they convert complex organic substrates into short-chain fatty acids and other metabolites that support larval growth **(Gold *et al*., 2018; Lalander *et al*., 2015)**.

Insects generally harbor various microorganisms in their guts that play a key role in various aspects of their physiology **(Jang & Kikuchi, 2020)**. They either come from ingested feed or represent a natural microcenosis of their digestive tract. Many microorganisms can survive the conditions in the intestines and can even reproduce **(Engel & Moran, 2013)**. Subsequently, they can be excreted in large quantities into the residues **(Wynants *et al*., 2019)**. Therefore, it is highly likely that the components of the larval gut and the waste microbiome are interconnected **(Sheng *et al.*, 2024)**.

In the case of spoiled diets, we also noted an increase in the number of microscopic filamentous fungi, while the most significant increase was detected in larvae fed with spoiled couscous (5.33 log CFU.g^{-1}). Raw carrot peels in touch with the soil may be one of the sources of micromycetes. However, under realistic circumstances, the composition of food waste might vary significantly, therefore it is important to consider the widespread presence of fungi and their metabolites. The development of potentially toxic fungi such as *Aspergillus*, *Penicillium* or *Fusarium* can be considered a problem. These fungi are capable of producing mycotoxins, which are extremely stable secondary metabolites that are dangerous to the health of both humans and animals and persist through a variety of processing stages, including heat treatment **(Daou *et al*., 2021; Bullerman *et al*., 2007)**. If FW substrates support the growth of such toxigenic fungi during storage, it could compromise the safety of BSFL-derived products and limit their applicability in feed or food systems.

Table 5 Counts of analysed microbiological parameters in *Hermetia illucens* larvae reared on different types of food waste obtained by means of plate dilution method (mean value ± standard deviation, *n*=4)

Variant	**Microbial count [log CFU.g^{-1}]**				
	TMC	**ENT**	**SB**	**FFM**	**Y**
P-I	6.18 ± 0.30	6.86 ± 0.15	6.89 ± 0.65	3.43 ± 0.07	3.83 ± 0.64
P-F	7.39 ± 0.20	7.02 ± 0.11	5.04 ± 0.62	2.15 ± 0.30	5.79 ± 0.35
P-S	7.21 ± 0.09	7.08 ± 0.21	5.60 ± 0.43	3.00 ± 0.15	5.48 ± 0.49
C-I	6.76 ± 0.41	5.66 ± 0.26	5.54 ± 1.03	3.64 ± 0.07	3.94 ± 0.08
C-F	6.74 ± 0.21	6.94 ± 0.38	4.93 ± 0.10	1.27 ± 0.38	5.78 ± 0.23
C-S	7.28 ± 0.06	7.66 ± 0.23	5.83 ± 0.30	5.33 ± 0.42	5.05 ± 0.36
R-I	7.05 ± 0.70	5.42 ± 1.28	4.28 ± 0.91	3.09 ± 0.40	4.01 ± 0.12
R-F	7.57 ± 0.02	7.84 ± 0.05	3.60 ± 0.09	2.15 ± 0.24	6.11 ± 0.07
R-S	7.49 ± 0.05	7.60 ± 0.08	6.12 ± 0.06	3.03 ± 0.86	4.94 ± 0.41

Legend: TMC – total microbial count; ENT – Enterobacteriaceae; SB – sporulating bacteria; FFM – microscopic filamentous fungi; Y – yeasts; P-* – variant fed by egg pasta with milk; C-* – variant fed by mixture of hydrated couscous, boiled egg, fresh spinach, and carrot peels; R-* – variant fed by boiled rice with thawed peas; *-I – larvae before the experiment; *-F – freshly prepared diet; *-S – spoiled diet

Enterobacteriaceae and other bacteria identification

Enterobacteriaceae, one of the most important groups in health safety consideration, rose in all diet variants. The diversity and abundance of this bacterial group in larvae varied with diet type and condition, with spoiled diets showing a significantly broader range of species. The results (**Table 6**) showed that the most frequently occurring bacteria of this family, regardless of the characteristics of the feed, were *Klebsiella pneumoniae* and *Morganella morganii*. The variants fed with couscous-based feed showed the greatest species diversity. As the riskiest, in the context of potential human consumption of larvae, due to their pathogenic potential and association with foodborne illnesses, we consider *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Morganella morganii*. All these species were recorded with the highest frequency in the larvae from spoiled variants, emphasizing the risk of microbial contamination from suboptimal feed conditions. Moreover, new research has demonstrated that a risk assessment of edible insects should also consider the prevalence of antibiotic-resistant genes and antibiotic-resistant pathogens in the supply chain **(Garofalo *et al*., 2019)**.

Table 6 Identification of Enterobacteriaceae representatives detected in *Hermetia illucens* larvae applied in bioconversion process of different types of food waste using MALDI-TOF MS system.

Bacteria	**P-I**	**P-F**	**P-S**	**C-I**	**C-F**	**C-S**	**R-I**	**R-F**	**R-S**
Citrobacter freundii					+	+			
Enterobacter cloaceae			+	+		+			
Enterobacter putida			+	+		+			
Erwinia sp.					+				
Escherichia coli						+			
Hafnia alvei		+			+				
Klebsiella aerogenes		+				+			
Klebsiella pneumoniae	+	+	+	+		+	+	+	+
Klebsiella vavicola		+							
Kluyvera georgiana								+	+
Moellerella wisconsensis		+	+		+	+			+
Morganella morganii	+	+	+	+	+	+		+	+
Pantotea agglomerans					+				
Proteus mirabilis		+	+		+	+			+
Pseudomonas fluorescens					+	+			
Pseudomonas putida			+		+				
Serratia liquefaciens			+						
Serratia marcescens				+					

Legend: P-* – variant fed by egg pasta with milk; C-* – variant fed by mixture of hydrated couscous, boiled egg, fresh spinach, and carrot peels; R-* – variant fed by boiled rice with thawed peas; *-I – larvae before the experiment; *-F – freshly prepared diet; *-S – spoiled diet; "+" – positive finding; colors – the darker color of the field indicates a higher frequency of occurrence within the experimental repetitions (*n*=4) of the variant

Whereas certain microbes in and on the larvae may pose a concern to the safety of animal feed or possibly human food, and the residue may be harmful to the environment and public health **(Lalander *et al.*, 2013)**, numerous studies have demonstrated that inoculating beneficial microorganisms to biowaste can improve the performance of the BSFL process **(Xiao *et al*., 2018; Yu *et al*., 2011; Zheng *et al*., 2012)**. The identification of potentially beneficial strains (e.g., *Pseudomonas fluorescens*), in our study, also highlights the potential for employing these microbes for such a beneficial application if contamination risks are mitigated. These findings underline the importance of exploring the practical advantages of beneficial bacteria species.

Raw edible insects are known to generally contain high numbers of mesophilic aerobes, bacterial endospores or spore-forming bacteria, Enterobacteriaceae, lactic acid bacteria, psychrotrophic aerobes and fungi, and potentially harmful species (i.e. pathogenic, mycotoxigenic and spoilage microbes) **(Garofalo *et al.*, 2019)**. When assessing microbiological risk, numerous genera including *Bacillus*, *Proteus*, *Providencia*, and *Morganella*, some of which were present in our study, should receive special attention. They include organisms that have been characterized as opportunistic pathogens, drug-resistant, or highly morbid **(Raimondi *et al.*, 2020)**. Several studies have focused on reducing the microbial contamination of edible insects by applying treatments such as starvation, rinsing, thermal treatments, chilling, drying, fermentation, and marination, both alone and, sometimes, in combination. Although these studies show that various heat treatments were the most efficient methods for reducing microbial numbers, they also highlight the need for species-specific mitigation strategies **(Garofalo *et al.*, 2019)**. **Van Looveren *et al*. (2023)** claim that contamination with Enterobacteriaceae can be reduced to less than 1 log by applying a 60 °C treatment for 10 minutes. The success of some treatments in lowering microbial contamination of this novel feed or potential food is further supported by **Garofalo *et al.* (2019)**. According to their observation, processed edible insects typically had lower microbial counts than fresh insects; however, in general, heat treatment raises the financial demands.

CONCLUSION

This study underscores the potential of BSFL (*H. illucens*) as an efficient tool for food waste bioconversion, with implications for sustainable waste management and protein production. The results demonstrate that feed composition and quality significantly influence larval biomass yield, nutritional content, and microbiological safety. Fresh couscous, egg, spinach, and carrot peel-based diets yielded the highest bioconversion efficiency and protein content, whereas spoiled diets were associated with higher microbial loads and potential safety concerns. We consider it important that future studies focus on post-harvest decontamination methods and further characterization of microbial dynamics to improve the safety profile of *H. illucens* larvae as a sustainable resource.

From a nutritional perspective, it cannot be conclusively stated that better microbiological quality of food waste always leads to higher protein accumulation. It is possible that a certain level of microbial activity positively influences the final protein content of the larvae. This highlights the complexity of the interplay between feed quality, microbial activity, and larval nutritional output, requiring a balanced approach to optimize both bioconversion performance and product safety.

Our findings also emphasize the importance of implementing robust microbial safety protocols to maximize the benefits of BSFL while mitigating risks. Future research should explore the scalability of these processes and develop strategies to enhance the microbial safety of BSFL-derived products for animal feed or human consumption.

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