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Seasonal variability of the HO.RE.CA. food leftovers employed as a feeding substrate for black soldier fly (*Hermetia illucens* L.) larvae and effects on the rearing performance

Giuseppe Montevecchi [a, b, *], Laura Ioana Macavei [a, 1], Elena Zanelli [a], Giacomo Benassi [c], Giulia Pinotti [a], Sara D'Arco [a], Silvia Buffagni [a], Francesca Masino [a, b], Lara Maistrello [a, b], Andrea Antonelli [a, b]

[a] *Department of Life Science, University of Modena and Reggio Emilia, Via Amendola 2 (Pad. Besta), Reggio Emilia, 42122, Italy*

[b] *BIOGEST - SITEIA Interdepartmental Centre, University of Modena and Reggio Emilia, Piazzale Europa 1A, Tecnopolo di Reggio Emilia, Reggio Emilia, 42124, Italy*

[c] *Kour Energy S.r.l., Via Martiri di Cervarolo 74/10, Reggio Emilia, 42122, Italy*

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ABSTRACT

The SCALIBUR project (Horizon, 2020) aimed to explore innovative solutions, including the use of black soldier fly larvae, for the bio-urban waste management.

This research work describes the evaluation of the variability in water, proteins, fat, ashes, and carbohydrates present in the HO.RE.CA. food leftovers which were withdrawn from a local canteen over a 12-month period and the relationship with (i) the growth parameters of the larvae, (ii) the percentage of substrate reduction and the percentage of frass separated through the mechanical sieve at the end of the rearing process.

HO.RE.CA. food leftovers are overall a suitable feeding substrate for larval rearing. Water contained in the HO.RE.CA. food leftovers was sufficient for larval rearing without resorting to further addition. As for water content, a seasonal trend was not observed, on the contrary, it was proved to be totally random. However, high amount of water (>80%) was correlated with higher larval mortality rate.

The larval weight was significantly correlated to the amount of protein ($r = 0.80$; $p \leq 0.001$) present in the substrate, and to a lesser extent to the amount of fat ($r = 0.43$; $p \leq 0.05$). The feed conversion rate and bioconversion rate were both in agreement with literature data.

The statistical test did not show any significant correlation between the amount of water contained in the initial fresh HO.RE.CA. food leftovers and the percentage of substrate reduction and the percentage of frass separated through the mechanical sieve at the end of the rearing process.

* Corresponding author. Department of Life Science, BIOGEST - SITEIA Interdepartmental Centre, University of Modena and Reggio Emilia, Reggio Emilia, Emilia-Romagna, 42124, Italy.

E-mail address: giuseppe.montevecchi@unimore.it (G. Montevecchi).

1 Current address: Bioecology S.r.l., Via della Corte 4, Corte Tegge (RE), 42025, Italy.

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1. Introduction

To cope with the management of urban bio-waste, the SCALIBUR project (Horizon, 2020) proposes to explore innovative solutions, including the use of black soldier fly larvae (BSFL), *Hermetia illucens* (Diptera, Stratiomyidae) for the bioconversion of Hotel-Restaurants-Catering (HO.RE.CA.) food leftovers.

BSF is a non-pest insect, commonly present in temperate to tropical zones worldwide, which can be reared on a wide range of residual or decaying organic waste, such as fruit and vegetables (Barbi et al., 2020; Hadj Saadoun et al., 2020), animal products (Hadj Saadoun et al., 2020), and even chicken manure (Miron et al., 2019; Bortolini et al., 2020).

In this framework, the use of BSFL represents a sustainable solution, capable of addressing the organic waste problem and efficiently delivering a large amount of biomass rich in protein, fat, and chitin (Caligiani et al., 2018; Liu et al., 2019; Montevecchi et al., 2020). The components obtained from larval fractionation find application in numerous sectors ranging from feed (Schiavone et al., 2018; Biasato et al., 2019; Dabbou et al., 2020) and food (Montevecchi et al., 2021) to agricultural materials (Setti et al., 2020; Nuvoli et al., 2021; Barbi et al., 2021).

A highly frequented canteen or cafeteria is an abundant source of food leftovers, which can be analyzed to gain insight into the composition and quality of food residues in the HO.RE.CA. sector. These commercial businesses accumulate a wide range of food leftovers, which include leftovers from the kitchen, such as prepared but unserved food, partially used ingredients, perishable food, and expired or spoiled food, as well as plate scrapings. However, the quality of food residues might vary in relation to several factors: i) availability of fresh products such as fruit and vegetables; ii) requests for different types of food in cold and hot seasons; iii) expectations of canteen patrons.

Although the biochemical mechanisms of humans' seasonal preferences for particular types of food are not yet fully understood, some relationships are ascertained. In particular, individuals (especially males), require a greater quantity of energy foods in the winter period. Conversely, the intake of fruit, vegetables, eggs, meat, and cereals follows a seasonal pattern of consumption (Stelmach-Mardas et al., 2016; Folwarczny et al., 2022).

How the reasons mentioned above are reflected in the quality and quantity of food leftovers in a canteen is not at all easy to hypothesize. As a consequence, the variability of canteen food leftovers might have implications for the BSFL performance when used as a year-round substrate for sustainable production.

This research study aimed at investigating the variability of key components of HO.RE.CA. food leftovers (coming from kitchen only, excluding expired or spoiled food), collected from a local canteen over a 12-month period, in relation to i) the growth parameters of the BSFL; ii) the percentage of substrate reduction and the percentage of frass separated through the mechanical sieve at the end of the rearing process.

2. Materials and methods

2.1. Insects

The black soldier fly (*Hermetia illucens*) larvae were provided by the Applied Entomology Laboratory of the University of Modena and Reggio Emilia (Italy) where a permanent BSF colony is reared.

2.2. Equipment

The equipment used for the substrate initial treatment and the rearing chamber were all designed and realized within the SCALIBUR project.

2.3. Sampling and larval rearing

Kitchen leftovers were collected from a local canteen (CIRFOOD s.c., Reggio Emilia, Italy). Ten batches were collected between July 2020 and July 2021. In November 2020, two batches were collected on the same day and processed separately to verify the repeatability of the experiments. Each batch weighed approximately 40 kg and included leftovers from the kitchen, such as prepared but unserved food, partially used ingredients, and perishable food. Expired or spoiled food and plate scrapings were not collected.

After a thorough grinding using a two shafts shredder, each batch material was evenly mixed through a homogenizer and introduced in plastic trays together with 5-days old BSFL, using a larval density of 5 larvae/cm^2 and approximately 0.70–0.76 g of feeding substrate/larva, all administered at the beginning of the experiment. Three replicates were performed for each batch of HO.RE.CA. food leftovers collected.

The larval rearing was performed in a climatic chamber at 27.0 ± 0.5 °C and 70 ± 10% relative humidity. When the first pupae were observed, the rearing procedure was concluded, and the larval biomass was separated from the frass through a mechanical sieve, if possible; in the other cases the separation was done manually. For each replicate, based on five random samples (20 larvae each), the average weight of collected BSFL was recorded and the larvae were suppressed by blanching at 90 °C for 60 s.

The percentage of substrate reduction (SR%) was calculated based on the reduction in wet weight of the fresh feeding substrate using Eq. (1).

$$SR\% = (\text{substrate } g_{WM} - \text{residue } g_{WM}/\text{substrate } g_{WM}) \times 100 \quad \text{(Eq. 1)}$$

The mechanically separated frass (SF%) was assessed as the percentage of frass separated through the mechanical sieve, according to Eq. (2).

$$\text{SF\%} = (\text{separated frass } g_{WM} - \text{residual frass } g_{WM}/\text{separated frass } g_{WM}) \times 100 \quad \text{(Eq. 2)}$$

2.4. Larval performance

Larval rearing performance was assessed by measuring the larval average weight (g) using a weighing scale. Larval mortality was estimated as the percentage of dead specimens. Feed conversion rate (Eq. (3)) and bioconversion rate (Eq. (4)) were calculated based on wet weight:

$$\text{Feed conversation rate (FCR)} = \text{Substrate } g_{WM}/(\text{End larval biomass } g_{WM} - \text{Start larval biomass } g_{WM}) \quad \text{(Eq. 3)}$$

$$\text{Bioconversion rate (\%)} = (\text{End larval biomass } g_{WM} - \text{Start larval biomass } g_{WM})/\text{Substrate } g_{WM}) \times 100 \quad \text{(Eq. 4)}$$

2.5. Methods of analysis

2.5.1. HO.RE.CA. food leftovers characterization

Representative samples from each batch of fresh HO.RE.CA. food leftovers used as a feeding substrate were oven-dried at 60 °C – in order to avoid degradation of sugars, enzymes and other proteins – until constant weight to evaluate dry matter and moisture content.

Then, the samples were ground, homogenized, and subjected to the determination of: crude protein content using a Kjeldahl apparatus; fat using a Soxhlet apparatus; and ashes through incineration at 550 °C up to constant weight. Total carbohydrates (including starch and fibers) were calculated by difference.

2.5.2. Statistical analysis

Univariate and multivariate analyses were carried out on the data set. Statistical differences among the samples were assessed by analysis of variance (one-way ANOVA) based on three replicates. When a significant effect (at least $p \leq 0.05$) was shown, comparative analyses were carried out by the post-hoc Tukey's test.

All variables were subjected to a study of the linear correlations.

Principal component analysis (PCA) of the autoscaled values was also carried out. All statistical tests were performed using Statistica v8.0 software (former Stat Soft Inc., now TIBCO Software Inc., Palo Alto, USA).

3. Results and discussion

Table 1 shows the values of the proximate composition of HO.RE.CA. food leftovers and the growth performance of the BSF larvae fed with them. All the parameters analyzed showed statistically significant differences ($p \leq 0.001$) among the different batches of samples collected in different periods of the year.

Regarding the moisture content present in the HO.RE.CA. food leftovers, a seasonal trend was not observed. If this statement were confirmed by collecting a greater amount of data and larger samples, this would imply that in a canteen, the different seasonal availability of fruit and vegetable waste would not significantly affect the overall composition of leftovers. What emerges clearly is that the quantity of water present in the HO.RE.CA. food leftovers is sufficient for larval rearing without resorting to further water addition.

The amount of protein varied between 9.46% and 21.41% with an average value of 17.03% and a median of 18.09%. The amount of protein in the substrate can influence performance of the BSF larvae since these nutrients are essential for their proper growth (≥15% DM proteins) (Lalander et al., 2019; Gold et al., 2020).

The amount of fat in the substrate varied from a minimum of 6.70% to a maximum of 16.34%, with an average value of 10.84% and a median of 11.27%. The role of fatty substances during growth is still under study. In some experiments of the present work, they tended to stratify on the surface in the rearing trays. Some authors have hypothesized that the lipid layer that stratifies on top of the

Table 1
Proximate analysis of substrate batches. The one-way ANOVA is expressed as p_{values}. ***$p \leq 0.001$. Tukey's test results are reported. Different superscript letters identify factors that are significantly different ($p \leq 0.05$).

Date	Dry matter (%)	Moisture (%)	Protein (%)	Ashes (%)	Fat (%)	Total Carbohydrates (%)	Protein:Carbohydrate
p_{value}	***	***	***	***	***	***	
29/07/2020	$16.77^{e} \pm 0.12$	$83.23^{a} \pm 0.12$	$21.41^{a} \pm 0.26$	$5.321^{cd} \pm 0.002$	$11.31^{abc} \pm 0.08$	$61.96^{bc} \pm 0.29$	1:2.89
03/08/2020	$19.63^{d} \pm 0.32$	$80.37^{b} \pm 0.32$	$21.34^{a} \pm 0.22$	$5.880^{bcd} \pm 0.004$	$8.34^{bc} \pm 0.62$	$64.44^{bc} \pm 0.62$	1:3.02
21/10/2020	$15.70^{e} \pm 0.20$	$84.30^{a} \pm 0.20$	$16.19^{e} \pm 0.14$	$6.22^{bcd} \pm 0.05$	$9.92^{abc} \pm 3.38$	$67.67^{b} \pm 3.34$	1:4.18
12/11/2020	$22.30^{c} \pm 0.26$	$77.70^{c} \pm 0.26$	$19.69^{b} \pm 0.17$	$7.45^{b} \pm 0.06$	$9.45^{bc} \pm 2.21$	$63.40^{bc} \pm 1.75$	1:3.22
12/11/2020 bis	$23.03^{bc} \pm 0.06$	$76.97^{cd} \pm 0.06$	$17.78^{d} \pm 0.16$	$6.823^{bc} \pm 0.003$	$13.62^{ab} \pm 1.40$	$61.78^{bc} \pm 1.40$	1:3.47
15/01/2021	$23.20^{bc} \pm 0.20$	$76.80^{cd} \pm 0.20$	$9.46^{h} \pm 0.08$	$2.962^{f} \pm 0.002$	$10.24^{abc} \pm 3.45$	$77.34^{a} \pm 3.45$	1:8.18
25/02/2021	$19.43^{d} \pm 1.89$	$80.57^{b} \pm 1.89$	$14.24^{f} \pm 0.13$	$3.40^{ef} \pm 0.57$	$6.70^{c} \pm 1.87$	$75.66^{a} \pm 1.39$	1:5.31
05/05/2021	$24.57^{b} \pm 0.31$	$75.43^{d} \pm 0.31$	$18.39^{c} \pm 0.16$	$4.76^{de} \pm 0.07$	$16.34^{a} \pm 0.54$	$60.51^{c} \pm 0.47$	1:3.29
20/05/2021	$22.43^{c} \pm 0.72$	$77.57^{c} \pm 0.72$	$19.46^{b} \pm 0.15$	$6.09^{bcd} \pm 1.68$	$14.32^{ab} \pm 0.88$	$60.13^{c} \pm 1.81$	1:3.09
02/07/2021	$28.37^{a} \pm 0.71$	$71.63^{e} \pm 0.71$	$12.32^{g} \pm 0.11$	$11.572^{a} \pm 0.003$	$8.13^{bc} \pm 3.81$	$67.98^{b} \pm 3.81$	1:5.52

substrate can reduce the evaporation rate, thus maintaining a high-water content in the growth medium (Lopes et al., 2020). Furthermore, the high fat content makes the mobility of the larvae within the substrate less favorable.

The ash content was extremely variable ranging from a minimum of 2.96% to a maximum of 11.57%, with an average value of 6.05% and a median of 5.88%. It is very likely that it is closely related to the quantity of salt (composed primarily of sodium chloride) used as a condiment that is discarded along with the food. An excessive amount of salt has caused an unfavorable effect on larval weight and pupation ratio (Cho et al., 2020). Some authors have suggested keeping the amount of macro and micro-nutrients under strict control. However, this is a very complex practice given their variety and different concentrations in HO.RE.CA. food leftovers (Gold et al., 2020).

The calculation of carbohydrates was carried out by difference. This does not allow to establish the quantity of simple sugars compared with the complex ones, as well as the real quantity of hydrolysable biopolymers (such as starch) compared with the fibrous fractions (such as cellulose, hemicelluloses, and non-polysaccharidic fibers) that are indigestible even by the BSF larvae. However, fibrous fractions can represent a prebiotic source for the microbiota of the larval gut, thus resulting in a positive effect on larval growth (Fuso et al., 2021). The maximum carbohydrate value was 77.34%, while the minimum value was 60.13%, with an average of 66.09% and a median of 63.83%.

The protein to carbohydrate ratio was also calculated (Cammack and Tomberlin 2017). All protein/carbohydrate values were off balance towards the carbohydrate side, ranging from a minimum of 1:2.89 to values around 1:5.50. In only one case, the ratio was higher than 1:8.00; the average value was 1:4.22, while the median value was 1:3.38.

Larval growth performances showed average larval weight values with a minimum of 0.12 g (10.7 days of trails) and a maximum of 0.19 g (11.0 days of trails), average and median values of 0.16 g (Table 2). This leads to a coefficient of variation between the minimum value and the maximum value of 31.93%, which results in a significant difference in terms of the growth performance of the larvae for the same time.

Larval mortality showed values below 20% in most cases, while the value exceeded 40% in only two cases (Table 2). Food conversion rate showed values between 4.23 and 8.69, while only one value was higher than 10 (13.67). The mean value was 6.68 and the median 5.37. The bioconversion rate, that measures the conversion efficiency of the substrate by the larvae, showed a maximum value of 24.05% and only one value below 10%. The mean value was 17.63% and the median was 18.95%. Although the ANOVA showed significant differences, trends attributable to the season were not evident for both conversion indices. In a recent study (Gold et al., 2020), bioconversion rates of 15.3% and 22.7% have been found for canteen waste and vegetable canteen waste, respectively. In a previous study (Nyakeri et al., 2017), the authors have found a bioconversion rate of 20.8%, which is in line with some of the values found in the present study.

The substrate reduction showed values between a minimum of 75.23% and a maximum of 95.54%, with a mean value of 86.49% and median of 88.42%. The analysis of figures for mechanically separated frass was more complex. In three cases its consistency did not allow for mechanical separation, so the frass was separated manually. In the other cases the minimum value was 45.74% and the maximum 93.91%, with an average value of 76.81% and a median of 82.26%.

In order to establish which are the parameters that most influence the larval weight, an evaluation of the linear correlations was carried out. Only significant correlations (at least $p \leq 0.05$) were discussed, along with the non-significant ones made regarding the research study aims. The larval weight was significantly correlated to the quantity of proteins present in the substrate ($r = 0.80$; $p \leq 0.001$), and negatively correlated to the quantity of carbohydrates ($r = -0.85$; $p \leq 0.001$). However, the quantities of proteins and carbohydrates in the substrate were each other inversely correlated with a high significance ($r = -0.79$; $p \leq 0.001$). Significantly lower protein contents have been found in BSF larvae reared on fruit by-products, while the protein content has increased when the larvae have been fed using higher protein legume waste (Fuso et al., 2021).

Regarding fat content, similar relationships were found. Indeed, fat was slightly positively correlated with larval weight ($r = 0.43$; $p \leq 0.05$), while it was negatively correlated with the carbohydrate content ($r = -0.68$; $p \leq 0.001$). Although there are

Table 2
Average larval weight, larval mortality, feed conversion rate, bioconversion rate, substrate reduction, and mechanically separated frass. The one-way ANOVA is expressed as p_{values}. ***$p \leq 0.001$. Tukey's test results are reported. Different superscript letters identify factors that are significantly different ($p \leq 0.05$).

Date	Average larval Weight (g)	Larval mortality (%)	Feed conversion rate	Bioconversion rate (%)	Substrate Reduction (%)	Mechanically separated frass (%)
p_{value}	***	***	***	***	***	***
29/07/2020	$0.164^{d} \pm 0.002$	$42.88^{a} \pm 0.11$	$13.67^{a} \pm 1.07$	$7.66^{d} \pm 0.60$	$92.02^{ab} \pm 0.80$	$45.74^{d} \pm 0.92$
03/08/2020	$0.184^{b} \pm 0.002$	$9.17^{e} \pm 0.03$	$4.89^{e} \pm 0.04$	$21.25^{ab} \pm 0.15$	$79.54^{de} \pm 2.20$	Manual separation
21/10/2020	$0.153^{e} \pm 0.002$	$22.32^{c} \pm 0.12$	$6.89^{bc} \pm 0.21$	$15.91^{c} \pm 0.65$	$75.23^{e} \pm 2.51$	Manual separation
12/11/2020	$0.163^{d} \pm 0.004$	$0.00^{i} \pm 0.00$	$4.84^{d} \pm 0.10$	$21.51^{ab} \pm 0.41$	$90.55^{ab} \pm 2.03$	$83.46^{b} \pm 1.20$
12/11/2020 bis	$0.162^{d} \pm 0.003$	$0.00^{i} \pm 0.00$	$4.51^{e} \pm 0.04$	$21.61^{ab} \pm 0.05$	$91.35^{ab} \pm 2.91$	$82.65^{b} \pm 1.06$
15/01/2021	$0.119^{g} \pm 0.001$	$4.99^{g} \pm 0.03$	$7.36^{b} \pm 1.07$	$16.07^{c} \pm 1.99$	$79.29^{de} \pm 0.90$	$80.93^{b} \pm 0.80$
25/02/2021	$0.140^{f} \pm 0.002$	$41.78^{b} \pm 0.21$	$8.69^{b} \pm 0.02$	$11.43^{d} \pm 0.08$	$95.54^{a} \pm 1.73$	$93.91^{a} \pm 1.10$
05/05/2021	$0.17^{c} \pm 0.01$	$16.87^{d} \pm 0.09$	$5.28^{cd} \pm 0.48$	$19.44^{bc} \pm 1.74$	$88.57^{bc} \pm 2.16$	$83.64^{b} \pm 0.92$
20/05/2021	$0.194^{a} \pm 0.003$	$5.41^{f} \pm 0.04$	$4.23^{e} \pm 0.07$	$24.05^{a} \pm 0.29$	$84.00^{cd} \pm 1.86$	Manual separation
02/07/2021	$0.16^{d} \pm 0.01$	$3.78^{h} \pm 0.03$	$5.00^{e} \pm 0.48$	$20.10^{ab} \pm 1.85$	$88.77^{bc} \pm 3.05$	$67.34^{c} \pm 1.05$

not unanimous conclusions in the literature, the fat content can positively influence the growth performance of the BSF larvae because it increases the energy density of the feed (Oonincx et al., 2015).

An interesting correlation was found between the total moisture content in the substrate and the high larval mortality (r = 0.63; $p \leq 0.001$). This might be due to an excess of liquid together with a lack of nutrients. In particular, it seemed that the critical threshold was set around 80%. In fact, values higher than 80% of moisture present in the substrate were associated with a higher larval mortality. Recent studies have shown similar results (Lalander et al., 2020; Bekker et al., 2021).

The conversion indices, food conversion rate and bioconversion rate, which are inversely correlated with each other (r = −0.96; $p \leq 0.001$) because of the mathematical formula, showed interesting correlations. In particular, the food conversion rate was positively correlated with substrate moisture content (r = 0.81; $p \leq 0.001$) and with larval mortality (r = 0.83; $p \leq 0.001$), and negatively correlated with the days of trials (r = −0.48; $p \leq 0.05$). A higher larval mortality, due to the high moisture content and therefore to a lack of nutrients, were both factors predisposing to a higher (less efficient), feed conversion rate. In addition, the higher the days of trials the higher the efficiency of conversion. Correlations of the opposite sign, but of similar magnitude, were shown by the bioconversion rate.

The statistical test did not show any significant correlation between the amount of water contained in the initial fresh HO.RE.CA. food leftovers and the SR% nor the SF% at the end of the rearing process, while SR% and SF% were each other correlated (r = 0.67; $p \leq 0.001$). Finally, the food conversion rate was negatively correlated with SF% (r = −0.68; $p \leq 0.01$). This correlation suggested that the higher the substrate conversion rate, the easier it was to mechanically separate the frass from the larval mass.

3.1. Principal component analysis

Autoscaled data were processed via principal component analysis (PCA). The principal component 1 (PC1; 34.83% of the total variance) was mainly described by the bioconversion rate and dry matter of the substrates with positive sign, while the moisture content of the substrate, feed conversion rate and larval mortality characterized the PC1 with negative loadings (Fig. 1a). The PC2 (25.60% of the total variance) was mainly characterized by the protein content and larval weight with positive loadings and the carbohydrate content and the percentage of mechanically separate frass with negative loadings on this PC. Larval weight also showed an inverse correlation with the total carbohydrate content.

The score plot of the PC1 vs PC2 (Fig. 1b) shows a cluster of substrate samples collected during wintertime (in blue) characterized by a high amount of carbohydrates. However, substrate samples collected during summertime (in red) are not grouped into a single cluster, being characterized by different moisture quantity. The samples collected on the same day Nov 12, 2020 showed that there is a good repeatability in the results.

PC3 (14.21% of the total variance) was mainly characterized by the SR% and the SF%, both with a positive loading (Fig. 2a). It turns out that the two batches collected during wintertime are placed in different positions in the plane (Fig. 2b), as well as the substrate batches collected during summertime. In fact, there was no correlation between the SR% and the SF% with the initial moisture content. This confirmed that the abundance of fat and protein had a positive effect on larval weight.

4. Conclusions

The investigation of the variability of the key components of HO.RE.CA. food leftovers showed that the protein content of the rearing substrate is an essential parameter for optimizing larval weight gain, with fat content having a lesser effect. HO.RE.CA. food leftovers are overall a suitable feeding substrate for larval rearing. The food conversion rate and the bioconversion rate are both in line with literature data.

The water contained in the HO.RE.CA. food leftovers is sufficient for BSFL rearing without requiring additional moisture, unlike drier substrates. While there is no seasonal trend in the substrate water content, this parameter is correlated with the type of food leftovers collected over a limited timeframe. However, the high amount of water (>80%) was correlated with a higher larval mortality rate.

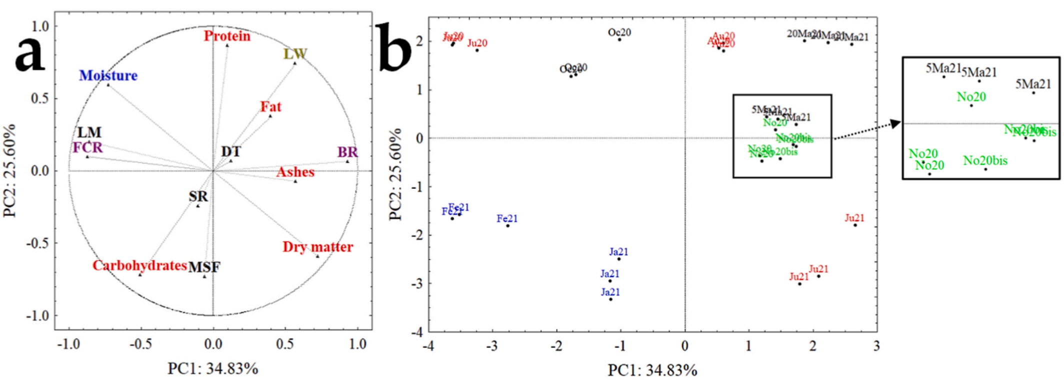

Fig. 1. a) Loading plot of the PC1 vs. PC2 with the respective variances; **b**) PC1 vs PC2 score plot. LW: larval weight; LM: larval mortality; DT: days of trial; SR: percentage of substrate reduction; MSF: mechanically separated frass; FCR: food conversion rate; BR: bioconversion rate.

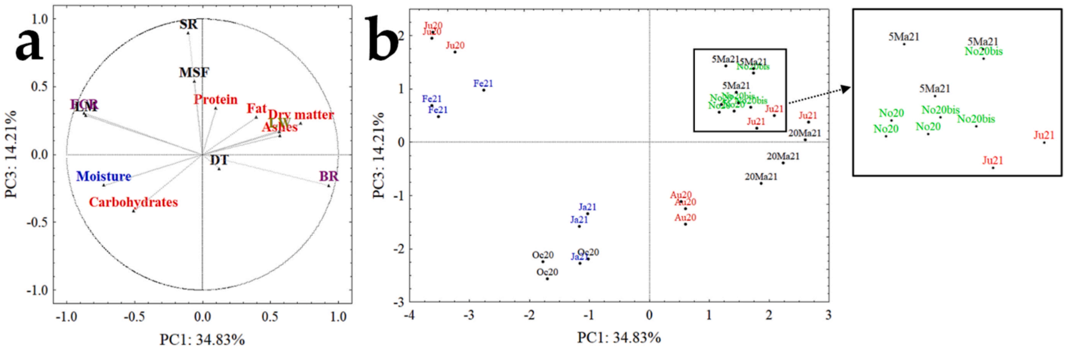

Fig. 2. a) Loading plot of the PC1 vs. PC3 with the respective variances; **b**) PC1 vs PC3 score plot. LW: larval weight; LM: larval mortality; DT: days of trial; SR: percentage of substrate reduction; MSF: mechanically separated frass; FCR: food conversion rate; BR: bioconversion rate.

At the end of the larval growth process, the residual moisture plays a fundamental role in the separability of the frass. However, neither the percentage of substrate reduction nor the mechanically separated frass were statistically correlated to the initial water quantity contained in the HO.RE.CA. food leftovers.

CRediT author statement

Giuseppe Montevecchi, conceptualization, data curation, funding acquisition, project administration, supervision, roles/writing - original draft, **Laura Ioana Macavei**, conceptualization, data curation, supervision, writing - review & editing, **Elena Zanelli**, formal analysis, investigation, roles/writing - original draft, **Giacomo Benassi**, investigation, resources, **Giulia Pinotti**, formal analysis, **Sara D'Arco**, formal analysis, **Silvia Buffagni**, formal analysis, **Francesca Masino**, methodology, resources, **Lara Maistrello**, methodology, project administration, writing - review & editing, **Andrea Antonelli**, funding acquisition, methodology, project administration, writing - review & editing.

All authors have read and agreed with the submitted version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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