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ORIGINAL PAPER

Impact of a Microbial and Physical Predigestion of Food Waste on the Black Soldier Fly *Hermetia illucens* (Linnaeus, 1758) Larvae Growth and Nutritional Composition

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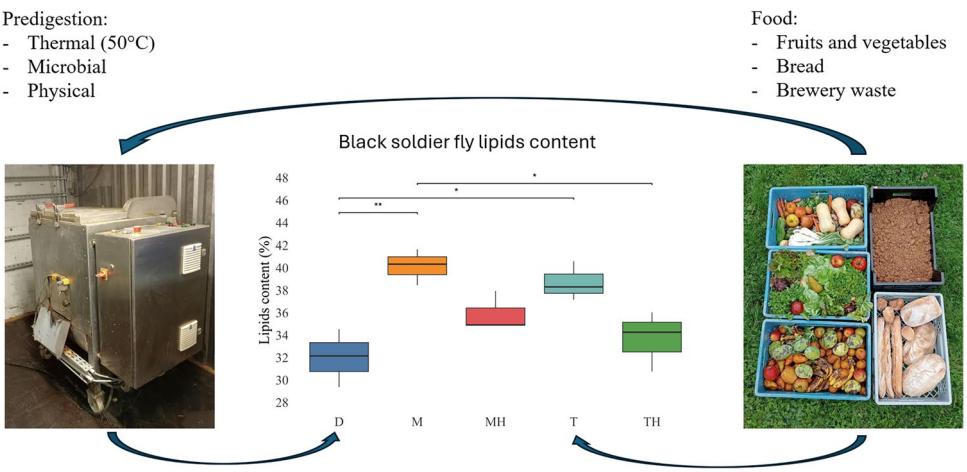
Abstract

The ability of the black soldier fly *Hermetia illucens* (L. 1758) to convert biowaste is well established, and research is increasingly focused on optimizing rearing conditions and improving the nutritional profile of larvae. Our study examined the impact of physical (thermal and grinding) and microbial predigestion of a diet on the development (growth and survival rate) and composition (lipids, fatty acids, ash, and proteins) of black soldier fly larvae. We compared the effects of different pretreatments on a single diet to evaluate the potential use of a digester in black soldier fly farming for agro-industrial purposes. This innovative method shows that grinding and digesting a mixture of unsold food and brewery waste (fruits, vegetables, bread, and brewer's spent grain) using thermophilic microorganisms can produce white larvae weighing 278 mg in 12 days, with 30.66% crude protein while significantly reducing lipid content and saturated fatty acids. These new method and results present promising prospects for biowaste valorization and large-scale insect farming.

Graphical Abstract



Keywords Pretreatment · Edible insect · Protein · Lipid

Introduction

Worldwide, food loss and food waste (FLW) represent roughly one-third of the food intended for human consumption, corresponding to 28% of the world's agricultural

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land [1, 2]. Poor management of organic waste can harm ecosystems (eutrophication, acidification) through various forms of pollution, notably phosphorus and nitrogen compounds [3–5]. Through land use change, agriculture, food modification and transport processes, and waste treatment, FLW is a significant source of greenhouse gases, notably carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) [6]. Regarding CO_2 equivalent, FLW-related greenhouse gas emissions account for 8% of global emissions [7]. In addition, organic waste can lead to health problems for humans and livestock alike [8]. Unfortunately, the threats posed by FLW exacerbate the issues of poverty, malnutrition, and food insecurity in developing countries [9].

There are several ways to process biowaste. Composting, or aerobic decomposition, enables the production of plant fertilizers from organic matter [10]. Anaerobic digestion or methanization is another biowaste treatment method for energy production [11]. Biowaste can also produce edible insects [12, 13]. An emerging practice in various countries is using black soldier flies larvae (BSFL), *Hermetia illucens* (L. 1758) (Diptera: Stratiomyidae), for the valorization of organic matters, including unsold food, kitchen waste, food by-products, and even manure and meat [14–19]. The voracity of BSFL allows them to efficiently process large quantities of organic matter while transforming it into high-quality protein and lipid sources [20]. The ease of rearing BSF and the scalability of farming facilities have enabled the development of both small-scale artisanal operations and large industrial farms, in both developing and developed countries [21]. These larvae can be used in various agronomic and industrial applications, such as animal feed (poultry, pigs, and fishes), biofuel, and fertilizer [22–25].

For yield improvement, studies have explored whether different food pretreatments could enhance the production of BSF larvae [26]. The main challenge of pretreatment is to break down lignocellulosic compounds to reduce the indigestible portion of the food [27–30]. Among the pretreatment methods, we can mention physical treatments such as grinding and heating, chemical treatments as alkaline, acidic, or oxidizing agents, and biological treatments as the use of microorganisms [26]. Physical treatments, such as grinding or blending, make the substrate easier to consume and release nutrients. The benefits of these treatments depend on the type of food and the size to which it is ground [26]. On the other hand, thermal treatments generally appear to improve larval growth and reduce lipid content compared to non-heated foods [31, 32]. Finally, the use of lignocellulosic bacteria enables BSF to degrade better cellulose-containing compounds [33–35] and even enhances their development [36].

Thus, our work aimed to determine the effect of predigestion of various substrates on the following parameters of black soldier fly larvae: larval growth, survival rate, lipid content, fatty acid profile, protein, and ash content.

Materials and Methods

Food Predigestion

A mixture of unsold food and brewery waste collected from local stores composed of 50% of fruits and vegetables (Table 1), 25% of bread and 25% of brewer's grains was used to undergo five different pretreatments (n = 5) before being offered to the larvae as food. The standard (CF) group (n = 5) contained chicken feed (Chicken Pellet, AVEVE, Leuven, Belgium) which has become the reference substrate for the black soldier fly [37]. The control group was used to compare larval growth and survival rate to ensure that the experiment was conducted properly.

For the digester condition (D) rice husks hosting a mix of thermophilic microorganisms (Actinomycetales, Caryophanales, Cellvibrionales, Clostridiales, Desulfobacterales, Eubacteriales, Micrococcales, Pseudomonadales, Sphingobacteriales) was used into a digester (biowaste upcycling, Waterloo, Belgium) that allows for grinding and heating food; the food was ground and heated at 50 °C with microorganism during 24 h. The choice of 50 °C for 24 h is inspired by the thermophilic phase of composting (50–60 °C), which is known to activate thermophilic microbial communities (Actinomycetales, Clostridiales, Pseudomonadales) and

Table 1 List of foods used to prepare the diets

Ingredients	Weight (kg) into digester	Weights (kg) for all other groups	Percentage
Romanesco cabbages	0.23	0.08	0.40%
Bok choy	0.60	0.21	1.04%
Blueberries	0.75	0.26	1.30%
Saddlery	0.85	0.29	1.47%
Green cabbage	0.90	0.31	1.55%
Endive	0.90	0.31	1.55%
Leek white	0.95	0.33	1.64%
Apples	1.50	0.52	2.59%
Kiwi	1.50	0.52	2.59%
Pomegranate	1.65	0.57	2.85%
Grapes	2.46	0.85	4.25%
Green beans	2.56	0.88	4.42%
Citrus	3.40	1.17	5.87%
Bell pepper	5.35	1.85	9.24%
Tomatoes	5.35	1.85	9.24%
Bread	14.48	5.00	25.00%
Brewer's grains	14.48	5.00	25.00%
Total	57.90	20.00	100.00%

their thermostable enzymes (cellulases, hemicellulases, proteases) [38, 39]. At this temperature, mesophilic and pathogenic bacteria are inhibited, while thermophiles are favored, enhancing predigestion [38, 40]. This treatment increases nutrient bioavailability without the need for harsher conditions. For the other conditions, food was ground with a blender (Steel fruit crusher 1100 W, WilTec, Eschweiler, Germany before treatment, M = incorporation of microorganisms and not heated; MH = incorporation of microorganisms and heated in the WTB oven (Binder GmnH, Tuttlingen, Germany at 50 °C for 24 h; T = no incorporation and microorganisms and not heated; TH = no incorporation of microorganisms and heated in the oven at 50 °C for 24 h. Distilled water was used after each treatment to compensate for evaporated water. To ensure that each group was in similar conditions, 1 ml of this same distilled water was added to each experimental condition's five replicates.

BSFL Rearing

The experiment was conducted in a dark room at a temperature and humidity of 26.17 °C ± 0.27 and 73.64% ± 7.23 (data logger; MCH – 383 SD, Lutron, Taiwan). The BSFL used in this experiment came from a lineage maintained since 2017 at the Functional and Evolutionary Entomology breeding facility of Gembloux Agro-Bio Tech (ULiège, Belgium). Populations of 100 larvae, 5 days old (0.0039 ± 0.0004 g), were manually counted and weighed in each replicate (n = 5). The larvae were grown in plastic containers (17.20 × 11.50 × 6.00 cm, AVA, Temse, Belgium) covered with a transparent lid with ventilation made using a mosquito net (1 × 1 cm) as proposed by Hoc et al. [41, 42].

The larvae were fed with 0.1 g of dry matter per day per larva for 12 days (0.1 g × 12 × 100 = 120 g) [43]. To determine the food's dry matter content (30.98% ± 1.65%) before treatment, 15 g of food (n = 5) was analyzed using an MA150 moisture analyzer (Sartorius, Göttingen, Germany). For the much drier chicken feed (85.35% ± 4.87), the same procedure was repeated using 1 g of feed. A mixture of 367 g of food and chicken feed (69% moisture) was placed in each container of 100 larvae. Twenty larvae per container were cleaned, dried, and weighed every 3 days for 12 days. After the 12th day, each container was inspected daily until a prepupa was detected. From then on, the containers with prepupae were emptied from their substrates. All living larvae were counted, washed with water, dried with a towel, and weighed before being stored at − 20 °C.

Chemical Analyzes

First, the larvae from each container were freeze-dried in a FreeZone6 for 72 h (Labconco Corp., Kansa City, MO, USA) before being ground into a fine homogeneous powder using a grinder (IKA A10, Staufen, Germany) following the manufacturer's protocol. The dry matter of each sample was determined on 0.1 g of sample (n = 2) using the same infrared balance mentioned previously (Sartorius, Göttingen, Germany).

The nitrogen content of the larvae was obtained using the Kjeldahl method. For each sample, 400 mg was weighed on nitrogen-free paper (Kern ABJ 320-4NM, Kern, Balingen, Germany). The samples were digested in 42 × 350 mm tubes using a Kjeldahl digester (DKL 20 Automatic Digestion Unit, Velp Scientifica, Usmate Velate, Italy) with 96% concentrated sulfuric acid (Acros Organics Sulfuric Acid, Thermo Fisher Scientific, Massachusetts, USA) and a catalyst in tablet form (Kjeldahl tablets, Sigma-Aldrich, Missouri, USA) containing copper sulfate and potassium sulfate. After digestion, 40 mL of 98.5% concentrated sodium hydroxide (Sodium hydroxide for analysis, Thermo Fisher Scientific, Massachusetts, USA) and 30 mL of distilled water were brought to a boil in each tube. The ammonia was distilled into a 4% solution of boric acid (Boric acid 99.5%, Thermo Fisher Scientific, Massachusetts, USA), and the ammonium borate was titrated using a 0.05 M sulfuric acid (Sulfuric acid 0.05 M, Thermo Fisher Scientific, Massachusetts, USA) to determine the nitrogen content. The N-to-protein conversion factor was 4.76 for the BSF and 5.83 for the diet to determine the protein content of each sample [44, 45].

The Folch method [46], as used by Carpentier et al. [47], was used to extract lipids from one gram for the substrate and 0.8 g for BSF (n = 3) of each group. The composition of fatty acids was estimated by gas chromatography coupled with a flame ionization detector, according to the protocol of Hoc et al. [41, 42].

Ash content was determined by incinerating 0.8 g of each sample in a muffle furnace, as proposed by Hoc et al. [41, 42]. The furnace was heated to 550.0 °C at a constant rate of 50.0 °C for 30 min and maintained for four hours at 550.0 °C.

Statistical Analyses

Statistical analysis and graphical illustrations were done using RStudio version 2023.9.1.494 [48]. The distribution shape was analyzed using the Shapiro–Wilk test, and for each of our analyses, the significance threshold was set at 5%.

The comparison of groups with a non-parametric distribution was performed using a Kruskal–Wallis test followed by a Dunn's test with Bonferroni correction using the Dunn.test package [49]. The graphical representation of larval growth based on different substrates was created using the ggplot2, ggsignif, and Rmisc packages [50–52]. The comparison was performed using an ANOVA test followed by a Tukey post

hoc test for the groups with a normal distribution. To compare the complete fatty acid profile, a Permutational Analysis of Variance (PERMANOVA) was conducted using an Euclidean distance matrix and 999 permutations with the vegan package [53]. Finally, the fatty acid profile was visualized using a Principal Component Analysis (PCA).

Results

The influence of pretreatment of the substrate did not show significant differences in larval weight at the beginning of the experiment (Kruskal–Wallis $\chi^2(4) = 4.00$, p-value > 0.05), and day 3 (Kruskal–Wallis $\chi^2(4) = 4.90$, p-value > 0.05). On day 6, the Kruskal–Wallis test suggested a difference between the groups (Kruskal–Wallis $\chi^2(4) = 9.75$, p-value = 0.045). However, post-hoc comparisons performed using Dunn's test, corrected with the Bonferroni method, did not reveal any significant differences between the groups (Dunn test – Bonferroni p-value > 0.05). However, on day 9 (Kruskal–Wallis $\chi^2(4) = 10.50$, p-value = 0.033), TH (0.291 ± 0.009) group individual weight was significantly higher than D (0.271 ± 0.005) group (Dunn test – Bonferroni p-value = 0.014). Finally, larval weight on day 12 again highlighted the pretreatment influence on larval growth (Kruskal–Wallis $\chi^2(4) = 16.85$, p-value = 0.002). These differences involved the MH and M (Dunn test – Bonferroni p-value = 0.031), D and MH (Dunn test – Bonferroni p-value = 0.034), TH and M (Dunn test – Bonferroni p-value = 0.014), and TH and D (Dunn test – Bonferroni p-value = 0.015) groups (Fig. 1). On the 12th day, each larval group had a very high average weight, ranging from 0.278 to 0.307 g, with the CF group reaching 0.326 g.

It took a minimum of 14 ± 0 days to obtain a fully black larva in each of the replicates of group TH, and up to 14.8 ± 0.45 days for group D. As for the CF group, it only took 13 ± 0 days. The different pretreatments significantly impacted the larval survival rate on the final day (Kruskal–Wallis $\chi^2(4) = 12.66$, p-value = 0.01). These differences were noted between 92.2 ± 1.3% (D) and 98.4 ± 1.5% (TH) (Dunn test – Bonferroni p-value = 0.043) and 92.2 ± 1.3% (D) and 98.6 ± 2.6% (T) (Dunn test – Bonferroni p-value = 0.013) (Fig. 2). The survival rate of the CF group was 93.2 ± 3.27%.

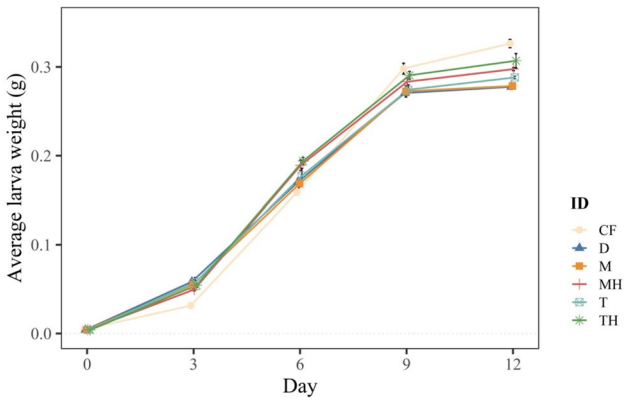

Fig. 1 Average *H. illucens* larva weight (n = 20) every 3 days over 12 days on the same substrate subjected to different pretreatments. *CF* chicken feed; *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

No significant differences (ANOVA F(4, 10) = 0.88, p-value > 0.05) were found in the substrate lipid content at the beginning of the experiment, with values ranging from 3.28% ± 1.43 (MH) to 5.62% ± 1.85 (TH). Similarly, no significant differences (ANOVA F(4, 10) = 0.97, p-value > 0.05) were observed in substrates at the end of the experiment, ranging from 5.20% ± 1.59 (TH) to 6.66% ± 0.84 (T). The lipid content of BSF was significantly influenced by the type of pretreatment (ANOVA F(4, 10) = 7.58 p-value = 0.004). The most notable difference was between 32.03 ± 2.58 (D) and 40.16 ± 1.60 (M) (Tukey HSD p-value = 0.006) (Fig. 3).

At first glance, the PERMANOVA appears to show a significant effect (PERMANOVA F(4,10) = 10.09, $R^2 = 0.801$, p = 0.001) of the pretreatment on the fatty acid profile of BSF; however, there is no significant difference (Post-hoc pairwise p-value > 0.05) in the pairwise comparisons (Fig. 4).

However, univariate analyses revealed high levels of saturated fatty acids ranging from 77.29 ± 1.10% (T) and 82.08 ± 0.85% (MH) (ANOVA F(4, 10) = 18.89 p-value < 0.001) and conversely a low quantity of unsaturated fatty acids from 17.92 ± 0.85% (MH) to 22.71 ± 1.10% (T) (ANOVA F(4, 10) = 18.89 p-value < 0.001). Monounsaturated fatty acids ranged from 2.91 ± 0.19% (M) to 3.57 ± 0.08% (T) (ANOVA F(4, 10) = 11.08 p-value = 0.001) and polyunsaturated fatty acids 8.55 ± 0.38% (MH) and 12.25 ± 0.35% (D) (ANOVA F(4, 10) = 24.17 p-value < 0.001).

The most abundant fatty acids were lauric acid (C12) from 56.97% ± 1.04 (T) to 61.97% ± 1.41 (MH), palmitic acid (C16) from 9.72% ± 0.20 (TH) to 10.20% ± 0.22 (T), and linoleic acid (C18:2n6 cis) from 7.32% ± 0.35 (MH) to 11.18% ± 0.20 (D) (Table 2). As in the substrates, the least abundant fatty acid was pentadecylic acid (C15), found only in the T group at a concentration of 0.08% ± 0.01. Unsaturated fatty acids represent approximately 80% of all fatty acids, while monounsaturated and polyunsaturated fatty acids each account for around 10%.

The ash content of the different substrates at the beginning (MH = 3.12% ± 0.04 and D = 3.45% ± 0.03; p-value > 0.05) was not influenced by the various pretreatment. No significant differences in ash content (TH = 2.67% ± 0.36 and D = 3.46% ± 0.20; p-value > 0.05) were observed between the different groups of BSF reared on these substrates either.

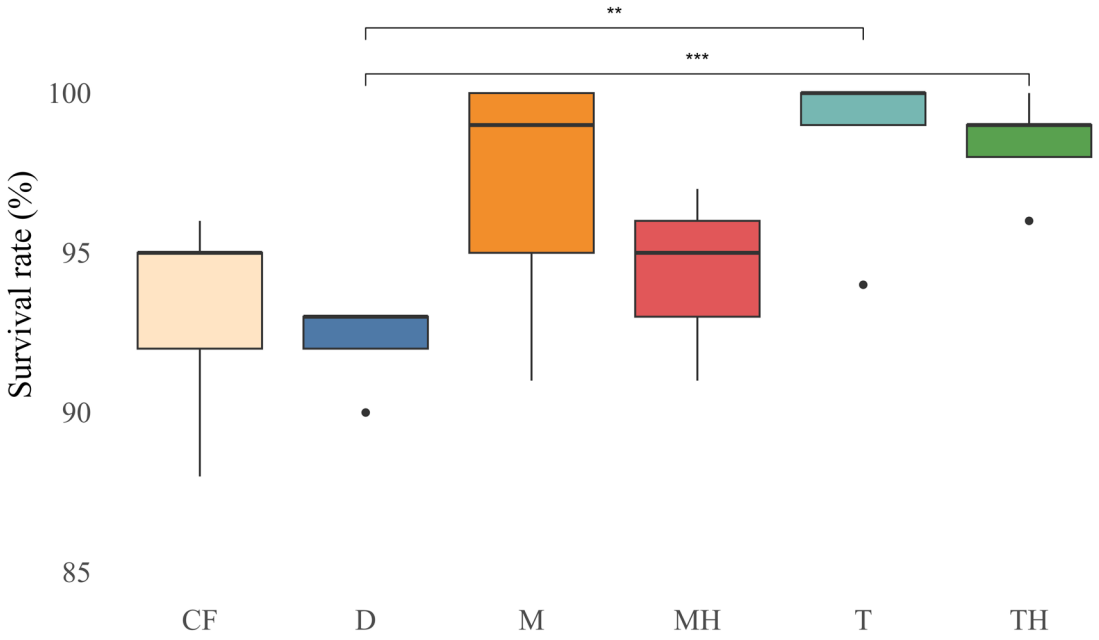

Fig. 2 *H. illucens* larval survival rate depending on the food pretreatment. *CF* chicken feed; *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

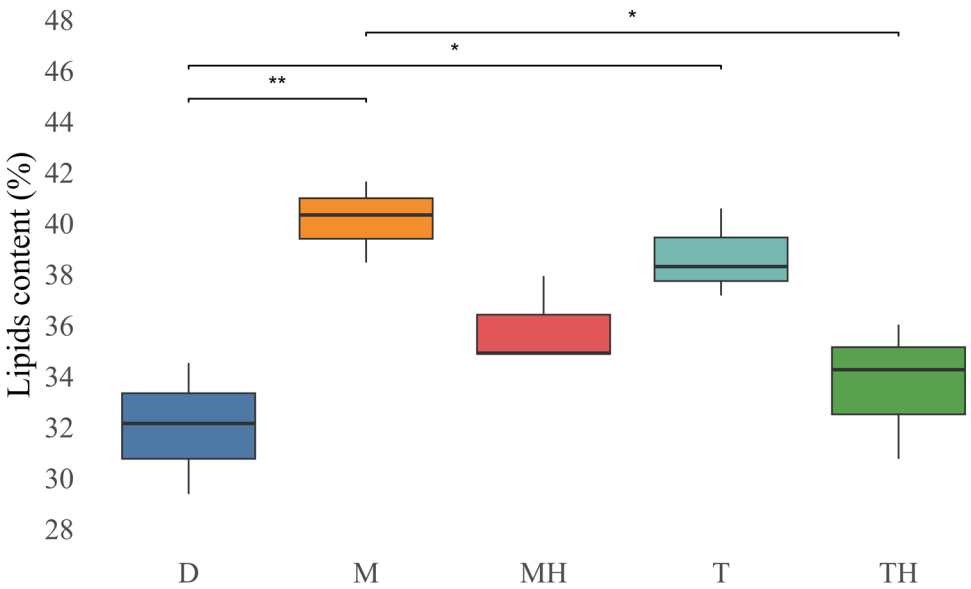

Fig. 3 Final larval lipids content depending on the food pretreatment. *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

The protein content of the different substrates at the beginning (M = 17.08% ± 0.06 and T = 20.70% ± 0.80; p-value < 0.001) varied significantly depending on the type of treatment (Fig. 5).

However, on the final day of development, the protein content did not significantly vary between the different BSF groups from 30.38% ± 1.16 (M) and 31.49% ± 4.14 (T) (ANOVA F(4, 10) = 0.07 p-value > 0.05) or between the various substrates from 21.51% ± 2.15 (M) to 23.75% ± 2.05 (T) (ANOVA F(4, 10) = 0.50 p-value > 0.05) (Fig. 6).

Discussion

Food loss and waste are challenges for our future that require institutional, academic, and industrial investments starting today. New waste valorization methods must be developed, and existing ones must be improved. Black soldier fly larvae have repeatedly demonstrated their ability to valorize biowaste efficiently. In the current study, we focused on the influence of physical and microbial

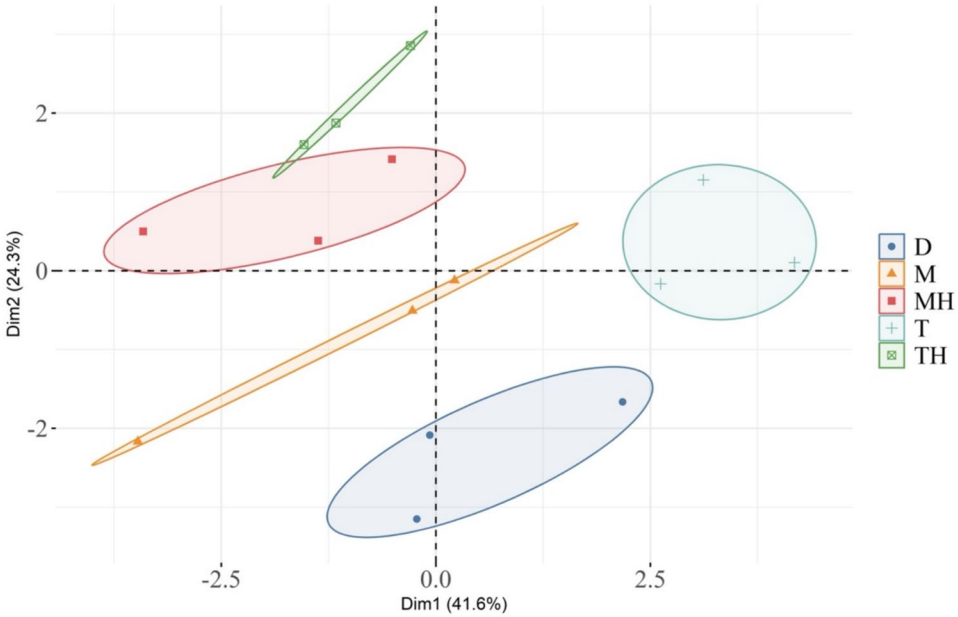

Fig. 4 Representation of PCA scores based on the fatty acid profiles of larvae fed the same substrate subjected to different pretreatments. *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

Table 2 Fatty acid profile of the different treatments

Fatty acid	D	M	MH	T	TH	Statistical analyses	p-value
C10	1.14 ± 0.08	1.07 ± 0.11	1.24 ± 0.08	1.09 ± 0.15	1.10 ± 0.10	F = 1.231	0.358
C12	58.44 ± 1.13	60.77 ± 1.45	61.97 ± 1.40	56.97 ± 1.04	60.65 ± 0.61	F = 8.939	0.002
C14	7.26 ± 0.15	7.56 ± 0.07	8.04 ± 0.32	7.77 ± 0.16	8.45 ± 0.25	F = 14.167	< 0.001
C14:1	0.22 ± 0.04	0.19 ± 0.09	0.21 ± 0.05	0.19 ± 0.04	0.16 ± 0.03	F = 0.524	0.721
C15:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.02	0.00 ± 0.00	H = 13.80	0.008
C16:0	10.03 ± 0.33	9.76 ± 0.45	9.67 ± 0.48	10.20 ± 0.22	9.72 ± 0.19	F = 1.233	0.357
C16:1	2.16 ± 0.09	1.82 ± 0.28	1.95 ± 0.09	2.41 ± 0.28	1.77 ± 0.14	F = 5.454	0.014
C18:0	1.03 ± 0.13	1.08 ± 0.14	1.17 ± 0.07	1.18 ± 0.06	1.26 ± 0.06	F = 2.492	0.11
C18:1n9 cis & trans	7.46 ± 0.19	6.71 ± 0.33	7.22 ± 0.36	8.13 ± 0.30	7.61 ± 0.09	F = 10.993	0.001
C18:2n6 cis	11.18 ± 0.20	9.83 ± 0.51	7.32 ± 0.38	10.54 ± 0.81	8.00 ± 0.33	F = 34.09	< 0.001
C18:3n3	1.07 ± 0.15	1.21 ± 0.23	1.22 ± 0.05	1.44 ± 0.09	1.27 ± 0.10	F = 2.802	0.085
MUFAs	9.84 ± 0.31	8.72 ± 0.58	9.37 ± 0.47	10.73 ± 0.24	9.54 ± 0.14	F = 11.078	0.001
PUFAs	12.25 ± 0.35	11.04 ± 0.67	8.55 ± 0.38	11.98 ± 0.89	9.27 ± 0.42	F = 24.173	< 0.001
SFAs	77.91 ± 0.66	80.24 ± 0.92	82.08 ± 0.85	77.29 ± 1.10	81.19 ± 0.46	F = 18.889	< 0.001

predigestion of biowaste on the development and nutritional profile of black soldier fly larvae.

Our results did not reveal any significant weight gain or loss associated with the different pretreatments compared to the mixed feed that was neither heated nor inoculated with bacteria. However, on the 12th day, the heated groups, both with (MH) and without microorganisms (TH), had a significantly higher weight compared to the non-heated feed with bacteria (M) and the predigested feed (D). A study on the thermal pretreatment of substrates suggests a weight gain in larvae as the temperature increases, starting at 75 °C [31]. However, some results differ regarding the actual weight gain in larvae when treated at 90 °C, depending on the tested feed types [54]. One possible hypothesis for the slower development observed in the group fed with predigested food could be differences in bacterial populations. The results from Yu et al. [55] highlighted that supplementing a diet with bacteria from BSF enhances their growth. Supplementation with aerobic thermophilic bacteria and heat treatment may have drastically reduced the presence of beneficial bacteria for BSF. Another possible explanation could be the absence of lactic acid-fermenting bacteria compared to the other groups. The introduction of air, the presence of thermophilic bacteria, and their activation by heat within the digester may have hindered the proliferation of lactic acid bacteria, which seem to promote better BSF development

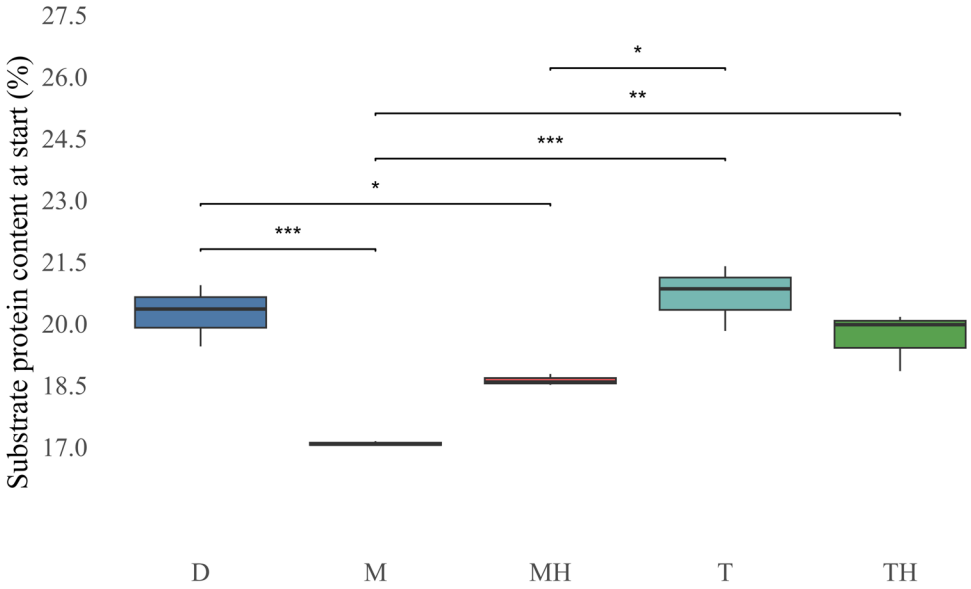

Fig. 5 Substrate lipids content at the beginning of the experiment. *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

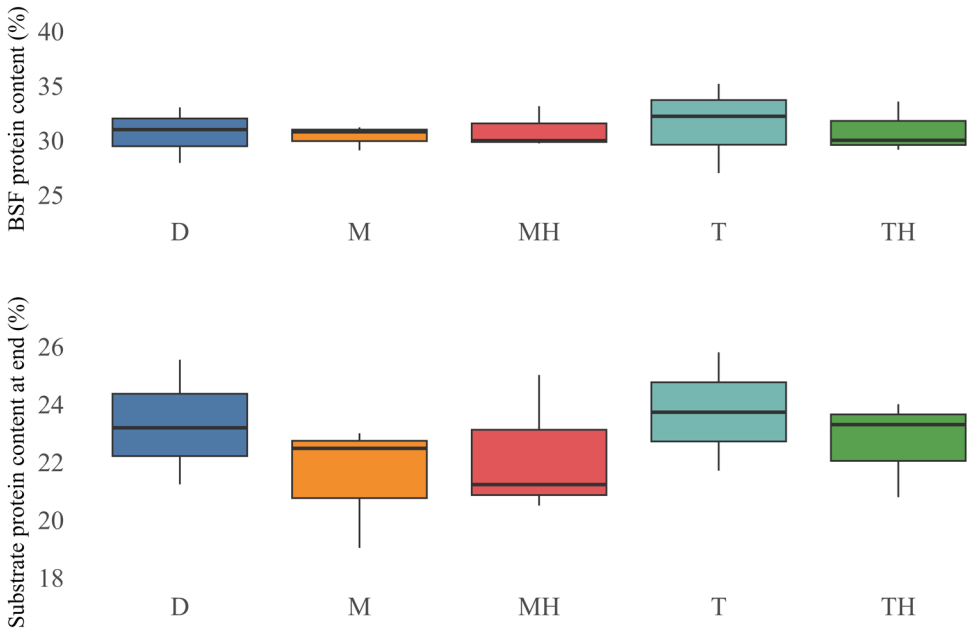

Fig. 6 Larval protein content (BSF) and diet protein content (SUB) at the end of the experiment. *D* digester; *M* microorganisms, *MH* microorganisms and heated; *T* no microorganisms and not heated; *TH* heated

[56, 57]. These two hypotheses could also partly explain why the group fed predigested food had a lower survival rate. We acknowledge the importance of microbial community analyses, such as 16S rRNA gene sequencing, to better understand the specific shifts in bacterial populations caused by the pretreatment. However, this study focused primarily on assessing the physiological effects of thermal and microbial pretreatment on larval performance and composition for an agro-industrial aim. While we did not perform sequencing analyses in the present work, we recognize this as a limitation and suggest that future studies should investigate microbial dynamics more deeply to validate the hypothesized mechanisms behind improved bioconversion. Another factor contributing to the lower survival rate could be the reduced water retention capacity of the feed caused by grinding, heat, and bacterial enzymatic activity [58, 59]. Once rehydrated to the same percentage as the other substrates, the predigested substrate appeared slightly more watery and less viscous. Therefore, it could be beneficial to rehydrate the predigested substrate to achieve a specific consistency rather than aiming for the same moisture percentage as the original feed.

Concerning the lipid profile, heat treatment and the predigestion of the substrate reduce the lipid content of the larvae feeding on it. The TH group had 6.46% less lipids than the M group, and the D group had 8.13% and 6.67% less lipids

than the M and T groups. Several studies have shown that thermal pretreatment of the substrate, with or without pressure, can reduce the lipid percentage in larvae [31, 32]. This result is consistent with our data, although the pretreatment duration differed. This decrease in lipid percentage could be explained by bacterial populations in the diet affected by thermal treatment. Studies have highlighted that inoculating a culture of bacteria from BSF eggs and larvae can result in higher lipid levels [60]. This suggests that the larvae's needs extend beyond the macronutrients in their diet to include the bacteria present [61]. Another explanation could be that the degradation of lignocellulosic compounds by the microorganisms led to various carbohydrates, which may be metabolized to different extents into fats [47]. Thermophilic aerobic bacteria have proven effective in reducing starch and cellulose during kitchen waste treatment [62]. This latter hypothesis could also explain the observed differences in the fatty acid profiles depending on the pretreatment type. Our results highlighted that predigestion slightly reduces the percentage of saturated fatty acids in favor of unsaturated fatty acids. Regarding the percentages of these fatty acid types, our values appear relatively similar to those reported by Liew et al. [32].

Ash content in the larvae was very similar to that of the substrate. Black soldier fly larvae are known for bioaccumulating minerals, particularly calcium [63]. Additionally, there were no significant differences between the groups, which was expected since the feed was the same, with only the pretreatment varying.

The significant difference in protein content in the substrate on day 0 is likely due to the analysis being performed on a single sample. Measuring the substrate on the 1st day as a pseudo-replicate results in very low variance, which likely explains the significance of the results. However, this measurement ensures no outliers are present at the start of the experiment. This difference faded by the final day of development and did not appear in the protein content of the larvae. The results found in the literature regarding the positive impact of thermal pretreatment vary, likely due to differences in how the various feed materials degrade, as well as variations in larval density and feeding rate across different experiments [32, 54].

The use of common unsold food and brewery waste (50% fruits and vegetables, 25% brewer's spent grains, 25% bread) and the digester allowed the production of larvae with a highly suitable nutritional profile for BSF farming (32.03% lipids, 30.66% proteins, and 3.46% ash). Compared to commonly used feeds for BSF (fruits and vegetables, brewer's spent grains, food waste, poultry feed, poultry manure), the observed ranges are comparable, with 23–43% proteins, 22–40% lipids, and ash content ranging from 7% to over 11% [64–66]. Moreover, the digester's ability to reduce the lipid content and saturated fatty acids in the larvae while achieving a development time of 12 days, a survival rate of 92%, and an average weight of 278 mg per larva presents promising prospects for large-scale BSF production. Due to the reduction in lipid content, it seems more appropriate to use this type of digester for animal feed purposes rather than biofuel production [67]. In animal nutrition, the use of defatted BSF allows for better results and increases the inclusion rate in the diet [68, 69]. The BSF is increasingly utilized in animal nutrition for poultry [70], fish [41, 42, 71], and pigs [23] that is why it is important to provide insects with a good protein-to-lipid ratio. Therefore, it would be pertinent to prioritize this larva production method in animal feed and study its effects, based on inclusion rates, on various species. Another advantage of using an aerobic digester is its ability to dehydrate food, potentially improving storage. It would be valuable to explore the use of the digester over an extended period to fully dehydrate food and study its storage viability and impact on larval development. A key factor in the use of such a machine inevitably lies in its cost, including both its initial price and energy consumption. Dedicated studies have focused on evaluating the life cycle assessment of BSFL farming systems, and these works highlight that electricity consumption is one of the main environmental and economic concerns [72–74]. In this context, the integration of a digester, such as the one used in our study, would indeed warrant a more in-depth evaluation of its energy and economic impacts, especially with a view toward industrial-scale implementation. We therefore consider that life cycle analysis (including both energy and economic aspects) represents a crucial research avenue to confirm the sustainability of the process and to optimize its large-scale application. Beyond its economic aspect, which would require more in-depth studies, the sourcing of supplies also plays an important role in its environmental sustainability [75]. Additionally, investigating the introduction of bacterial strains from the substrate or the BSF's digestive system could further optimize this approach.

Now that the use of physical and microbial predigestion has shown promising results in BSF farming, it would be interesting to study the impact of such digestion on the frass produced by the insects, as frass can be a plant fertilizer [76]. Numerous studies on various crop types (leafy vegetables, fruits, cereals, and forage crops) have demonstrated the positive effects of frass application, including enhanced root development, vegetative growth, and increased yields [77]. The use of a digester, by partially mimicking the thermophilic phase of composting, could further enhance frass quality by stimulating microbial enzymatic activity and improving nutrient availability [78, 79]. Therefore, a promising direction for future research would be to assess how substrate predigestion influences the bioavailability of nutrients in BSFL frass and its subsequent agronomic effects on a range of plant species. Although this seems straightforward,

sorting organic matter conducted upstream to feed the larvae ensures that no non-organic pollutants are introduced into agricultural or forestry systems [80]. However, one of the challenges frass faces during composting is its high moisture content, which prevents the production of fully mature compost [76, 81]. An interesting perspective would be conducting large-scale growth tests to compare the final moisture content of frass from pre-treated substrates with untreated ones. If the moisture content proves to be lower, this could be advantageous for composting [82] and for the combustion [83] of organic waste. On the other hand, wet organic waste could be effectively used to produce biogas [84, 85], making it worthwhile to study whether such predigestion of substrates enhances the frass's potential for biogas production.

Conclusion

This study provided valuable insights into the production of black soldier fly larvae. The ability of substrate predigestion to modulate the lipid content, particularly the saturated fatty acid profile of the larvae, offers promising opportunities for insect protein production. It would be worthwhile to explore the use of other microorganisms during the predigestion process to enhance this technique. Large-scale trials to evaluate the potential of insect proteins in animal nutrition would be a feasible and intriguing perspective. Finally, examining the potential applications of the frass produced using this method would be another area of interest.

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Data Availability Data can be requested directly from the reference author (Hugo Luttenschlager): hluttenschlager@uliege.be.

Declarations

Competing Interests Hugo Luttenschlager and Nicolas Deville are funded by the Walloon Region as part of the ASTIPPOR project. Sebastien Finet supplied the recycler we worked with. There are no other financial or material affiliations that could be interpreted as a conflict of interest.

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