Black soldier fly (*Hermetia illucens*) larvae processing as a strategy for solid waste reduction

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Nowadays, the total amount of bio-waste produced by food and beverage chains is huge due to the rise of the world population and the lack of adequate strategies to cope with this problem. The larvae of the black soldier fly (BSF), *Hermetia illucens* (Diptera, Stratiomyidae) can be reared on a wide variety of substrates, such as HO.RE.CA. (Hotel-Restaurant-Catering) solid waste, as well as agricultural- and food-processing solid residues. In this scenario, bioconversion of leftovers through BSF represents a sustainable solution, capable of addressing the solid waste issue and efficiently supplying a large amount of protein biomass rich in fats (Spranghers *et al*, 2017; Meneguz *et al*, 2018; Montevecchi *et al*, 2019).

The present report describes some of the main outcomes of the research, including the evaluation of i) the effectiveness of substrate reduction by BSF larvae ii) the management of the unpleasant smells released during larvae growth. The experiments aim at evaluating the reduction of the organic solid waste.

Three replicates were performed for each lot of HO.RE.CA. solid waste. The waste reduction was evaluated using a specific quantity of HO.RE.CA. solid waste (Table 1) and 100 young BSF larvae for each replicate. Experiments were performed in a climatic chamber at 27.0 ± 0.5 °C and 70 ± 10% RH. At the end of the experiment, when the insects reached the desired development stage, residual substrate (frass) was separated, weighed and stored in freezer for other analytical determinations. Samples of both initial and residual HO.RE.CA. solid waste were oven dried at 60 °C, grinded, homogenized, and subjected to the following analytical determinations: i) ash content; ii) crude protein content (Kjeldahl); and iii) lipid content (Soxhlet).

A minor but disturbing issue is represented by the strong and unpleasant odors released by the complex biological system under investigation: HO.RE.CA. solid waste (growth substrate), commensal microbial flora, and BSF larvae. The strategy planned to reduce the smells included the addition of several materials with adsorbent properties, to the frass: finely ground grape stalks (212-500 µm), coarsely ground grape stalks (500-850 µm), activated charcoal, and zeolitite were added (6% w/w). To evaluate the volatiles released, eight sampling were performed every other day after larvae inoculation. Headspace-solid phase microextraction (HS-SPME) was used to extract volatiles. A gas chromatograph (GC) coupled with a mass spectrometer (MS), equipped with a carbowax-like column, was used to carry out a semi-quantitative analysis of volatiles and verify the effectiveness of the adsorbent materials. Peak identification was performed by comparing mass spectra with those of pure reference standards and those present in software libraries (Nist 14 and Wiley).

Results indicate that HO.RE.CA. solid waste can be conveniently reduced by the action of BSF larvae (Table 1). The solid waste reduction was always higher than 63% w/w considering the BSF larval maturity and higher than 69% w/w considering the prepupal development stage. The waste reduction index (WRI, that includes the time variable) showed that higher performances were obtained using HO.RE.CA. solid waste, if compared to the control substrate (Gainesville diet). Instead, the best WRI values were obtained when grape stalks were added to the solid waste.

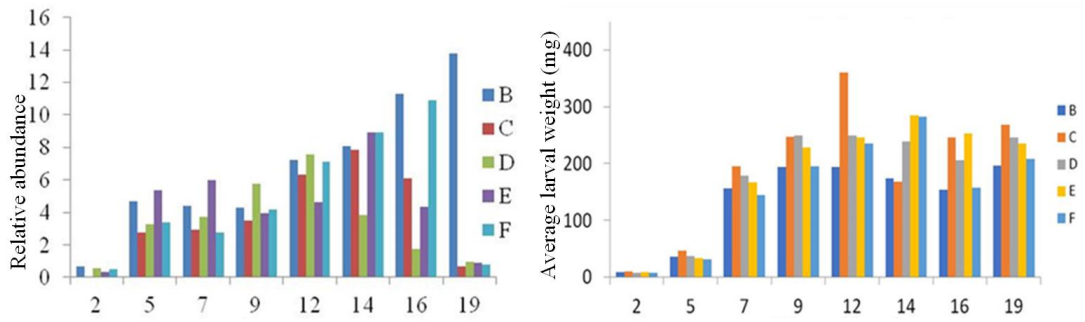
HS-SPME-GC-MS allowed volatile compounds to be grouped into four categories: i) malodorous volatile compounds (short and medium chain fatty acids, phenolic substances, and thioethers), ii) low boiling liquids (solvents), iii) aromatic compounds (mainly spices and citrus-like scents) already present in HO.RE.CA. leftovers, and iv) products deriving from various fermentation processes.

All the adsorbent materials tested showed similar aptitude to retain many of the volatile compounds responsible for unpleasant odors. As an example, the trend of isovaleric acid is reported (Fig. 1a). This short chain fatty acid is one of the responsible of the malodorous smell. At the end of the experiment the capacity of the different absorbent materials of retaining isovaleric acid was similar, while its concentration in the control was clearly more intense. Furthermore, no inhibitory effects on the larval growth were detected by adding any of the adsorbent materials (Fig. 1b), although finely ground grape stalks ensured a higher larval growth compared to the other substrates. However, grape stalks need to be dried, grinded, and sieved, thus worsening the life cycle assessment of the process. The actual possibility of employment therefore remains to be assessed.

Table 1. Results of BSF performances under different solid waste compositions

	Average larvae weight (g)	Initial substrate (g)	Final substrate (g)	Final day	Substrate reduction (%)	WRI	Humidity (%)	Dry residue (%)	Ashes (% d.w.)	Proteins (% d.w.)	Lipids (% d.w.)	Carbohydrates (% d.w.)
I lot				*(Prepupal stage)*			54.14	45.86	12.54	21.76	20.83	44.87
Fresh substrate		430.0	249.7	19	41.93	2.21						
Dry + Rehydrated >	0.245	400.0	145.2	39	75.69	1.63						
Dry + Rehydrated =	0.295	430.0	227.2	28	69.05	1.56						
Dry + Rehydrated <	0.225	350.0	107.1	42	73.65	1.65						
II lot				*(Prepupal stage)*			62.93	37.07	3.95	21.02	33.19	41.84
Zeolitite	0.282	350.0	100.5	38	71.29	1.79						
Activated charcoal	0.285	350.0	105.5	28	69.84	2.51						
Grape stalks (finely ground)	0.360	350.0	106.4	24	69.59	2.90						
Grape stalks (coarsely ground)	0.311	350.0	98.5	28	71.86	2.57						
Control (no adsorbent material)	0.270	350.0	95.2	42	72.81	1.73						
Control diet		350.0	100.4	30	71.31	2.38						
III lot				*(Larval stage)*			57.06	42.94	12.48			
HORECA -1 add	0.249	300.0	102.0	12	66.00	5.50						
HORECA -2 add	0.251	300.0	100.6	12	66.45	5.54						
HORECA -4 add	0.250	300.0	108.2	12	63.94	5.33						
Control -1 add		300.0	147.2	10	50.94	5.09						
Control -2 add		300.0	149.0	10	50.35	5.03						
Control -4 add		300.0	171.0	10	43.01	4.30						

Figure 1. **a)** Trend of the isovaleric acid concentration using various adsorbent materials and **b)** effects of the adsorbent materials on larval growth. B: Substrate consisting of HO.RE.CA. solid waste only (HSW); C: HSW + finely ground stalks; D: HSW + coarsely ground stalks; E: HSW + activated carbon; F: HSW + zeolitite



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