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Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae

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1 1 Effect of rearing substrate on growth performance, waste reduction
2 2 efficiency and chemical composition of black soldier fly (*Hermetia illucens*)
3 3 larvae†

5 5 RUNNING TITLE: Rearing substrate effects on performance and nutritional
6 6 composition of black soldier fly

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ABSTRACT

BACKGROUND: Wastes can be used as rearing substrate by black soldier fly (BSF) larvae, the latter being exploitable as protein source in animal feed. This research aimed to assess the influence of four rearing substrates [Trial 1 (organic wastes): a mixture of vegetable and fruit (VEGFRU) vs a mixture of fruits only (FRU); Trial 2 (agro-industrial by-products): brewery (BRE) vs winery (WIN) by-products] on BSF larvae development, waste reduction efficiency, and nutritional composition.

RESULTS: If respectively compared to FRU and WIN, VEGFRU and BRE larvae needed less time to reach the prepupae stage (22.0, 22.2, 20.2 and 8.0 days of trial, respectively) and had higher protein content (229.7, 257.3, 312.9 and 395.7 g kg⁻¹ DM). The waste reduction index ranged from 2.4 (WIN) to 5.3 g d⁻¹ (BRE). BRE larvae showed the lowest saturated and the highest polyunsaturated fatty acids proportions (612.4 and 260.1 g kg⁻¹ total fatty acids, respectively).

CONCLUSION: Vegetable and fruit wastes and winery by-products can be used as rearing substrates for BSF larvae mass production. Brewery by-products led to very promising larvae performances and nutritional composition. However, given BRE limited availability, low BRE dietary inclusion levels could be used with the purpose of increasing larvae performances.

Keywords: organic waste, agro-industrial by-product, *Hermetia illucens*, animal feed, crude protein, fatty acid profile

INTRODUCTION

The world population is estimated around 7.3 billion, with a growth rate of about 83 million per year. This increase will generate an increment of food demand with a consequent rise in waste and by-products production.¹ Urgent and innovative solutions are needed for the management of the waste streams (WS) that nowadays are estimated around 1.3 billion and 100 million tons per year in the world and in the European Union, respectively.^{1,2} Furthermore, the EC Directive No 2008/98 unequivocally establishes the order of priority in the choice of WS treatment, the first being their reuse and the last their landfill disposal.

Some WS could be valorized through the recovery of the residual bio-elements they contain, with a cost reduction both for the industry (disposal cost) and the environment (pollution).³ The use of insects in the bioconversion of WS constitutes a new approach and an interesting example of sustainable circular economy. This bioconversion can generate new elements such as proteins and lipids for animal feeds,^{4,5,6,7} biodiesel,⁸ high value products as chitin⁹ or anti-microbial peptides.¹⁰

Processed proteins from seven insect species have recently been approved for aquafeed by the EC Regulation No 2017/893, which also lists the licensed rearing substrates. Among authorized species, black soldier fly (BSF; Diptera: Stratiomyidae) is one of the most promising and researches recently aimed to increase knowledge on optimal rearing substrates for larvae and prepupae. In this respect, BSF has shown great flexibility as it can be used to reduce volume and add value to various wastes.^{8,11,12} The available literature has highlighted that BSF life cycle and nutritional composition are noticeably influenced by the rearing substrate,^{13,14} with the crude protein (CP) content of the larvae ranging from about 317 to 630 g kg⁻¹ dry matter (DM).^{7,15,16}

In 2014, around 90 million tons of slaughter and vegetable WS were produced in Europe (EUROSTAT(http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_wasge&n&lang=en)). Considering the Italian context, 54% of the total production of waste

and agro-industrial by-products is generated by the manufacturing of vegetable products.¹⁷ About 1.5 million tons of winery by-products and 406 tons of brewery by-products are produced every year (EUROSTAT(<http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tag00034&plugin=1>; EU Report (https://www.brewersofeurope.org/site/media-centre/index.php?doc_id=905&class_id=31&detail=true)).¹⁸

The aim of this research was to evaluate the effects of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector as rearing substrates for BSF larvae on their development, waste reduction efficiency and chemical composition.

MATERIAL AND METHODS

Two trials were carried out at the Experimental Facility of the Department of Agricultural, Forest and Food Sciences (DISAFA; University of Torino, Torino, Italy).

Rearing substrates

In Trial 1, two organic wastes were compared:

- Vegetable-fruit waste (VEGFRU) obtained from a street market (Torino, Italy) and containing a mixture of vegetables and fruits (celery 43.4%, oranges 28.9% and peppers 27.7%);

- Fruit waste (FRU) obtained from a fruit market (Torino, Italy) and containing fruits only (apples 47.8%, oranges 15.5%, apple leftovers 13.8%, strawberries 7.1%, mandarins 4.8%, pears 4.1%, kiwis 3.4%, bananas 1.9% and lemons 1.6%).

In Trial 2, two agro-industrial by-products were used:

- Winery by-product (WIN) obtained during the wine making process, before the alcohol extraction, from a private distillery (Distilleria Santa Teresa dei Fratelli Marolo S.R.L., Alba (CN), Italy) and containing grape seeds, pulp, skins, stems and leaves;

111 - Brewery by-product (BRE) obtained during beer production (IFN 5-00-517 Barley
112 brewers grains wet) from a private brewery ("Birrificio dei Santi", Castelnuovo Don
113 Bosco (AT), Italy).

114 Each substrate was ground with a 3 mm die meat mincer (FTS136; Fama Industrie
115 S.r.l., Rimini, Italy) and carefully mixed.

116 A sample of each substrate was freeze-dried and frozen at -80°C for further
117 chemical analysis, while the remaining was stored at -20°C until it was fed to the
118 larvae.

119
120 **BSF eggs**

121 BSF eggs laid on corrugated cardboards for less than 24 h, were purchased from a
122 private company (CIMI S.r.l., Cervasca (CN), Italy). The cardboards with the eggs
123 were immediately transported to the DISAFA Experimental Facility. The cardboards
124 were put onto plastic boxes (25cm × 33cm × 12cm) which contained whole rye
125 thoroughly mixed with water (60% moisture) as rearing substrate for the newborn
126 larvae. The plastic boxes were placed into climatic chambers under controlled
127 environmental conditions (T: 27±0.5°C; RH: 70±5%; 24:0 L:D photoperiod). The
128 eggs hatched approximately three days after oviposition.

129
130 **Experimental design and calculations**

131 *Larvae development and waste reduction efficiency*

132 Six-day-old larvae were used in both trials. In each trial, for the evaluation of
133 larvae development (weight and length) and waste reduction efficiency, six
134 replicates of 100 larvae were weighed (KERN PLE-N v. 2.2; KERN & Sohn GmbH,
135 Balingen-Frommern, Germany; d: 0.001) and assigned to each rearing substrate.
136 The method reported by Harnden and Tomberlin¹⁹ was used to count the larvae. For
137 each replicate, the larvae were placed into plastic containers (10cm × 17.5cm ×
138 7cm), directly on the rearing substrate (100 g per replicate). The containers were
139 covered with a perforated cap with a black nylon grid and placed in a climatic

chamber under controlled environmental conditions (T: $27 \pm 0.5^\circ\text{C}$; RH: $70 \pm 5\%$; 24:0 L:D photoperiod).

Each replicate was monitored daily to control the quantity of available feed. If needed, as reported by Harnden and Tomberlin,¹⁹ 50 g of substrate per replicate was added in all replicates at the same time.

To avoid the effect of handling on the considered dependent variables,¹³ weight and length data were collected every four days until the appearance of the first prepupae, thereafter every day for the relative substrate. Thirty larvae were randomly sampled for three consecutive times from each container to measure weight and length. As measurement was not destructive, the larvae were re-introduced into the containers between two consecutive samplings. The sampled larvae were individually cleaned, dried with a paper towel and weighed, and photographed orthogonally (Lumix G1; Panasonic Corporation, Kadoma, Osaka, Japan) with a metric scale (mm). The images were analyzed with ImageJ software package (v. 1.50b) to record larvae length (i.e., from mouthpart to the bottom of the last abdominal segment).

For each container, weight and length data collection ended when 30% of the larvae reached the prepupae stage. The prepupae were removed from the containers. The remaining 70% of the larvae were hand-counted, washed, dried with a paper towel and individually weighed and photographed. The total final biomass (larvae + prepupae) and the residual rearing substrate were also weighed.

The following parameters were then calculated:

– larvae mortality (LM)

$$\text{LM} = [\text{initial number of larvae} - (\text{final number of larvae} + \text{number of prepupae})] / \text{initial number of larvae} * 100;$$

– growth rate (GR),²⁰ readapted for this research substituting prepupa body weight (g) with larva body weight (g)

$$\text{GR} = (\text{larva average final body weight (g)} - \text{larva initial body weight (g)}) / \text{days of trial (d)};$$

169 - substrate reduction (SR)²¹
170 $SR = [(distributed\ substrate\ (g) - residual\ substrate\ (g)) / distributed\ substrate$
171 $(g)] * 100;$
172 - waste reduction index (WRI)²⁰
173 $WRI = [(W - R) / W] / days\ of\ trial\ (d) * 100$
174 where W = total amount of rearing substrate distributed during the trial (g); R =
175 residue substrate (g);
176 - efficiency of conversion of digested food (ECD)²⁰
177 $ECD = total\ final\ biomass\ (g) / (total\ feed\ distributed\ (g) - residual\ substrate\ (g))$
178 where total final biomass = larvae + prepupae; residual substrate = undigested
179 food + excretory products.
180 Parameters related to waste reduction efficiency (SR, WRI and ECD) were
181 calculated on a fresh matter basis.

183 *Larvae nutritional composition*

184 For each trial, a second set of six replicates per rearing substrate was
185 simultaneously prepared with the aim to rear a sufficient amount of larvae to be
186 analyzed for their proximate composition and fatty acid - FA - profile. Five hundred
187 hand-counted 6-day-old larvae were placed into plastic containers of bigger size
188 (25cm × 33cm × 15cm) than those used for the larvae development and waste
189 reduction efficiency test, following the same relationships between (i) number of
190 larvae / container size surface, and (ii) amount of administered feed / larvae
191 density. The larvae were not handled until the appearance of the first prepupa.
192 Then, each container was checked daily and the identified prepupae were removed.
193 The trial ended when the 30% of the larvae reached the prepupae stage. The
194 remaining larvae were then manually separated from the residual rearing substrate,
195 washed, slightly dried with paper towel, weighed and frozen at -80°C until being
196 freeze-dried.

Chemical analyses of rearing substrates and larvae

Samples of freeze-dried rearing substrates and larvae were ground using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland). They were analyzed for DM, ash, CP and EE following AOAC International methods as detailed in Gasco *et al.*⁵ For the determination of the CP of whole BSF larvae, in addition to the conventional nitrogen-to-protein (N-factor) conversion factor of 6.25, the more accurate N-factor of 4.67 suggested by Janssen *et al.*²² was used. Neutral detergent fiber (NDF) was analyzed according to Van Soest *et al.*²³ Acid detergent fiber and acid detergent lignin (ADF and ADL) were determined according to method no. 973.18 of AOAC International.²⁴ The residual nitrogen in ADF (ADFN) was determined according to method no. 984.13 of AOAC International.²⁴ Chitin (CHI, g kg⁻¹ DM) was estimated as: ash free ADF (g kg⁻¹) – ADFN * N-factor (g kg⁻¹).⁹ Gross energy (GE) was determined using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). The FA composition of substrates and larvae was assessed.²⁵ The results were expressed as g kg⁻¹ of total detected fatty acids (TFA) (Table 2).

Statistical analyses

The statistical analysis of data was performed using IBM SPSS Statistics v. 20.0 for Windows. The two trials were considered separately. Larvae weights and lengths were subjected to a Two-Way Mixed ANOVA. The Shapiro-Wilk test was used to verify if the dependent variables were normally distributed for each combination of the groups of within- (test day, considered as a repeated measure) and between- (rearing substrate) subjects factors. The Levene's test was used to verify the homogeneity of variances for each combination of the groups of within- and between-subjects factors. The Mauchly's test was used to verify the assumption of sphericity; if such an assumption was violated, the Greenhouse-Geisser or the Huynh-Feldt correction (in cases of estimates of sphericity lower or higher than 0.75, respectively) was applied to correct the degrees of freedom of the F-distribution. Final larvae weights and lengths (average weight and length of the

leftover 70% larvae after removing the 30% of prepupae) were further subjected to independent samples Student's *t*-tests to assess differences between rearing substrates. Differences in terms of larvae growth performances, waste reduction efficiency, proximate composition and FA profile between substrates were also assessed using independent-samples Student's *t*-tests. The Kruskal-Wallis test was used to compare the time needed by the larvae to reach the prepupae stage. Significance was declared at $P<0.05$.

RESULTS

Growth performances and waste reduction efficiency of the BSF larvae

The effect of the rearing substrate on the development of BSF larvae over time is reported in Figure 1 (Trial 1) and Figure 2 (Trial 2). In both trials, results from the Two-Way Mixed ANOVA showed that, for larvae development (weight and length), rearing substrate, test day and their interaction were highly significant ($P<0.001$), VEGFRU and BRE performing better than FRU and WIN, respectively. In Trial 1, no differences were observed for larvae weight (mean $\pm$ SD: 0.004 ± 0.0002 g) at the beginning of the trial (day 0; 6 days-old larvae) (Figure 1A). Differences appeared after 4 days of trial, with a higher weight in the VEGFRU larvae (0.055 ± 0.0084 g) compared to the FRU larvae (0.037 ± 0.0058 g). Such trend was maintained at each test day until day 16 (VEGFRU: 0.148 ± 0.0103 g; FRU: 0.120 ± 0.0094 g) when VEGFRU larvae started to enter in the prepupae stage. The final weight of the larvae did not show differences between the two rearing substrates. At the beginning of Trial 1, VEGFRU and FRU larvae showed length values of 5.7 ± 1.32 mm and 5.3 ± 1.17 mm, respectively ($P<0.05$; Figure 1B). VEGFRU larvae continued showing higher length values than FRU larvae until the

last statistical assessment (day 16). VEGFRU and FRU larvae achieved a final length of 17.7 ± 0.46 mm and 17.8 ± 0.51 mm, respectively ($P > 0.05$). At the beginning of Trial 2, no differences were observed between WIN and BRE for larvae weight (0.007 ± 0.0011 g) (Figure 2A). Remarkable differences were reported after 4 days of trial, with a higher weight in the BRE larvae compared to the WIN larvae (0.092 ± 0.0063 and 0.017 ± 0.0018 g, respectively). The final weight of the larvae (reached after 8 and 26 days of trial for BRE and WIN, respectively) did not show differences between treatments. The mean length of 6-day-old larvae (day 0) was 6.5 ± 1.36 and 6.4 ± 1.24 mm for BRE and WIN, respectively ($P > 0.05$; Figure 2B). After 4 days of trial, differences in larvae length were highlighted, with recorded values of 15.1 ± 1.84 mm (BRE) and 8.7 ± 1.23 mm (WIN). Dynamic of growth and waste reduction efficiency parameters are reported in Table 1. In Trial 1, VEGFRU larvae showed lower LM and time needed to reach the prepupae stage, as well as higher ECD than FRU larvae. In Trial 2, BRE larvae showed lower LM, time needed to reach the prepupae stage and SR, and contemporarily higher total final biomass, GR, WRI and ECD than WIN larvae.

271

272 ***Proximate and fatty acid compositions of the rearing substrates***

273 The proximate and FA compositions of the rearing substrates are reported in Table
274 2 and Table 3, respectively.

275 In Trial 1, VEGFRU showed lower values of DM and NSC and higher contents of ash,
276 CP, NDF and ADF than FRU, while comparable EE and ADL contents were found. In
277 Trial 2, WIN showed higher DM, ash, NDF, ADF, and ADL contents and lower CP and
278 NSC contents than BRE. VEGFRU and FRU showed similar GE values which were
279 lower than those obtained in the second trial for WIN and BRE.

280 Total FA ranged from 10.04 (FRU) to 82.47 g kg⁻¹ DM (BRE). VEGFRU showed
281 higher total polyunsaturated fatty acids (PUFA) and lower total monounsaturated
282 fatty acids (MUFA) than FRU. WIN had higher MUFA and lower SFA when compared
283 to BRE. Linoleic acid (C18:2 n6) was the most abundant FA in all substrates.

284

285 ***Proximate and fatty acid compositions of the BSF larvae***

286 The proximate and FA compositions of the BSF larvae are reported in Table 4 and
287 Table 5, respectively.

288 Concerning Trial 1, ash, CP and ADF values in the VEGFRU larvae were higher than
289 those in FRU larvae. Conversely, the FRU larvae showed higher DM, EE and NDF
290 contents than the VEGFRU larvae. In Trial 2, the WIN larvae showed lower DM and
291 CP contents when compared to the BRE larvae, while all the other parameters
292 showed an opposite trend.

293 Considering the FA composition of the larvae, FRU larvae showed higher TFA than
294 VEGFRU larvae. On the contrary, in Trial 2 similar TFA contents were observed for
295 BRE and WIN larvae. Significant differences between treatments were observed in
296 both trials for almost all considered FA groups and individual FA. PUFA were higher
297 in VEGFRU and BRE larvae when compared to FRU and WIN larvae, respectively,
298 while an opposite trend was observed for SFA. The most represented individual FA
299 in BSF larvae from all treatments was C12:0, which showed higher amounts in FRU
300 and WIN when compared to VEGFRU and BRE, respectively. C18:1 c9 and C18:2 n6
301 were the most represented unsaturated FA in all treatments.

302

303 **DISCUSSION AND CONCLUSIONS**

304 Our study investigated, through 2 trials, the effects of different rearing substrates
305 on development, waste reduction efficiency, and nutritional composition of BSF
306 larvae.

307 VEGFRU and BRE larvae showed higher weights after 4 days from the beginning of
308 the trial, had lower mortality and needed less time to reach the prepupae stage
309 than FRU and WIN larvae, respectively. Such results were obtained in spite of
310 comparable GE values found in Trial 1 for VEGFRU and FRU and in Trial 2 for WIN
311 and BRE substrates, and can be at least partly ascribed to the higher CP and

moisture contents of VEGFRU and BRE, confirming the results obtained by other authors.^{26,27}

The need for high dietary moisture content could be ascribed to the morphology of the mouthparts of BSF larvae, which resembles the characteristics of scavenger insects.^{28,29} This kind of macerating mouth apparatus allows BSF larvae to scrape off the food from the feeding surface. By softening the feed solids, increased dietary moisture content makes easier for the larvae to feed.³⁰

The results obtained in our trials could be also reflective of possible differences between rearing substrates in terms of the content of nutrients other than CP (e.g., ether extract, structural and non-structural carbohydrates, amino acids) and/or in terms of nutrient digestibility. In both trials, the EE content of substrates (<90 g kg⁻¹ DM) was far below the 200-260 g kg⁻¹ found by Nguyen *et al.*^{12,13} to have detrimental effects for the survival of BSF larvae and adults. BRE larvae showed very good performances despite the high structural carbohydrates content of the relative rearing substrate (NDF: 447 g kg⁻¹ DM; ADF: 225 g kg⁻¹ DM). Such a result clearly demonstrates that BSF larvae are also able to efficiently bioconvert wastes and by-products characterized by high fiber content, thanks to the presence, in the digestive tract of the insect, of intestinal bacteria able to degrade cellulose.³¹ The amino acid composition of the rearing substrates was not analyzed in our trials, and little literature is available concerning the effects of dietary amino acids on development and nutritional composition of BSF larvae.^{32,33} Studying the nutritional composition of BSF prepupae reared on different organic waste substrates, Sprangers *et al.*³² showed that the amino acid content of the prepupae had narrow ranges, particularly when compared to the noticeable differences found in the amino acid composition of the rearing substrates. Concerning nutrient digestibility, to the best of our knowledge no studies are currently available. Further studies are necessary to deepen these aspects for the optimization of BSF feeding and nutrition.

340 In Trial 2 the differences in larvae growth performances between treatments were
341 very pronounced. We may speculate that the GE of the WIN substrate was not fully
342 available for the larvae. The methodology used to grind the WIN substrate could
343 have influenced the availability of the oil present inside the grape seeds. Indeed, a
344 3-mm grinder was used, and this size could not have completely milled the seeds.
345 Moreover, the WIN substrate could have contained substances unsuitable for the
346 BSF larvae development. Indeed, winery by-products usually contain high levels of
347 polyphenols.³⁴ It is known that plants use polyphenolic compounds to protect
348 themselves from herbivore insect attacks.³⁵ Some studies also showed how the
349 grape seeds can accumulate high doses of pesticides and insecticides used in wine
350 grapevines management.^{35,36}
351 Hard *et al.*³⁷ reported that larvae rearing density affects competition for food, low
352 densities usually leading to highest larvae weights. This was also reflected in our
353 trial, as no (Trial 1) or slight (Trial 2) differences were observed for the total final
354 biomass despite the differences found in LM between treatments. The observed LM
355 for VEGFRU was lower than that reported by Nguyen *et al.*¹³ using a vegetable and
356 fruit rearing substrate.
357 In both trials, the differences highlighted in terms of LM and ECD were closely
358 connected, and treatments leading to lower mortality allowed obtaining the best
359 performances in terms of ECD. BRE larvae reduced a lesser quantity of substrate
360 compared to WIN larvae; nevertheless, the WRI was higher in the BRE larvae as
361 they took less time to reach the prepupae stage, which is also confirmed by the
362 higher GR results. The SR was particularly high (above 65%) in Trial 1, showing the
363 great potential of BSF larvae in the degradation of vegetable and fruit wastes.^{12,13}
364 Overall, the BRE larvae showed the best ECD combined with the absolute highest
365 total final biomass production and the shortest developmental period.
366 The time needed by the larvae to reach the prepupae stage seemed to influence
367 their chitin content. Such results agree with the findings of Diener *et al.*¹¹ who

368 reported how small larvae grown in 42 days showed a chitin level higher than
369 heavy larvae grown in 16 days.

370 In both trials, substrates containing the highest CP and moisture contents (VEGFRU
371 and BRE) allowed obtaining BSF larvae with the highest CP level, which is
372 consistent with the results obtained by other authors.^{26,27} Consistently with the
373 findings of Janssen *et al.*,²² the use of the conventional N-factor of 6.25 led to a CP
374 overestimation of about 25%.

375 Despite comparable EE values of the rearing substrates, FRU larvae showed higher
376 EE content than VEGFRU larvae, probably as a consequence of the higher NSC level
377 of FRU.³⁸ Insects have the ability to convert carbohydrates into lipids.^{32,39} Insects
378 store lipids for two reasons. Firstly, as energy reserve for the adult stage.¹⁴
379 Secondly because, as insect body presents an open blood system and a high
380 surface compared to volume and the combination of these two factors could be a
381 problem for the loss of water and the drying out process, lipids allow them to avoid
382 transpiration and store non-imbibed water.⁴⁰ However, the influence of the NSC
383 content of substrates on the EE content of BSF larvae should be further
384 investigated as higher NSC in BRE substrate did not lead to higher EE content in
385 BSF larvae in Trial 2.

386 The FA composition of the rearing substrates did not directly affect the larvae FA
387 composition, which was also influenced by carbohydrates (starch and sugars),
388 confirming other researches.^{26,32} Being of vegetable origin, all rearing substrates
389 had PUFA as the most abundant FA group. Notwithstanding, as typically observed
390 for Diptera, the BSF larvae FA profile was dominated by SFA, mainly C12:0 (which
391 showed the absolute highest values among individual detected FA), C14:0, C16:0
392 and C18:0.^{32,41} The high presence of SFA in insects is connected with cold-
393 adaptation.⁴² Indeed, larvae from some species showed a SFA decrease from
394 summer to autumn while PUFA increased highlighting a correlation between the
395 change in FA composition and the temperatures due to seasonal change.^{42,43,44} BSF
396 is a sub-tropical species growing with high temperatures (27-32°C) and the difficult

adaptation to low temperatures was demonstrated by the lowest BSF survival rate at about 16°C.⁴⁵ We can argue that the high SFA presence could be ascribed to BSF adaptation to the sub-tropical climate. In particular the high content of lauric acid (melting point: 43.2°C) could preserve BSF larvae from lipid oxidation and allow them to survive at temperatures above 40°C.⁴¹ Consistent with other findings,^{7,15,32} C18:1 n7 was the main represented MUFA in the larvae, while C18:2 n6 and C18:3 n3 were the main represented PUFA n6 and PUFA n3, respectively. The low quantity of recovered n3 PUFA in the larvae could represent a problem if insect meals are intended to be used for animal feed. Indeed, researches highlighted a decrease in nutritional product quality with the inclusion of insect meals in animal diets especially when full-fat meals are used.^{5,6,46} Nevertheless, BSF larvae can be enriched in n3-PUFA through the substrate.^{33,47} Authors^{10,48} reported that C12:0 is a good inhibitor of bacteria strains and could be of great interest in the reduction of the use of antibiotics in animal feeding.^{10,49,50} In this context, BSF larvae reared on organic wastes resulted very interesting with up to 574 g kg⁻¹ TFA of lauric acid.

Especially in Southern Europe, the large availability of vegetable and fruit wastes (mainly from markets and supermarkets) may allow the development of a BSF larvae mass production, enabling as well to obtain economic and environmental benefits from the sustainable management of organic wastes. Regarding the considered agro-industrial by-products, the use of winery by-products as rearing substrate for BSF larvae could be conditioned, both from a technological and economical point of view, by the need of preliminarily **processing to** remove or **reduce polyphenols**, pesticides and insecticides contents, which could exert a negative influence on the growth performance of the larvae. Remarkable positive results were obtained in terms of overall development time, growth performances and nutritional composition for the larvae reared on brewery by-products, which should therefore be considered promising rearing substrates. However, as brewery by-products are characterized by a more limited availability than vegetables, fruits

or winery by-products, it could be advisable to use BRE at low dietary inclusion levels with the purpose of increasing BSF larvae performances.

Overall, our results show that the performance and chemical composition of BSF larvae are largely affected by the chemical composition of the provided substrate. This clearly demonstrates that insects, like farm animals, have nutritional requirements which have to be met for optimal performance.

The performances obtained in our bench top trials may vary when transferred to an industrial scale. For instance, the large volumes of waste used as well as the high larvae concentration could result in environmental oxygen depletion and heat production.⁵¹ Attention should then be placed to the airflow to guarantee appropriate rearing conditions. In addition, to optimize land use, insect breeding should exploit the verticality of the breeding structure. However, this can lead to the stratification of temperature and particular attention must be given to an adequate air circulation to guarantee a homogeneous temperature in all parts of the building. At industrial level, the production system would also require a constant supply of substrates, possibly with a fairly constant chemical composition, as to obtain BSF larvae with relatively constant nutrient profile.

Future studies should be designed to assess the nutritional requirements of BSF larvae and to evaluate other agro-industrial by-products, as well as the effect of mixing different organic wastes and agro-industrial by-products, to obtain optimal BSF larvae performances in terms of development, waste reduction efficiency and nutritional composition.

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REFERENCES

1. van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, Vantomme P, *Edible Insects – Future Prospects for Food and Feed Security*. FAO

Forestry Paper 171. Food and Agriculture Organization of the United Nations, Roma, pp. 59-66 (2013).

2. Salomone R, Saija G, Mondello G, Giannetto A, Fasulo S, Savastano D, Environmental impact of food waste bioconversion by insects : Application of Life Cycle Assessment to process using *Hermetia illucens*. *J Clean Prod* **140**:890–905 (2017).

3. Arancon RAD, Lin CSK, Chan KM, Kwan TH, Luque R, Advances on waste valorization: new horizons for a more sustainable society. *Energy Science & Engineering* **1**:53–71 (2013).

4. Biasato I, De Marco M, Rotolo L, Renna M, Lussiana C, Dabbou S, Capucchio MT, Biasibetti E, Costa P, Gai F, Pozzo L, Dezzutto D, Bergagna S, Martínez S, Tarantola M, Gasco L, Schiavone A, Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens. *J Anim Physiol Anim Nutr* **100**:1104–1112 (2016).

5. Gasco L, Henry M, Piccolo G, Marono S, Gai F, Renna M, Lussiana C, Antonopoulou E, Mola P, Chatzifotis S, *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. *Anim Feed Sci Technol* **220**:34–45 (2016).

6. Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, Prearo M, Capucchio MT, Biasato I, Biasibetti E, De Marco M, Zoccarato I, Gasco L, Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *J Anim Sci Biotechnol* **8**:57, 1–13 (2017).

7. Barragan-Fonseca KB, Dicke M, van Loon JJA, Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. *J Insects Food Feed* **3**(2):105-120 (2017).

8. Surendra KC, Olivier R, Tomberlin JK, Jha R, Kumar S, Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renew Energy* **98**:197–

- 484 202 (2016).
- 485 9. Finke MD, Estimate of chitin in raw whole insects. *Zoo Biol* **26**:105–115 (2007).
- 486 10. Spranghers T, Joris M, Vrancx J, Ovyne A, Eeckhout M, De Clercq P, De Smet S,
487 Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia*
488 *illucens* L.) prepupae for weaned piglets *Anim Feed Sci Technol* **235**:33–42
489 (2018).
- 490 11. Diener S, Zurbrugg C, Tockner K, Conversion of organic material by black
491 soldier fly larvae: establishing optimal feeding rates. *Waste Manag Res* **27**:603–
492 610 (2009).
- 493 12. Nguyen TTX, Tomberlin JK, Vanlaerhoven S, Ability of Black Soldier Fly
494 (Diptera: Stratiomyidae) larvae to recycle food waste. *Environ Entomol* **44**:406–
495 410 (2015).
- 496 13. Nguyen TTX, Tomberlin JK, Vanlaerhoven S, Influence of resources on *Hermetia*
497 *illucens* (Diptera: Stratiomyidae) larval development. *J Med Entomol* **50**:898–
498 906 (2013).
- 499 14. Tomberlin JK, Sheppard DC, Joyce JA, Selected life-history traits of Black
500 Soldier Flies (Diptera: Stratiomyidae) reared on three artificial diets. *Ann*
501 *Entomol Soc Am* **95**:379–386 (2002).
- 502 15. Barroso FG, de Haro C, Sánchez-Muros MJ, Venegas E, Martínez-Sánchez A,
503 Pérez-Bañón C, The potential of various insect species for use as food for fish.
504 *Aquaculture* **422–423**:193–201 (2014).
- 505 16. Wang YS, Shelomi M, Review of Black Soldier Fly (*Hermetia illucens*) as animal
506 feed and human food. *Foods* **6**:91 (2017).
- 507 17. Segrè A, Falasconi L, *Il libro nero dello spreco in Italia: il cibo*. Edizioni
508 Ambiente, Milano, pp 1–124 (2011).
- 509 18. Briggs DE, Boulton CA, Brookes PA, Steven R, *Brewing: science and practice*.
510 Woodhead Publishing Limited and CRC Press, Boca Raton, pp. 1–863 (2004).
- 511 19. Harnden LM, Tomberlin JK, Effects of temperature and diet on black soldier fly,
512 *Hermetia illucens* (L.) (Diptera: Stratiomyidae), development. *Forensic Sci Int*

- 513 **266**:109–116 (2016).
- 514 20. Leong SY, Kuty SRM, Malakahmad A, Tan CK, Feasibility study of biodiesel
515 production using lipids of *Hermetia illucens* larva fed with organic waste. *Waste*
516 *Manage* **47**:84–90 (2016).
- 517 21. Tschirner M, Simon A, Influence of different growing substrates and processing
518 on the nutrient composition of black soldier fly larvae destined for animal feed. *J*
519 *Insects Food Feed* **1**:1–12 (2015).
- 520 22. Janssen RH, Vincken JP, van den Broek LAM, Fogliano V, Lakemond CMM,
521 Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*,
522 *Alphitobius diaperinus*, and *Hermetia illucens*. *J Agric Food Chem* **65**:2275–2278
523 (2017).
- 524 23. Van Soest PJ, Robertson JB, Lewis BA, Methods for dietary fiber, neutral
525 detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J*
526 *Dairy Sci* **74**:3583–3597 (1991).
- 527 24. AOAC International, Official methods of analysis of AOAC International. 16th ed.
528 Gaithersburg, MD: Association of Official Analytical Chemists (2000).
- 529 25. Renna M, Gasmi-Boubaker A, Lussiana C, Battaglini LM, Belfayez K, Fortina R,
530 Fatty acid composition of the seed oils of selected *Vicia* L. taxa from Tunisia. *Ital*
531 *J Anim Sci* **13**:308–316 (2014).
- 532 26. Oonincx DGAB, van Broekhoven S, van Huis A, van Loon JJA, Feed conversion,
533 survival and development, and composition of four insect species on diets
534 composed of food by-products. *PLoS ONE* **10**:1–20 (2015).
- 535 27. Cammack JA, Tomberlin JK, The impact of diet protein and carbohydrate on
536 select life-history traits of the Black Soldier Fly *Hermetia illucens* (L.) (Diptera:
537 Stratiomyidae). *Insects* **8**,56:1–14 (2017).
- 538 28. Purkayastha D, Sarkar S, Roy P, Kazmi AA, Isolation and morphological study
539 of ecologically-important insect "*Hermetia illucens*" collected from Roorkee
540 compost plant. *Pollution* **3**:453–459 (2017).
- 541 29. Kim W, Bae S, Park H, Park K, Lee S, Choi Y, Han S, Koh Y, The larval age and

- mouth morphology of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Int J Indust Entomol* **21**:185–187 (2010).
30. Banks IJ, To assess the impact of black soldier fly (*Hermetia illucens*) larvae on faecal reduction in pit latrines. Doctoral Thesis, London School of Hygiene Tropical Medicine (2014). Available at: <http://researchonline.lshtm.ac.uk/1917781/> (last accessed: 20 April 2018).
31. Kim E, Park J, Lee S, Kim Y, Identification and physiological characters of intestinal bacteria of the Black Soldier Fly, *Hermetia illucens*. *Korean J Appl Entomol* **53**:15–26 (2014).
32. Spranghers T, Ottoboni M, Klootwijk C, Olyn A, Deboosere S, De Meulenaer B, Michiels J, Eeckhout M, De Clercq P, De Smet S, Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J Sci Food Agric* **97**:2594–2600 (2017).
33. Liland NS, Biancarosa I, Araujo P, Biemans D, Bruckner CG, Waagbø R, Torstensen BE, Lock EJ, Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS ONE* **12(8)**: e0183188 (2017).
34. Garavaglia J, Markoski MM, Oliveira A, Marcadenti A, Grape seed oil compounds: biological and chemical actions for health. *Nutr Metab Insights* **9**:59–64 (2016).
35. Lattanzio V, Lattanzio VM, Cardinali A, Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects, in *Phytochemistry: Advances in Research*, ed. by Imperato F. Research Signpost, Trivandrum, pp. 23–67 (2006).
36. Rose G, Lane S, Jordan R, The fate of fungicide and insecticide residues in Australian wine grape by-products following field application. *Food Chem* **117**:634–640 (2009).
37. Hard JJ, Bradshaw WE, Malarkey DJ, Resource- and density-dependent development in tree-hole mosquitoes. *Oikos* **54**:137–144 (1989).

38. Arrese EL, Soulages JL, Insect fat body: energy, metabolism, and regulation. *Annu Rev Entomol* **55**:207–225 (2010).

39. Inagaki S and Yamashita O, Metabolic shift from lipogenesis to glycogenesis in the last instar larval fat body of the silkworm, *Bombyx mori*. *Insect Biochem* **16**:327–331 (1986).

40. Downer RGH, Matthews JR, Patterns of lipid distribution and utilisation in insects. *Am Zool* **16**:733–745 (1976).

41. Ushakova NA, Brodskii ES, Kovalenko AA, Bastrakov AI, Kozlova AA, Pavlov ADS, Characteristics of lipid fractions of larvae of the black soldier fly *Hermetia illucens*. *Dokl Biochem Biophys* **468**:209–212 (2016).

42. Bennett VA, Lee RE, Modeling seasonal changes in intracellular freeze-tolerance of fat body cells of the gall fly *Eurosta solidaginis* (Diptera, Tephritidae). *J Exp Biol* **200**:185–192 (1997).

43. Atapour M, Moharramipour S, Barzegar M, Seasonal changes of fatty acid compositions in overwintering larvae of rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae). *J Asia-Pacific Entomol* **10**:33–38 (2007).

44. Kalyoncu L, Çetin H, Effects of low temperature on the fatty acid compositions of adult of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae). *Selçuk Tarım ve Gıda Bilimleri Dergisi* **31**:11–15 (2017).

45. Holmes LA, VanLaerhoven SL, Tomberlin JK, Lower temperature threshold of black soldier fly (Diptera: Stratiomyidae) development. *J Insects Food Feed* **2**:255–262 (2016).

46. Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, Gasco L, Nizza A, Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, carcass and meat traits. *J Anim Sci* **94**:639–647 (2016).

47. Barroso FG, Sánchez-Muros MJ, Segura M, Morote E, Torres A, Ramos R, Guil JL, Insects as food: Enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. *J Food Compos Anal* **62**:8–13 (2017).

- 600 48. Skrivanova E, Marounek M, Benda V, Brezina P, Susceptibility of *Escherichia*
601 *coli*, *Salmonella* sp. and *Clostridium perfringens* to organic acids and monolaurin.
602 *Vet Med (Praha)* **51**:81–88 (2006).
- 603 49. Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S,
604 Dezzutto D, Gai F, Dabbou S, Dalle Zotte A, Partial or total replacement of
605 soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets:
606 effect on growth performances, feed-choice, blood traits, carcass characteristics
607 and meat quality. *Ital J Anim Sci* **16**:93–100 (2017).
- 608 50. Schiavone A, Dabbou S, De Marco M, Cullere M, Biasato I, Biasibetti E,
609 Capucchio MT, Bergagna S, Dezzutto D, Meneguz M, Gai F, Dalle Zotte A, Gasco
610 L, Black soldier fly (*Hermetia illucens* L.) larva fat inclusion in finisher broiler
611 chicken diet as an alternative fat source. *Animal*. Accepted on December 13
612 2017 (2018).
- 613 51. Cheng JYK, Chiu SLH, Lo IMC, Effects of moisture content of food waste on
614 residue separation, larval growth and larval survival in black soldier fly
615 bioconversion. *Waste Manage* **67**:315–323 (2017).

Table 1: Dynamic of growth and waste reduction efficiency (on a fresh matter basis) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1			Trial 2		
	(organic wastes)			(agro-industrial by-products)		
	VEGFRU	FRU	<i>P</i>	WIN	BRE	<i>P</i>
Larvae mortality (%)	11.2±4.35	19.3±5.24	0.015	24.8±10.53	9.5±5.68	0.011
Total final biomass (g)	10.42±0.648	10.92±2.057	0.584	9.90±0.785	11.32±0.864	0.014
Time needed to reach prepupae stage (days of trial)	20.2±1.33	22.0±0.89	0.031	22.2±0.98	8.0±0.01	0.003
Growth rate (g d ⁻¹)	0.006±0.0018	0.007±0.0007	0.451	0.006±0.0009	0.014±0.0009	0.000
Substrate reduction (%)	65.2±5.54	70.8±8.39	0.129	53.0±5.28	42.5±8.41	0.027
Waste reduction index (g d ⁻¹)	3.2±0.26	3.2±0.41	0.952	2.4±0.32	5.3±1.05	0.000
Efficiency of conversion of digested food	0.07±0.009	0.05±0.011	0.004	0.06±0.002	0.14±0.034	0.000
VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.						

Table 2: Proximate composition (g kg⁻¹ dry matter, unless otherwise stated) of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector and used as rearing substrates by black soldier fly larvae.

	Trial 1		Trial 2	
	(organic wastes)		(agro-industrial by-products)	
	VEGFRU	FRU	WIN	BRE
Dry matter (g kg ⁻¹)	82.7	131.9	358.3	232.1
Ash	91.1	30.4	103.0	39.8
Crude protein	119.9	46.0	117.4	200.5
Ether extract	26.0	27.8	79.0	86.7
Neutral detergent fiber	178.0	139.3	566.4	447.1
Acid detergent fiber	110.5	91.1	462.4	225.3
Acid detergent lignin	12.9	13.1	323.5	62.1
Non-structural carbohydrates*	585.0	756.5	134.2	225.9
Gross energy (MJ kg ⁻¹ DM)	15.1	15.6	19.5	19.4

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

*Calculated as: 1000 – (crude protein + ether extract + ash + neutral detergent fiber).

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Table 3: Fatty acid composition (g kg⁻¹ total fatty acids, unless otherwise stated) of organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector and used as rearing substrates by black soldier fly larvae.

	Trial 1		Trial 2	
	(organic wastes)		(agro-industrial by-products)	
	VEGFRU	FRU	WIN	BRE
Total fatty acids (g kg ⁻¹ dry matter)	20.91	10.04	73.57	82.47
C12:0	0.73	3.52	0.93	0.80
C14:0	8.85	8.44	1.99	3.22
C16:0	184.90	192.71	100.49	252.48
C16:1 c9	5.89	5.96	4.43	1.75
C18:0	26.14	43.36	50.24	15.42
C18:1 c9	65.91	208.61	185.09	103.27
C18:1 c11	20.85	29.62	8.51	7.46
C18:2 n6	575.23	333.38	630.32	554.90
C18:3 n3	111.50	174.40	18.00	60.70
Saturated fatty acids	220.62	248.03	153.65	271.92
Monounsaturated fatty acids	92.65	244.19	198.03	112.48
Polyunsaturated fatty acids	686.73	507.78	648.32	615.60

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

Table 4: Proximate composition (g kg⁻¹ dry matter, unless otherwise stated) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1 (organic wastes)			Trial 2 (agro-industrial by-products)		
	VEGFRU	FRU	P	WIN	BRE	P
Dry matter (g kg ⁻¹)	219.6±10.22	282.9±6.57	0.000	265.4±5.93	290.8±6.96	0.000
Ash	129.8±6.50	72.2±2.22	0.000	145.7±6.67	73.0±1.89	0.000
Crude protein ¹	418.8±13.24	307.5±10.29	0.000	344.3±7.63	529.6±5.27	0.000
Crude protein ²	312.9±9.89	229.7±7.69	0.000	257.3±5.70	395.7±3.94	0.000
Ether extract	262.8±18.01	407.0±18.83	0.000	322.2±19.60	298.7±6.49	0.031
Neutral detergent fiber	170.9±16.49	197.9±13.48	0.011	177.3±13.08	87.0±9.89	0.000
Acid detergent fiber	113.1±20.09	93.4±3.55	0.014	98.5±10.16	64.8±9.17	0.000
Acid detergent lignin	14.9±7.75	8.9±2.47	0.104	44.8±17.80	8.3±9.35	0.001
Chitin ³	62.4±19.63	56.0±3.96	0.453	52.9±9.25	14.2±6.06	0.000
Chitin ⁴	75.2±19.7	65.5±3.53	0.283	64.5±9.48	27.0±6.59	0.000

VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

¹ Obtained using the nitrogen-to-protein conversion factor of 6.25.

² Obtained using the nitrogen-to-protein conversion factor of 4.67.

³ Calculated using the nitrogen-to-protein conversion factor of 6.25.

⁴ Obtained using the nitrogen-to-protein conversion factor of 4.67.

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Table 5: Fatty acid composition (g kg⁻¹ total fatty acids, unless otherwise stated) of black soldier fly larvae reared on organic wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector (mean ± SD; n = 6).

	Trial 1			Trial 2		
	(organic wastes)			(agro-industrial by-products)		
	VEGFRU	FRU	<i>P</i>	WIN	BRE	<i>P</i>
TFA (g kg ⁻¹ dry matter)	253.02±18.512	398.40±18.547	0.000	287.41±16.973	282.93±6.936	0.563
C12:0	520.61±17.505	574.32±11.060	0.000	346.91±16.840	323.73±9.277	0.014
C14:0	103.55±3.303	96.39±3.471	0.004	65.54±4.283	66.49±2.687	0.654
C16:0	138.95±7.338	130.57±3.846	0.040	189.36±7.434	204.15±5.772	0.003
C16:1 c9	33.57±3.606	37.45±0.956	0.046	60.63±4.718	29.45±2.639	0.000
C18:0	25.90±1.693	17.51±0.539	0.000	28.32±2.139	18.07±0.599	0.000
C18:1 c9	85.37±4.075	93.19±2.086	0.002	124.59±4.280	92.23±2.414	0.004
C18:1 c11	4.31±0.381	2.79±0.157	0.000	4.46±0.261	5.75±1.155	0.040
C18:2 n6	70.41±7.408	40.70±1.534	0.000	175.76±14.935	235.47±6.593	0.000
C18:3 n3	17.31±1.370	7.06±0.729	0.000	4.44±0.392	24.65±0.504	0.000
SFA	789.02±10.854	818.81±4.632	0.000	630.13±16.745	612.45±8.784	0.045
MUFA	123.26±6.829	133.44±2.773	0.013	189.68±6.220	127.43±5.354	0.000
PUFA	87.72±7.333	47.75±2.083	0.000	180.19±15.244	260.12±6.843	0.000

4 VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste; WIN: winery by-product; BRE: brewery by-product.

Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. P-value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars represent the standard error of the mean.

106x140mm (600 x 600 DPI)

Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. P-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

107x140mm (600 x 600 DPI)

Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. *P*-value: **P*<0.05, ***P*<0.01, ****P*<0.001. Error bars represent the standard error of the mean.

Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. *P*-value: **P*<0.05, ***P*<0.01, ****P*<0.001. Error bars represent the standard error of the mean.