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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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SCHOLARONE™  
Manuscripts

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3 1 **Effect of rearing substrate on growth performance, waste reduction**  
4 2 **efficiency and chemical composition of black soldier fly (*Hermetia illucens*)**  
5 3 **larvae†**  
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10 5 **RUNNING TITLE: Rearing substrate effects on performance and nutritional**  
11 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<sup>-1</sup> DM). The waste reduction index ranged from 2.4 (WIN) to 5.3 g d<sup>-1</sup> (BRE). BRE larvae showed the lowest saturated and the highest polyunsaturated fatty acids proportions (612.4 and 260.1 g kg<sup>-1</sup> 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.<sup>1</sup> 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.<sup>1,2</sup> 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).<sup>3</sup> 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,<sup>4,5,6,7</sup> biodiesel,<sup>8</sup> high value products as chitin<sup>9</sup> or anti-microbial peptides.<sup>10</sup>

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.<sup>8,11,12</sup> The available literature has highlighted that BSF life cycle and nutritional composition are noticeably influenced by the rearing substrate,<sup>13,14</sup> with the crude protein (CP) content of the larvae ranging from about 317 to 630 g kg<sup>-1</sup> dry matter (DM).<sup>7,15,16</sup>

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\\_wasgen&lang=en](http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_wasgen&lang=en))). Considering the Italian context, 54% of the total production of waste

1  
2  
3 82 and agro-industrial by-products is generated by the manufacturing of vegetable  
4  
5 83 products.<sup>17</sup> About 1.5 million tons of winery by-products and 406 tons of brewery  
6  
7 84 by-products are produced every year  
8  
9 85 (EUROSTAT([http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language](http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tag00034&plugin=1;)  
10 86 [=en&pcode=tag00034&plugin=1;](http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tag00034&plugin=1;) EU Report  
11  
12 87 ([https://www.brewersofeurope.org/site/media-](https://www.brewersofeurope.org/site/media-centre/index.php?doc_id=905&class_id=31&detail=true)  
13  
14 88 [centre/index.php?doc\\_id=905&class\\_id=31&detail=true](https://www.brewersofeurope.org/site/media-centre/index.php?doc_id=905&class_id=31&detail=true))).<sup>18</sup>

15  
16 89 The aim of this research was to evaluate the effects of organic wastes (vegetables  
17  
18 90 and fruits) and agro-industrial by-products (winery and brewery) generated by the  
19  
20 91 Italian food sector as rearing substrates for BSF larvae on their development, waste  
21  
22 92 reduction efficiency and chemical composition.

23  
24 93

## 94 **MATERIAL AND METHODS**

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26  
27 95 Two trials were carried out at the Experimental Facility of the Department of  
28  
29 96 Agricultural, Forest and Food Sciences (DISAFA; University of Torino, Torino, Italy).

30  
31 97

### 32 ***Rearing substrates***

33  
34  
35 99 In Trial 1, two organic wastes were compared:

36  
37 100 - Vegetable-fruit waste (VEGFRU) obtained from a street market (Torino, Italy) and  
38  
39 101 containing a mixture of vegetables and fruits (celery 43.4%, oranges 28.9% and  
40  
41 102 peppers 27.7%);

42  
43 103 - Fruit waste (FRU) obtained from a fruit market (Torino, Italy) and containing fruits  
44  
45 104 only (apples 47.8%, oranges 15.5%, apple leftovers 13.8%, strawberries 7.1%,  
46  
47 105 mandarins 4.8%, pears 4.1%, kiwis 3.4%, bananas 1.9% and lemons 1.6%).

48  
49 106 In Trial 2, two agro-industrial by-products were used:

50  
51 107 - Winery by-product (WIN) obtained during the wine making process, before the  
52  
53 108 alcohol extraction, from a private distillery (Distilleria Santa Teresa dei Fratelli  
54  
55 109 Marolo S.R.L., Alba (CN), Italy) and containing grape seeds, pulp, skins, stems and  
56  
57 110 leaves;

1  
2  
3 111 - Brewery by-product (BRE) obtained during beer production (IFN 5-00-517 Barley  
4 112 brewers grains wet) from a private brewery ("Birrificio dei Santi", Castelnuovo Don  
5 113 Bosco (AT), Italy).

6  
7  
8 114 Each substrate was ground with a 3 mm die meat mincer (FTS136; Fama Industrie  
9 115 S.r.l., Rimini, Italy) and carefully mixed.

10  
11  
12 116 A sample of each substrate was freeze-dried and frozen at -80°C for further  
13  
14 117 chemical analysis, while the remaining was stored at -20°C until it was fed to the  
15  
16 118 larvae.

17  
18 119

19  
20 120

### **BSF eggs**

21  
22 121 BSF eggs laid on corrugated cardboards for less than 24 h, were purchased from a  
23  
24 122 private company (CIMI S.r.l., Cervasca (CN), Italy). The cardboards with the eggs  
25  
26 123 were immediately transported to the DISAFA Experimental Facility. The cardboards  
27  
28 124 were put onto plastic boxes (25cm × 33cm × 12cm) which contained whole rye  
29  
30 125 thoroughly mixed with water (60% moisture) as rearing substrate for the newborn  
31  
32 126 larvae. The plastic boxes were placed into climatic chambers under controlled  
33  
34 127 environmental conditions (T: 27±0.5°C; RH: 70±5%; 24:0 L:D photoperiod). The  
35  
36 128 eggs hatched approximately three days after oviposition.

37  
38 129

39 130

### **Experimental design and calculations**

40  
41 131

#### *Larvae development and waste reduction efficiency*

42  
43 132 Six-day-old larvae were used in both trials. In each trial, for the evaluation of  
44  
45 133 larvae development (weight and length) and waste reduction efficiency, six  
46  
47 134 replicates of 100 larvae were weighed (KERN PLE-N v. 2.2; KERN & Sohn GmbH,  
48  
49 135 Balingen-Frommern, Germany; d: 0.001) and assigned to each rearing substrate.  
50  
51 136 The method reported by Harnden and Tomberlin<sup>19</sup> was used to count the larvae. For  
52  
53 137 each replicate, the larvae were placed into plastic containers (10cm × 17.5cm ×  
54  
55 138 7cm), directly on the rearing substrate (100 g per replicate). The containers were  
56  
57 139 covered with a perforated cap with a black nylon grid and placed in a climatic

1  
2  
3 140 chamber under controlled environmental conditions (T: 27±0.5°C; RH: 70±5%;  
4 141 24:0 L:D photoperiod).

5  
6 142 Each replicate was monitored daily to control the quantity of available feed. If  
7  
8 143 needed, as reported by Harnden and Tomberlin,<sup>19</sup> 50 g of substrate per replicate  
9  
10 144 was added in all replicates at the same time.

11  
12 145 To avoid the effect of handling on the considered dependent variables,<sup>13</sup> weight and  
13  
14 146 length data were collected every four days until the appearance of the first  
15  
16 147 prepupae, thereafter every day for the relative substrate. Thirty larvae were  
17  
18 148 randomly sampled for three consecutive times from each container to measure  
19  
20 149 weight and length. As measurement was not destructive, the larvae were re-  
21  
22 150 introduced into the containers between two consecutive samplings. The sampled  
23  
24 151 larvae were individually cleaned, dried with a paper towel and weighed, and  
25  
26 152 photographed orthogonally (Lumix G1; Panasonic Corporation, Kadoma, Osaka,  
27  
28 153 Japan) with a metric scale (mm). The images were analyzed with ImageJ software  
29  
30 154 package (v. 1.50b) to record larvae length (i.e., from mouthpart to the bottom of  
31  
32 155 the last abdominal segment).

33  
34 156 For each container, weight and length data collection ended when 30% of the  
35  
36 157 larvae reached the prepupae stage. The prepupae were removed from the  
37  
38 158 containers. The remaining 70% of the larvae were hand-counted, washed, dried  
39  
40 159 with a paper towel and individually weighed and photographed. The total final  
41  
42 160 biomass (larvae + prepupae) and the residual rearing substrate were also weighed.

43 161 The following parameters were then calculated:

44  
45 162 - larvae mortality (LM)

46  
47 163  $LM = [\text{initial number of larvae} - (\text{final number of larvae} + \text{number of prepupae})] /$   
48  
49 164 initial number of larvae \* 100;

50  
51 165 - growth rate (GR),<sup>20</sup> readapted for this research substituting prepupa body weight  
52  
53 166 (g) with larva body weight (g)

54  
55 167  $GR = (\text{larva average final body weight (g)} - \text{larva initial body weight (g)}) / \text{days of}$   
56  
57 168 trial (d);



1  
2  
3 169 - substrate reduction (SR)<sup>21</sup>  
4  
5 170  $SR = [(distributed\ substrate\ (g) - residual\ substrate\ (g)) / distributed\ substrate$   
6  
7 171  $(g)] * 100;$   
8  
9 172 - waste reduction index (WRI)<sup>20</sup>  
10  
11 173  $WRI = [(W - R) / W] / days\ of\ trial\ (d) * 100$   
12  
13 174 where W = total amount of rearing substrate distributed during the trial (g); R =  
14  
15 175 residue substrate (g);  
16  
17 176 - efficiency of conversion of digested food (ECD)<sup>20</sup>  
18  
19 177  $ECD = total\ final\ biomass\ (g) / (total\ feed\ distributed\ (g) - residual\ substrate\ (g))$   
20  
21 178 where total final biomass = larvae + prepupae; residual substrate = undigested  
22  
23 179 food + excretory products.  
24  
25 180 Parameters related to waste reduction efficiency (SR, WRI and ECD) were  
26  
27 181 calculated on a fresh matter basis.  
28

182

### 183 *Larvae nutritional composition*

31 184 For each trial, a second set of six replicates per rearing substrate was  
32  
33 185 simultaneously prepared with the aim to rear a sufficient amount of larvae to be  
34  
35 186 analyzed for their proximate composition and fatty acid - FA - profile. Five hundred  
36  
37 187 hand-counted 6-day-old larvae were placed into plastic containers of bigger size  
38  
39 188 (25cm × 33cm × 15cm) than those used for the larvae development and waste  
40  
41 189 reduction efficiency test, following the same relationships between (i) number of  
42  
43 190 larvae / container size surface, and (ii) amount of administered feed / larvae  
44  
45 191 density. The larvae were not handled until the appearance of the first prepupa.  
46  
47 192 Then, each container was checked daily and the identified prepupae were removed.  
48  
49 193 The trial ended when the 30% of the larvae reached the prepupae stage. The  
50  
51 194 remaining larvae were then manually separated from the residual rearing substrate,  
52  
53 195 washed, slightly dried with paper towel, weighed and frozen at -80°C until being  
54  
55 196 freeze-dried.

197

### 198 **Chemical analyses of rearing substrates and larvae**

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

213

### 214 **Statistical analyses**

215 The statistical analysis of data was performed using IBM SPSS Statistics v. 20.0 for  
216 Windows. The two trials were considered separately. Larvae weights and lengths  
217 were subjected to a Two-Way Mixed ANOVA. The Shapiro-Wilk test was used to  
218 verify if the dependent variables were normally distributed for each combination of  
219 the groups of within- (test day, considered as a repeated measure) and between-  
220 (rearing substrate) subjects factors. The Levene's test was used to verify the  
221 homogeneity of variances for each combination of the groups of within- and  
222 between-subjects factors. The Mauchly's test was used to verify the assumption of  
223 sphericity; if such an assumption was violated, the Greenhouse-Geisser or the  
224 Huynh-Feldt correction (in cases of estimates of sphericity lower or higher than  
225 0.75, respectively) was applied to correct the degrees of freedom of the F-  
226 distribution. Final larvae weights and lengths (average weight and length of the

227 leftover 70% larvae after removing the 30% of prepupae) were further subjected to  
228 independent samples Student's *t*-tests to assess differences between rearing  
229 substrates.

230 Differences in terms of larvae growth performances, waste reduction efficiency,  
231 proximate composition and FA profile between substrates were also assessed using  
232 independent-samples Student's *t*-tests.

233 The Kruskal-Wallis test was used to compare the time needed by the larvae to  
234 reach the prepupae stage.

235 Significance was declared at  $P < 0.05$ .

236

237

## RESULTS

### ***Growth performances and waste reduction efficiency of the BSF larvae***

239 The effect of the rearing substrate on the development of BSF larvae over time is  
240 reported in Figure 1 (Trial 1) and Figure 2 (Trial 2).

241 In both trials, results from the Two-Way Mixed ANOVA showed that, for larvae  
242 development (weight and length), rearing substrate, test day and their interaction  
243 were highly significant ( $P < 0.001$ ), VEGFRU and BRE performing better than FRU  
244 and WIN, respectively.

245 In Trial 1, no differences were observed for larvae weight (mean  $\pm$  SD:  
246  $0.004 \pm 0.0002$  g) at the beginning of the trial (day 0; 6 days-old larvae) (Figure  
247 1A). Differences appeared after 4 days of trial, with a higher weight in the VEGFRU  
248 larvae ( $0.055 \pm 0.0084$  g) compared to the FRU larvae ( $0.037 \pm 0.0058$  g). Such  
249 trend was maintained at each test day until day 16 (VEGFRU:  $0.148 \pm 0.0103$  g;  
250 FRU:  $0.120 \pm 0.0094$  g) when VEGFRU larvae started to enter in the prepupae stage.

251 The final weight of the larvae did not show differences between the two rearing  
252 substrates. At the beginning of Trial 1, VEGFRU and FRU larvae showed length  
253 values of  $5.7 \pm 1.32$  mm and  $5.3 \pm 1.17$  mm, respectively ( $P < 0.05$ ; Figure 1B).

254 **VEGFRU larvae** continued showing higher length values than FRU larvae until the

255 last statistical assessment (day 16). VEGFRU and FRU larvae achieved a final length  
256 of  $17.7\pm 0.46$  mm and  $17.8\pm 0.51$  mm, respectively ( $P>0.05$ ).

257 At the beginning of Trial 2, no differences were observed between WIN and BRE for  
258 larvae weight ( $0.007\pm 0.0011$  g) (Figure 2A). Remarkable differences were reported  
259 after 4 days of trial, with a higher weight in the BRE larvae compared to the WIN  
260 larvae ( $0.092\pm 0.0063$  and  $0.017\pm 0.0018$  g, respectively). The final weight of the  
261 larvae (reached after 8 and 26 days of trial for BRE and WIN, respectively) did not  
262 show differences between treatments. The mean length of 6-day-old larvae (day 0)  
263 was  $6.5\pm 1.36$  and  $6.4\pm 1.24$  mm for BRE and WIN, respectively ( $P>0.05$ ; Figure  
264 2B). After 4 days of trial, differences in larvae length were highlighted, with  
265 recorded values of  $15.1\pm 1.84$  mm (BRE) and  $8.7\pm 1.23$  mm (WIN).

266 Dynamic of growth and waste reduction efficiency parameters are reported in Table  
267 1. In Trial 1, VEGFRU larvae showed lower LM and time needed to reach the  
268 prepupae stage, as well as higher ECD than FRU larvae. In Trial 2, BRE larvae  
269 showed lower LM, time needed to reach the prepupae stage and SR, and  
270 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  $\text{kg}^{-1}$  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

1  
2  
3 312 moisture contents of VEGFRU and BRE, confirming the results obtained by other  
4 313 authors.<sup>26,27</sup>

5  
6 314 The need for high dietary moisture content could be ascribed to the morphology of  
7  
8 315 the mouthparts of BSF larvae, which resembles the characteristics of scavenger  
9  
10 316 insects.<sup>28,29</sup> This kind of macerating mouth apparatus allows BSF larvae to scrape  
11  
12 317 off the food from the feeding surface. By softening the feed solids, increased  
13  
14 318 dietary moisture content makes easier for the larvae to feed.<sup>30</sup>

15  
16 319 The results obtained in our trials could be also reflective of possible differences  
17  
18 320 between rearing substrates in terms of the content of nutrients other than CP (e.g.,  
19  
20 321 ether extract, structural and non-structural carbohydrates, amino acids) and/or in  
21  
22 322 terms of nutrient digestibility. In both trials, the EE content of substrates (<90 g  
23  
24 323 kg<sup>-1</sup> DM) was far below the 200-260 g kg<sup>-1</sup> found by Nguyen *et al.*<sup>12,13</sup> to have  
25  
26 324 detrimental effects for the survival of BSF larvae and adults. BRE larvae showed  
27  
28 325 very good performances despite the high structural carbohydrates content of the  
29  
30 326 relative rearing substrate (NDF: 447 g kg<sup>-1</sup> DM; ADF: 225 g kg<sup>-1</sup> DM). Such a result  
31  
32 327 clearly demonstrates that BSF larvae are also able to efficiently bioconvert wastes  
33  
34 328 and by-products characterized by high fiber content, thanks to the presence, in the  
35  
36 329 digestive tract of the insect, of intestinal bacteria able to degrade cellulose.<sup>31</sup> The  
37  
38 330 amino acid composition of the rearing substrates was not analyzed in our trials, and  
39  
40 331 little literature is available concerning the effects of dietary amino acids on  
41  
42 332 development and nutritional composition of BSF larvae.<sup>32,33</sup> Studying the nutritional  
43  
44 333 composition of BSF prepupae reared on different organic waste substrates,  
45  
46 334 Sprangers *et al.*<sup>32</sup> showed that the amino acid content of the prepupae had narrow  
47  
48 335 ranges, particularly when compared to the noticeable differences found in the  
49  
50 336 amino acid composition of the rearing substrates. Concerning nutrient digestibility,  
51  
52 337 to the best of our knowledge no studies are currently available. Further studies are  
53  
54 338 necessary to deepen these aspects for the optimization of BSF feeding and  
55  
56 339 nutrition.

1  
2  
3 340 In Trial 2 the differences in larvae growth performances between treatments were  
4  
5 341 very pronounced. We may speculate that the GE of the WIN substrate was not fully  
6  
7 342 available for the larvae. The methodology used to grind the WIN substrate could  
8  
9 343 have influenced the availability of the oil present inside the grape seeds. Indeed, a  
10  
11 344 3-mm grinder was used, and this size could not have completely milled the seeds.  
12  
13 345 Moreover, the WIN substrate could have contained substances unsuitable for the  
14  
15 346 BSF larvae development. Indeed, winery by-products usually contain high levels of  
16  
17 347 polyphenols.<sup>34</sup> It is known that plants use polyphenolic compounds to protect  
18  
19 348 themselves from herbivore insect attacks.<sup>35</sup> Some studies also showed how the  
20  
21 349 grape seeds can accumulate high doses of pesticides and insecticides used in wine  
22  
23 350 grapevines management.<sup>35,36</sup>  
24  
25 351 Hard *et al.*<sup>37</sup> reported that larvae rearing density affects competition for food, low  
26  
27 352 densities usually leading to highest larvae weights. This was also reflected in our  
28  
29 353 trial, as no (Trial 1) or slight (Trial 2) differences were observed for the total final  
30  
31 354 biomass despite the differences found in LM between treatments. The observed LM  
32  
33 355 for VEGFRU was lower than that reported by Nguyen *et al.*<sup>13</sup> using a vegetable and  
34  
35 356 fruit rearing substrate.  
36  
37 357 In both trials, the differences highlighted in terms of LM and ECD were closely  
38  
39 358 connected, and treatments leading to lower mortality allowed obtaining the best  
40  
41 359 performances in terms of ECD. BRE larvae reduced a lesser quantity of substrate  
42  
43 360 compared to WIN larvae; nevertheless, the WRI was higher in the BRE larvae as  
44  
45 361 they took less time to reach the prepupae stage, which is also confirmed by the  
46  
47 362 higher GR results. The SR was particularly high (above 65%) in Trial 1, showing the  
48  
49 363 great potential of BSF larvae in the degradation of vegetable and fruit wastes.<sup>12,13</sup>  
50  
51 364 Overall, the BRE larvae showed the best ECD combined with the absolute highest  
52  
53 365 total final biomass production and the shortest developmental period.  
54  
55 366 The time needed by the larvae to reach the prepupae stage seemed to influence  
56  
57 367 their chitin content. Such results agree with the findings of Diener *et al.*<sup>11</sup> who

1  
2  
3 368 reported how small larvae grown in 42 days showed a chitin level higher than  
4  
5 369 heavy larvae grown in 16 days.

6  
7 370 In both trials, substrates containing the highest CP and moisture contents (VEGFRU  
8  
9 371 and BRE) allowed obtaining BSF larvae with the highest CP level, which is  
10  
11 372 consistent with the results obtained by other authors.<sup>26,27</sup> Consistently with the  
12  
13 373 findings of Janssen *et al.*,<sup>22</sup> the use of the conventional N-factor of 6.25 led to a CP  
14  
15 374 overestimation of about 25%.

16  
17 375 Despite comparable EE values of the rearing substrates, FRU larvae showed higher  
18  
19 376 EE content than VEGFRU larvae, probably as a consequence of the higher NSC level  
20  
21 377 of FRU.<sup>38</sup> Insects have the ability to convert carbohydrates into lipids.<sup>32,39</sup> Insects  
22  
23 378 store lipids for two reasons. Firstly, as energy reserve for the adult stage.<sup>14</sup>  
24  
25 379 Secondly because, as insect body presents an open blood system and a high  
26  
27 380 surface compared to volume and the combination of these two factors could be a  
28  
29 381 problem for the loss of water and the drying out process, lipids allow them to avoid  
30  
31 382 transpiration and store non-imbibed water.<sup>40</sup> However, the influence of the NSC  
32  
33 383 content of substrates on the EE content of BSF larvae should be further  
34  
35 384 investigated as higher NSC in BRE substrate did not lead to higher EE content in  
36  
37 385 BSF larvae in Trial 2.

38  
39 386 The FA composition of the rearing substrates did not directly affect the larvae FA  
40  
41 387 composition, which was also influenced by carbohydrates (starch and sugars),  
42  
43 388 confirming other researches.<sup>26,32</sup> Being of vegetable origin, all rearing substrates  
44  
45 389 had PUFA as the most abundant FA group. Notwithstanding, as typically observed  
46  
47 390 for Diptera, the BSF larvae FA profile was dominated by SFA, mainly C12:0 (which  
48  
49 391 showed the absolute highest values among individual detected FA), C14:0, C16:0  
50  
51 392 and C18:0.<sup>32,41</sup> The high presence of SFA in insects is connected with cold-  
52  
53 393 adaptation.<sup>42</sup> Indeed, larvae from some species showed a SFA decrease from  
54  
55 394 summer to autumn while PUFA increased highlighting a correlation between the  
56  
57 395 change in FA composition and the temperatures due to seasonal change.<sup>42,43,44</sup> BSF  
58  
59 396 is a sub-tropical species growing with high temperatures (27-32°C) and the difficult



1  
2  
3 397 adaptation to low temperatures was demonstrated by the lowest BSF survival rate  
4  
5 398 at about 16°C.<sup>45</sup> We can argue that the high SFA presence could be ascribed to BSF  
6  
7 399 adaptation to the sub-tropical climate. In particular the high content of lauric acid  
8  
9 400 (melting point: 43.2°C) could preserve BSF larvae from lipid oxidation and allow  
10  
11 401 them to survive at temperatures above 40°C.<sup>41</sup> Consistent with other findings,<sup>7,15,32</sup>  
12  
13 402 C18:1 n7 was the main represented MUFA in the larvae, while C18:2 n6 and C18:3  
14  
15 403 n3 were the main represented PUFA n6 and PUFA n3, respectively. The low quantity  
16  
17 404 of recovered n3 PUFA in the larvae could represent a problem if insect meals are  
18  
19 405 intended to be used for animal feed. Indeed, researches highlighted a decrease in  
20  
21 406 nutritional product quality with the inclusion of insect meals in animal diets  
22  
23 407 especially when full-fat meals are used.<sup>5,6,46</sup> Nevertheless, BSF larvae can be  
24  
25 408 enriched in n3-PUFA through the substrate.<sup>33,47</sup> Authors<sup>10,48</sup> reported that C12:0 is  
26  
27 409 a good inhibitor of bacteria strains and could be of great interest in the reduction of  
28  
29 410 the use of antibiotics in animal feeding.<sup>10,49,50</sup> In this context, BSF larvae reared on  
30  
31 411 organic wastes resulted very interesting with up to 574 g kg<sup>-1</sup> TFA of lauric acid.  
32  
33 412  
34  
35 413 Especially in Southern Europe, the large availability of vegetable and fruit wastes  
36  
37 414 (mainly from markets and supermarkets) may allow the development of a BSF  
38  
39 415 larvae mass production, enabling as well to obtain economic and environmental  
40  
41 416 benefits from the sustainable management of organic wastes. Regarding the  
42  
43 417 considered agro-industrial by-products, the use of winery by-products as rearing  
44  
45 418 substrate for BSF larvae could be conditioned, both from a technological and  
46  
47 419 economical point of view, by the need of preliminarily **processing to** remove or  
48  
49 420 **reduce polyphenols**, pesticides and insecticides contents, which could exert a  
50  
51 421 negative influence on the growth performance of the larvae. Remarkable positive  
52  
53 422 results were obtained in terms of overall development time, growth performances  
54  
55 423 and nutritional composition for the larvae reared on brewery by-products, which  
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57 424 should therefore be considered promising rearing substrates. However, as brewery  
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59 425 by-products are characterized by a more limited availability than vegetables, fruits

1  
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3 426 or winery by-products, it could be advisable to use BRE at low dietary inclusion  
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5 427 levels with the purpose of increasing BSF larvae performances.

6  
7 428 Overall, our results show that the performance and chemical composition of BSF  
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9 429 larvae are largely affected by the chemical composition of the provided substrate.  
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11 430 This clearly demonstrates that insects, like farm animals, have nutritional  
12  
13 431 requirements which have to be met for optimal performance.

14  
15 432 The performances obtained in our bench top trials may vary when transferred to an  
16  
17 433 industrial scale. For instance, the large volumes of waste used as well as the high  
18  
19 434 larvae concentration could result in environmental oxygen depletion and heat  
20  
21 435 production.<sup>51</sup> Attention should then be placed to the airflow to guarantee  
22  
23 436 appropriate rearing conditions. In addition, to optimize land use, insect breeding  
24  
25 437 should exploit the verticality of the breeding structure. However, this can lead to  
26  
27 438 the stratification of temperature and particular attention must be given to an  
28  
29 439 adequate air circulation to guarantee a homogeneous temperature in all parts of  
30  
31 440 the building. At industrial level, the production system would also require a  
32  
33 441 constant supply of substrates, possibly with a fairly constant chemical composition,  
34  
35 442 as to obtain BSF larvae with relatively constant nutrient profile.

36  
37 443 Future studies should be designed to assess the nutritional requirements of BSF  
38  
39 444 larvae and to evaluate other agro-industrial by-products, as well as the effect of  
40  
41 445 mixing different organic wastes and agro-industrial by-products, to obtain optimal  
42  
43 446 BSF larvae performances in terms of development, waste reduction efficiency and  
44  
45 447 nutritional composition.

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**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  $\pm$  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 $\pm$ 4.35	19.3 $\pm$ 5.24	0.015	24.8 $\pm$ 10.53	9.5 $\pm$ 5.68	0.011
Total final biomass (g)	10.42 $\pm$ 0.648	10.92 $\pm$ 2.057	0.584	9.90 $\pm$ 0.785	11.32 $\pm$ 0.864	0.014
Time needed to reach prepupae stage (days of trial)	20.2 $\pm$ 1.33	22.0 $\pm$ 0.89	0.031	22.2 $\pm$ 0.98	8.0 $\pm$ 0.01	0.003
Growth rate (g d <sup>-1</sup> )	0.006 $\pm$ 0.0018	0.007 $\pm$ 0.0007	0.451	0.006 $\pm$ 0.0009	0.014 $\pm$ 0.0009	0.000
Substrate reduction (%)	65.2 $\pm$ 5.54	70.8 $\pm$ 8.39	0.129	53.0 $\pm$ 5.28	42.5 $\pm$ 8.41	0.027
Waste reduction index (g d <sup>-1</sup> )	3.2 $\pm$ 0.26	3.2 $\pm$ 0.41	0.952	2.4 $\pm$ 0.32	5.3 $\pm$ 1.05	0.000
Efficiency of conversion of digested food	0.07 $\pm$ 0.009	0.05 $\pm$ 0.011	0.004	0.06 $\pm$ 0.002	0.14 $\pm$ 0.034	0.000

4 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<sup>-1</sup> 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 <sup>-1</sup> )	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 <sup>-1</sup> DM)	15.1	15.6	19.5	19.4

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

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

**Table 3: Fatty acid composition (g kg<sup>-1</sup> 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 <sup>-1</sup> 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<sup>-1</sup> 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	<i>P</i>	WIN	BRE	<i>P</i>
Dry matter (g kg <sup>-1</sup> )	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 <sup>1</sup>	418.8±13.24	307.5±10.29	0.000	344.3±7.63	529.6±5.27	0.000
Crude protein <sup>2</sup>	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 <sup>3</sup>	62.4±19.63	56.0±3.96	0.453	52.9±9.25	14.2±6.06	0.000
Chitin <sup>4</sup>	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.

<sup>1</sup> Obtained using the nitrogen-to-protein conversion factor of 6.25.

<sup>2</sup> Obtained using the nitrogen-to-protein conversion factor of 4.67.

<sup>3</sup> Calculated using the nitrogen-to-protein conversion factor of 6.25.

<sup>4</sup> Obtained using the nitrogen-to-protein conversion factor of 4.67.

**Table 5: Fatty acid composition (g kg<sup>-1</sup> 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 (organic wastes)			Trial 2 (agro-industrial by-products)		
	VEGFRU	FRU	<i>P</i>	WIN	BRE	<i>P</i>
TFA (g kg <sup>-1</sup> 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.

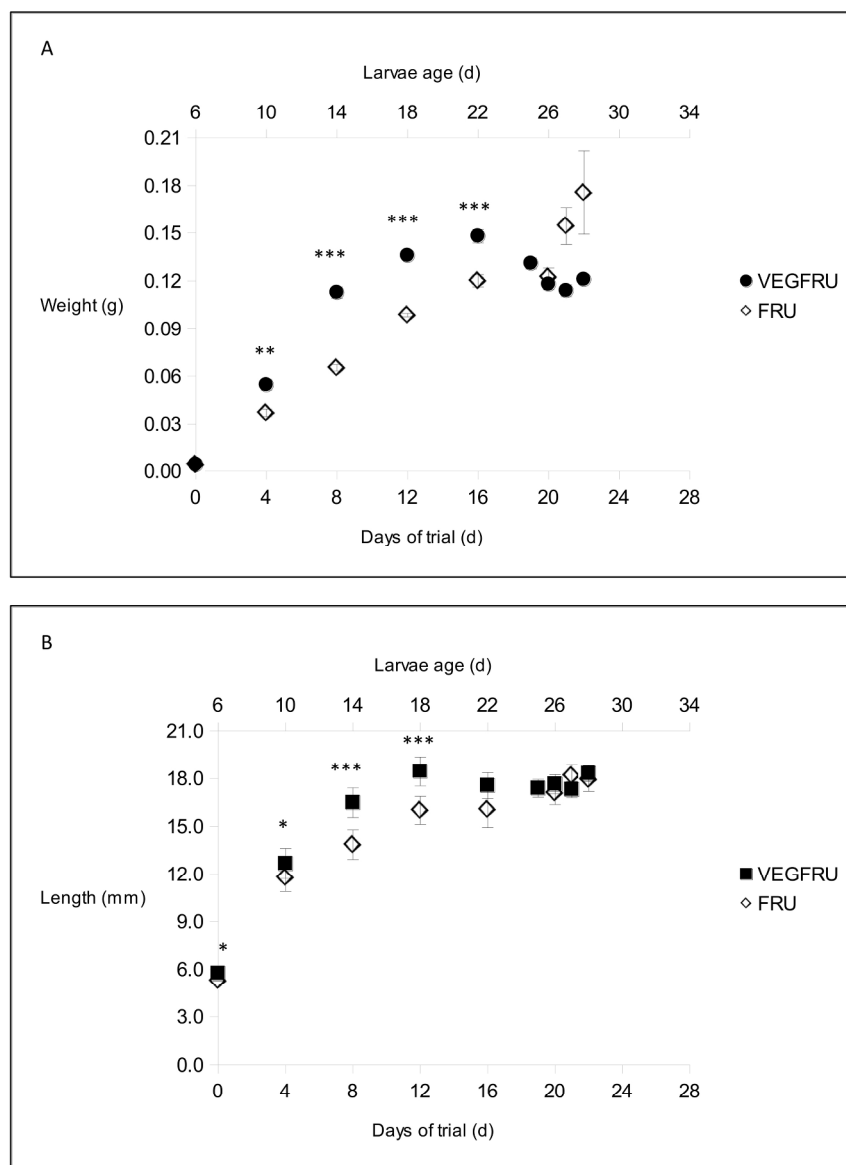


Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. P-value: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Error bars represent the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

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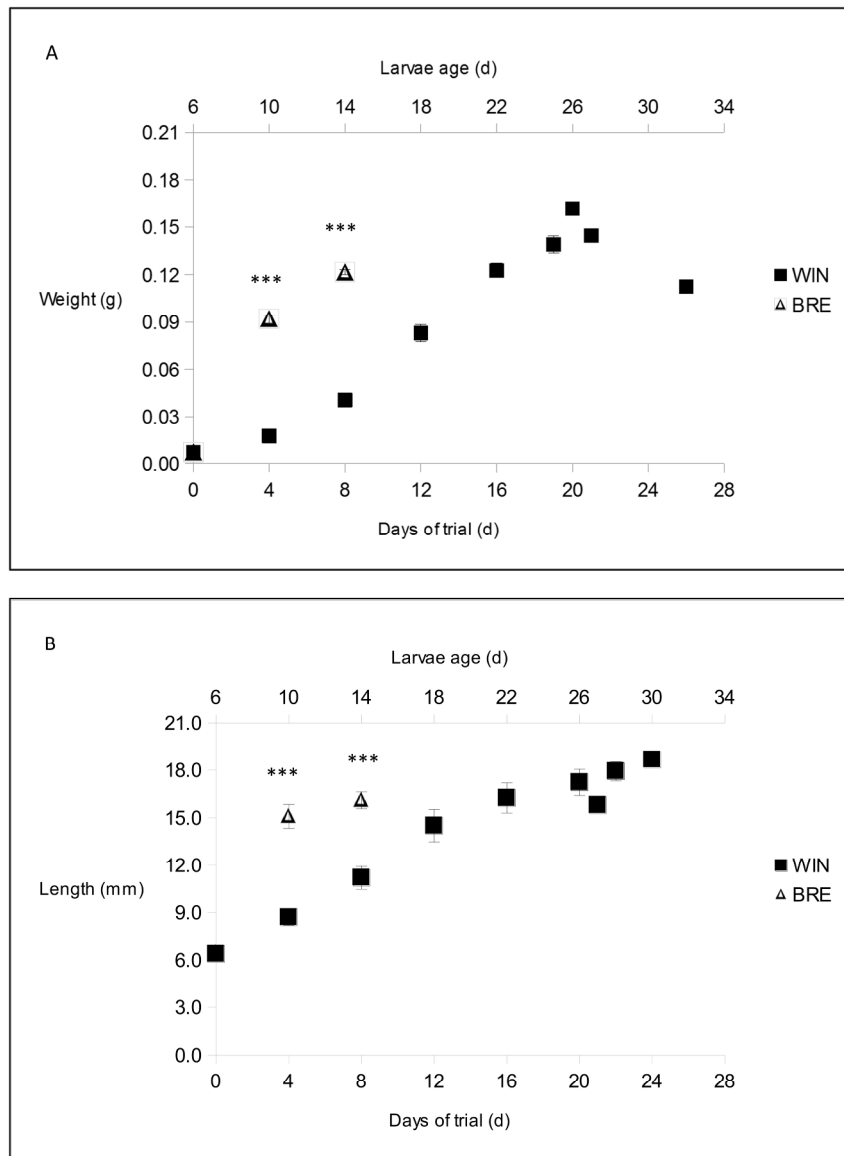


Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. P-value: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Error bars represent the standard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

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3 Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on  
4 organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste)  
5 **generated by the Italian food sector**. *P*-value: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001. Error bars  
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14 Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on  
15 agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) **generated by**  
16 **the Italian food sector**. *P*-value: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001. Error bars represent the  
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18 standard error of the mean.  
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