

Bioconversion of Lettuce, Potato and Pasta by Black Soldier Fly Larvae (*Hermetia illucens*) into high value insect biomass

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Abstract

The growing demand for sustainable alternative protein sources and effective food waste management highlights the potential of Black Soldier Fly Larvae (BSFL, *Hermetia illucens*). This study investigates the growth and bioconversion efficiency of BSFL reared on three substrates - lettuce, potatoes, and pasta. These substrates were subjected to pre-treatment methods such as slicing, blending and boiling. Larval performance was evaluated through wet and dry weight, length, and bioconversion rate (BCR). Pasta supported the highest insect biomass yield, with blending increasing larval weight to 77.6 mg and BCR to 14.8%, compared to 48.6 mg and 8.9% for whole pasta. Potatoes resulted in moderate growth, with boiling enhancing BCR from 7.4% to 9.2%. Lettuce, characterized by low nutrient content and high moisture, produced the poorest results, and blending slightly negatively affected BCR. The findings underline that both nutrient composition and pre-treatment of substrates are critical factors for maximizing BSFL growth and bioconversion outcomes.

Keywords: BSFL; food waste; substrate pre-treatment; bioconversion; *Hermetia illucens*

Introduction

The rapid growth of the world population is increasing the demand for food while simultaneously intensifying the problem of organic waste generation. Large amounts of food, 1.3 billion tons, are wasted annually, creating environmental and economic challenges¹. To feed the world population, the search for sustainable protein sources is essential. Insects, particularly Black Soldier Fly Larvae (BSFL), represent a promising solution of bio-waste management. The larvae can efficiently convert organic waste into protein- and fat-rich biomass that can be used in food production².

BSF larvae are capable of massively increasing their weight by the end of their growth process³. The bioconversion is however dependent on the varied nutritional value of the feeding substrate⁴. Most common BSFL diets consist of manure (poultry, swine, cow), kitchen waste, human feces, sludges and agricultural byproducts^{5,6}.

Once the waste processing is complete, the mature BSF larvae become a valuable product, as they can be used as feed for poultry, fish, and various commonly farmed animals. According to some estimates, such feed could partially replace fish meal thereby contributing to the restoration of natural fish populations in the oceans and simultaneously having a significantly positive effect on CO₂ emissions. This technology is suitable for both centralized and decentralized waste management systems^{3,4}. The larvae can be processed using various technologies (roasting, cooking, drying, grinding, pelletizing), which also extend their shelf life^{3,7}. The larvae are a high-quality source of proteins and fat, and can be processed into biopolymers and biofuels such as biodiesel^{8,9}. The BSFL processing of biowaste produces also a byproduct - frass. This can however be used as a fertilizer in agriculture, boosting organic matter, nitrogen and phosphorus in soil^{10,11}. Chitin in the frass even increases plant resistance against pathogens⁷.

In this study, lettuce, potatoes, and pasta were selected as representative feeding substrates for BSFL. These foods are among the most discarded food wastes due to their high consumption and perishability¹²⁻¹⁵. They also differ in nutritional composition. Lettuce is low in protein and energy density, and high in moisture¹⁶. Potatoes are starch-rich with low protein and moderate energy density, and pasta has a relatively higher protein content and energy density¹⁷⁻¹⁹. Each substrate allows also evaluation of how pre-treatment methods such as slicing, boiling, or blending affect BSFL growth. These substrates provide a relevant model system for assessing BSFL performance on common urban food waste streams.

Experimental part

Materials and Methods

The BSFL and experimental design

The BSF Larvae (*Hermetia illucens*) were sourced from the company Grinsect. The larvae were delivered as 5-DOLs (5-day-old larvae). The experiments were conducted in the summer of 2024. Each feeding tray was initially filled with approximately 10 000 5-DOL BSF larvae. The feeding trays were made of dark polypropylene (PP) plastic (size 60x40x12 cm). Feed substrate was added to the containers with BSFL usually every other day ensuring the container was filled to an acceptable level. To maintain the moisture of the feed and prevent the larvae from escaping the containers, a small amount of coconut fiber was always added on top of the feed. Each feeding substrate was tested in triplicate, with three independent trays prepared under identical conditions. The larvae were kept in a room without sunlight, with stable humidity (50% ± 20%), temperature of 23 ± 2 °C and non-stop air circulation. The experiment lasted until the point when pupating individuals (prepupae) first appeared in a container (after 20 days). At that point, the experiment was concluded.

Larvae were sampled regularly to determine their wet and dry weight. Measurements were performed gravimetrically. Fifteen randomly selected BSF larvae (5 from each tray) were taken from the containers, washed with distilled water and dried with paper towels. Their wet weight and dry weight were recorded with analytical scales. At the end of the experiment larvae were collected and photographed. Length of the larvae was measured with a software program ImageJ.

Bioconversion rate was calculated from total larval dry weight at the end of the experiment and the total dry weight of the feed substrate using the following formula:

$$BCR(\%) = \frac{\text{Total final larval weight (g, DM)}}{\text{Weight of feed substrate (g, DM)}} \times 100 \%$$

The larvae were sifted through meshes at the end of the experiment to separate them from most of the frass. Afterwards they were dried and weighed. The drying process occurred always at 105 °C for 4 hours.

Feed substrate

Each tray with BSFL was filled with feed substrate at the beginning of the experiment. Afterwards, the substrate was added to the trays when the old substrate disappeared, or the larvae did not feed on it anymore.

The feed substrates were lettuce, potatoes and pasta. These substrates were always purchased from a selected retail chain. The lettuce was prepared in two ways. The first method involved cutting it into pieces approximately 2×4 cm in size. The second method involved blending it in a smoothie blender until it was completely pureed.

The potatoes fed to the larvae were also prepared in two ways. In both cases, they were cut into pieces approximately 1×2×2 cm. In the first case, the potatoes were raw. In the second case, they were processed by being submerged in boiling water for 15 minutes, then cut to the desired size at room temperature.

The pasta used as feed was prepared in two ways. In the first case, it was boiled in water for 9 minutes. In the second case, it was boiled and then blended in a smoothie blender into a mash with consistency similar to mashed potatoes. The nutritional composition of the three substrates used (lettuce, potatoes, and pasta) is summarized in Table 1.

Table 1: Nutritional values of each feeding substrate.

Substrate	Protein (g/100 g)	Carbohydrates (g/100 g)	Fat (g/100 g)	Energy (kcal/100 g)	Fiber (g/100 g)	Moisture content (%)
Lettuce ¹⁶	1	2.1	0	14	1.1	96
Potatoes (raw) ¹⁷	2.1	17.5	0.09	77	2.1	79
Potatoes (boiled) ¹⁸	1.9	20.1	0.1	87	1.8	78
Pasta (boiled) ¹⁹	5.8	30.9	0.93	158	1.8	62

Statistical analysis

Results for final larval wet and dry weight, bioconversion rate, and larval length are presented as Mean \pm Standard deviation (SD). Prior to statistical analysis, normality of the data was assessed using the Shapiro–Wilk test and homogeneity of variances was evaluated using Levene's test. The assumptions required for ANOVA were considered satisfied for dry and wet weight, BCR, and length measurements ($p > 0.05$). Differences among feed substrates and treatments were evaluated using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's honestly significant difference (HSD) post-hoc test was applied for pairwise comparisons. Statistical significance was accepted at $p < 0.05$. Different letters for groups in Tukey's HSD indicate statistically significant differences between feed substrates and treatments. Statistical analyses were performed using OriginPro 2024 software. Intermediate wet and dry weight measurements were obtained from pooled larval samples collected from the three replicate trays and are therefore presented descriptively without statistical testing (Figure 1 and Figure 2).

Results

Final dry weight differed significantly among feeding substrates and substrate pre-treatments (one-way ANOVA, $F_{5,12} = 204.60$, $p < 0.0001$). The increase in larval dry weight over time across feeding substrates is shown in Figure 1. BSFL fed with pasta had the fastest increase in dry weight among the tested feed materials. They were followed in growth rate by larvae fed with potatoes, while the slowest dry weight increase was observed in larvae fed with lettuce. Tukey's HSD test separated the treatments into four statistical groups, with blended pasta $>$ whole pasta $>$ potatoes $>$ lettuce ($p < 0.05$) as shown in Table 2.

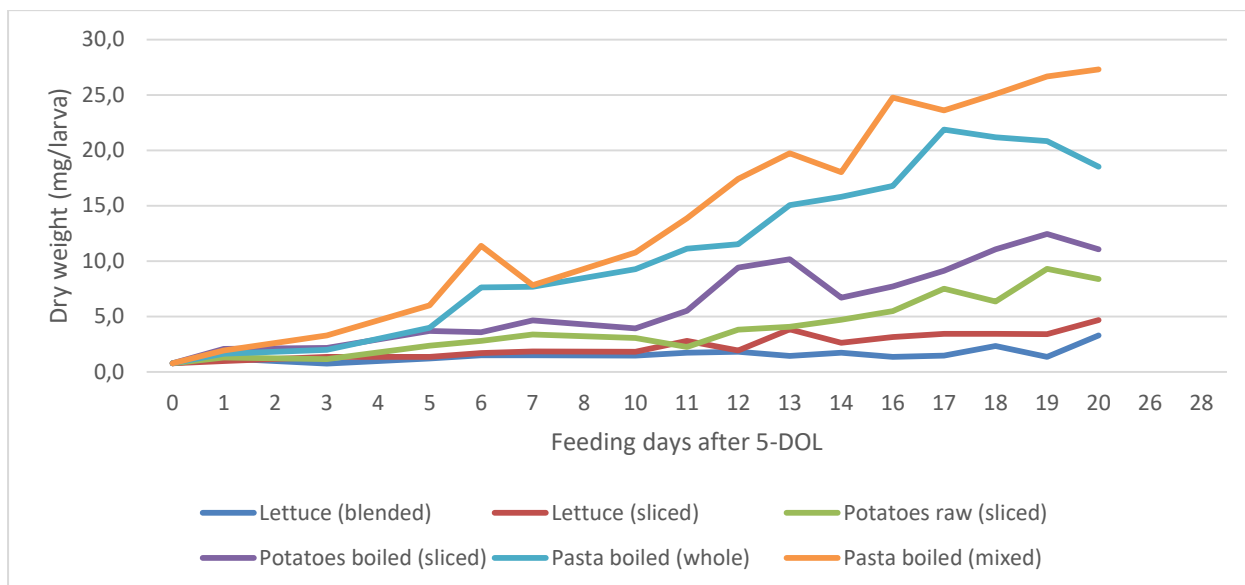


Figure: 1 Measured dry weight of BSF larvae fed with different substrates in time

Final wet weight also differed significantly among treatments (one-way ANOVA, $F_{5,12} = 55.39$, $p < 0.0001$). The same trend was observed as for dry weight, with the highest values for larvae fed with pasta (Figure 2). The highest wet and dry weight of BSFL at the end of the experiment was 77.6 mg/larva and 27.3 mg/larva for blended pasta, and the lowest was for blended lettuce at 21.4 mg/larva and 3.3 mg/larva. Larvae fed sliced lettuce reached 31.7 mg in wet weight. Those fed raw or boiled potatoes reached 47.7 mg and 43.5 mg, respectively, while larvae provided whole pasta reached 48.6 mg. Tukey's HSD test identified significant differences among the treatment groups ($p < 0.05$) as shown in Table 2.

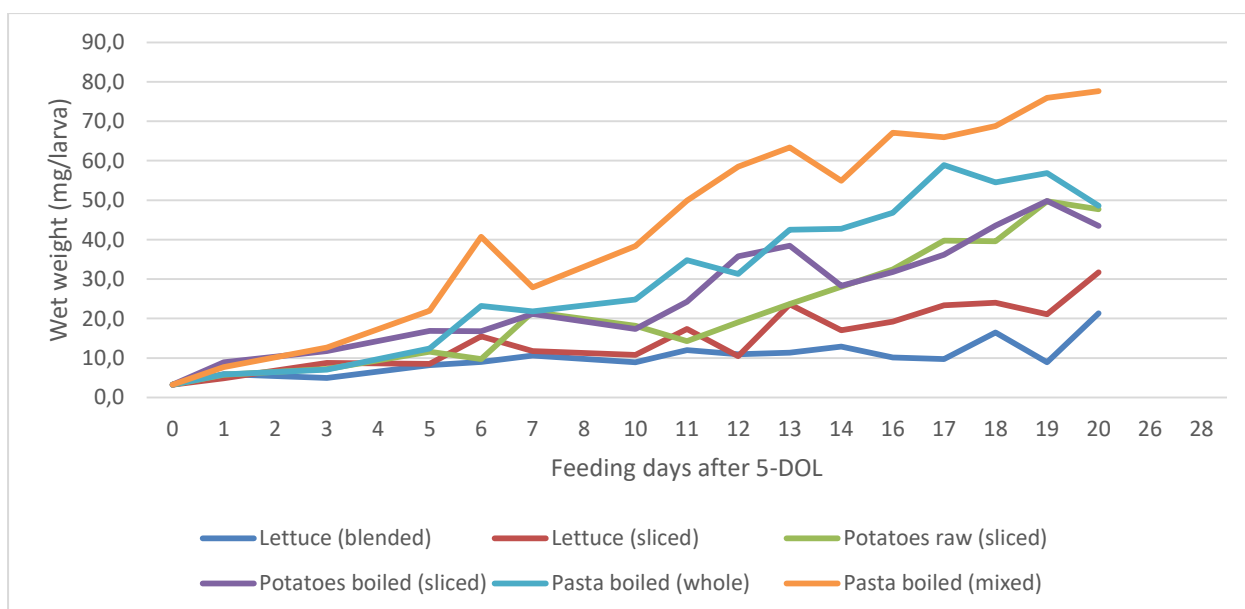


Figure: 2 Measured wet weight of BSF larvae fed with different substrates in time

The dry and wet weight at the beginning of the experiment and at the end are shown in Table 2 for each feed substrate with the groups from Tukey's HSD tests.

Table 2: Dry weight and wet weight at the beginning and at the end of the experiment (Mean ± SD) and their significance letters based on Tukey's HSD test.

	Initial	Final					
		Lettuce		Potato		Pasta	
		Blended	Sliced	Raw sliced	Boiled sliced	Boiled whole	Boiled blended
Dry weight (mg/larva)	0.8 ± 0.1	3.3 ± 0.6	4.7 ± 0.9	8.4 ± 0.8	11.1 ± 1.2	18.5 ± 0.8	27.3 ± 1.9
Groups (Tukey HSD)	-	A	A	B	B	C	D
Wet weight (mg/larva)	3.2 ± 0.3	21.4 ± 3.2	31.7 ± 5.6	47.7 ± 4.8	43.5 ± 4.8	48.6 ± 2.7	77.6 ± 4.9
Groups (Tukey HSD)	-	A	A	C	B	C	D

The ratio of dry to wet weight over time is shown in Figure 3, indicating that the percentage of dry matter in larval biomass was highest in larvae fed with pasta (33.7% and 30.0%), followed by potatoes (18.4% and 23.2%), and lowest in larvae fed with lettuce (15.3% and 15.9%). Overall, the share of dry matter in the biomass ranged between 11.0% and 38.9%. The larvae fed with lettuce did not grow as well as those fed with potatoes or pasta. Larvae fed with blended and sliced lettuce grew to 4.1 and 5.9 times their initial dry weight (DW), respectively. Those given raw potatoes and boiled potatoes grew to 10.5 and 13.9 times their initial DW, respectively. BSFL that were fed whole pasta grew to 23.1 times their initial DW and those fed with blended pasta grew the most, to 34.1 times their initial DW.

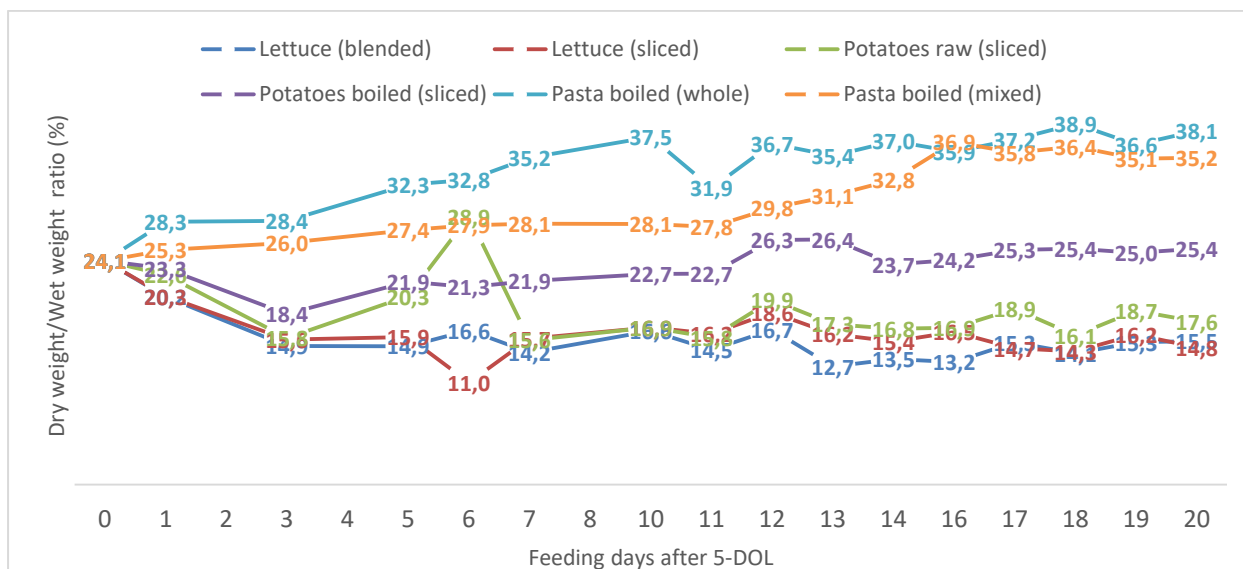


Figure: 3 Dry weight to wet weight ratio of BSF larvae fed with different substrates in time

Bioconversion rate (BCR)

The bioconversion rate of BSFL fed with different substrates is presented in Figure 4. It ranged for BSFL fed on different substrates from 7.4% to 14.8%. The highest BCR was achieved with blended pasta (14.8%), reflecting its higher protein and carbohydrate content. Blending the pasta improved

accessibility of the substrate to the larvae, which improved the BCR significantly from 8.9% to 14.8%. In contrast, raw potatoes had the lowest BCR, only 7.4%. Boiling increased the BCR to 9.2%. Lettuce resulted in intermediate BCR values of around 12% despite poor larval growth.

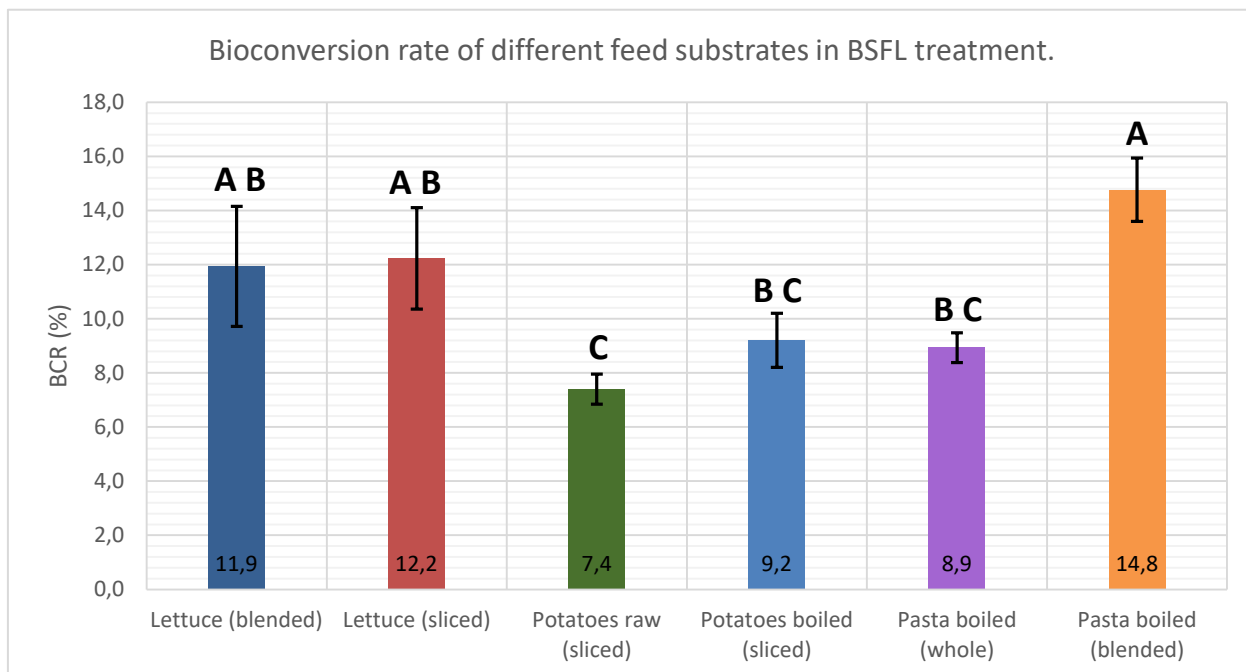


Figure: 4 Bioconversion rate of different feed substrates in BSFL treatment (mean, range, Tukey's HSD groups)

Length of the BSFL

The average final length of BSFL reared on each substrate is shown in Figure 5. Final length of the larvae ranged between 8.26 mm and 14.2 mm, depending on the feed substrate. On average, the smallest larvae were those fed with lettuce, then those with potatoes, and the largest were those fed with pasta.

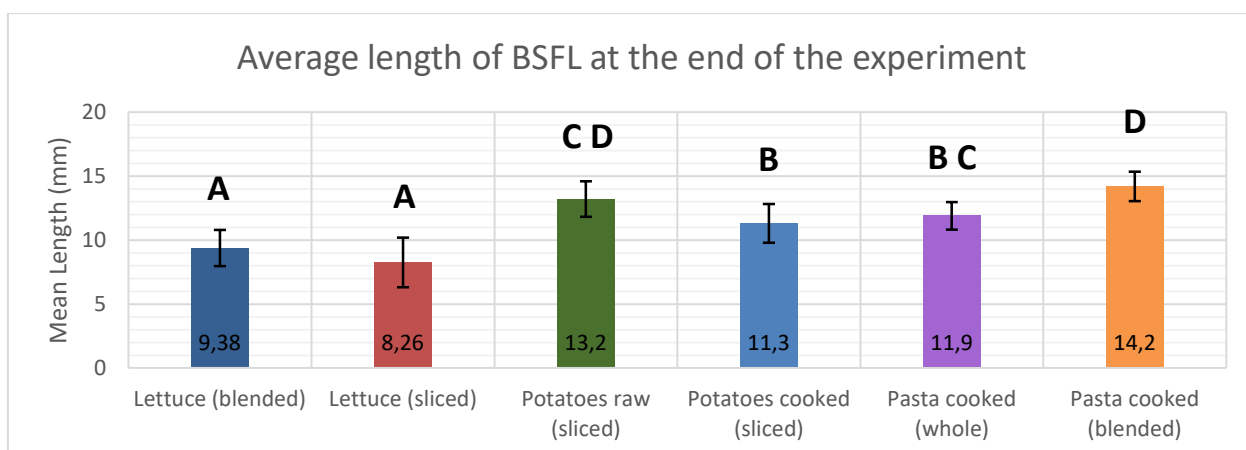


Figure: 5 Average length of BSF larvae at the end of the experiment (mean, range, Tukey's HSD groups)

Discussion

Previous studies identified that generally BSFL grown on substrates higher in proteins have a higher larval weight^{4,20}. This aligns with the results of our study. Our results confirm that the nutritional composition of the feeding substrate has a decisive effect on BSFL growth. Larvae fed with pasta which has the highest protein content of the three feeding substrates, grew the most in weight. On the other hand, larvae fed with lettuce characterized by low protein and high moisture grew the least in weight. The bioconversion rate further highlighted these differences.

Blending pasta increased the larval weight (48.6 mg to 77.6 mg) and BCR substantially (from 8.9% to 14.8%), indicating that substrate accessibility is an important factor for BSFL conversion efficiency. Boiling potatoes also improved the BCR from 7.4% to 9.2%, which indicates that thermal processing of potatoes enhances starch digestibility for the larvae. Lettuce resulted in intermediate BCR values (approximately 12%) despite the poor growth performance of BSFL. Although the fresh weight of lettuce provided to the larvae was comparable to that of pasta and potato, its substantially higher moisture content resulted in a much lower dry matter input. Since BCR was calculated on a dry matter basis, the relatively low amount of dry matter supplied by lettuce may explain why intermediate BCR values were obtained despite limited larval biomass production. The BCR values fall mostly within the range of approximately 2 to 33%^{1,21} and confirm that substrate composition and pre-processing have a strong influence on the efficiency of bioconversion.

Mechanical pretreatment is a widespread technology used in biogas production and composting. Reduced substrate particle size has previously positively correlated with biogas and methane yield^{22, 23}. However, a study²⁴ showed that the mechanical pretreatment of feeding substrate for BSFL, in their case decreasing almond hull particle size from 6 to 4 mm, decreased larval mass by 10%. The authors concluded that smaller particle size, and thus increased surface area of the substrate, could improve access to nutrients, but BSFL may benefit from better aeration due to the larger particle size. In our study the decreased particle size for lettuce might have hindered aeration of the BSFL and feed mixture, having a negative effect on larval growth. This could have been exacerbated by the water released from the blended lettuce, as indicated in a study²⁵. On the other hand, blending pasta had positive effect on larval growth. It shows that the positive effect of mechanical pre-treatment on larval growth is substrate dependent.

The main goal of thermal pre-treatment such as boiling of the substrate is to increase the solubility of the organic matter and improving biodegradability²⁵. This was confirmed in our study for potatoes as substrate.

These findings highlight both opportunities and constraints for BSFL bioprocessing. While pasta and potatoes are representative of carbohydrate-rich food residues that are frequently discarded by households, restaurants, and the food industry. Lettuce, on the other hand, represents high-moisture, low-nutrient vegetable waste. The poor performance of BSFL on lettuce alone suggests that such residues might be best used in combination with more nutrient-rich wastes to balance diet quality and moisture content.

A limitation of this study is that only three substrates were tested individually under controlled laboratory conditions. In practice, food waste is heterogeneous and mixed, and BSFL are known to perform differently on composite waste streams. Future work might thus address the BSFL performance fed with mixed substrate from the studied substrates.

Conclusions

This study demonstrated that BSFL growth depends on the nutritional quality and pre-processing of food substrates. Among lettuce, potatoes, and pasta, larvae fed with pasta achieved the highest biomass yield, followed by potatoes, while lettuce supported the lowest growth. Substrate pre-treatment also influenced outcomes: boiling improved the digestibility of potatoes, and blending increased the accessibility of nutrients in pasta but not in lettuce. These results suggest that carbohydrate- and protein-rich food residues such as pasta or potatoes can serve as effective substrates for BSFL biomass

production, while watery, nutrient-poor wastes like lettuce may require co-feeding with higher-quality substrates (higher energy density and protein content). By identifying the relationship between substrate composition, processing, and BSFL growth, this work contributes to the optimization of insect-based bioconversion strategies for sustainable waste management and protein production.

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Author Contributions

F.T.: Conceptualization, methodology, data curation, investigation, formal analysis, writing - original draft, visualization, project administration, funding acquisition; I.B.: conceptualization, supervision, writing - review and editing, project administration, funding acquisition.

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Biokonverzia šalátu, zemiakov a cestovín larvami vojačika čierneho (*Hermetia illucens*) na hodnotnú hmyziu biomasu

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Souhrn

Rastúci dopyt po udržateľných alternatívnych zdrojoch bielkovín a efektívnom nakladaní s potravinovým odpadom poukazuje na potenciál lariev muchy *Hermetia illucens* (BSFL). Táto štúdia skúma rast a efektívnosť biokonverzie BSFL chovaných na troch substrátoch – šaláte, zemiakoch a cestovinách. Tieto substráty boli predupravené metódami, ako je krájanie, mixovanie a varenie. Výkonnosť procesu spracovania substrátu larvami bola hodnotená na základe mokrej a suchej hmotnosti, dĺžky a miery biokonverzie (BCR). Cestoviny vykazovali najvyšší nárast biomasy, pričom mixovanie zvýšilo hmotnosť lariev na 77.6 mg a BCR na 14.8 % v porovnaní s 48.6 mg a 8.9 % pri celých cestovinách. Zemiaky ako substrát viedli k strednému rastu, pričom varenie zvýšilo BCR zo 7.4 % na 9.2 %. Šalát, charakteristický nízkym obsahom živín a vysokou vlhkosťou, vykazoval najmenší nárast hmyzej biomasy, pričom mixovanie mierne negatívne ovplyvnilo BCR. Zistenia ukazujú, že nutričné zloženie aj predúprava substrátov sú kľúčovými faktormi pre maximalizáciu rastu BSFL a výsledkov biokonverzie.

Kľúčová slova: BSFL; potravinový odpad; predúprava substrátu; biokonverzia; *Hermetia illucens*