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

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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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Manuscripts

1   **Effect of rearing substrate on growth performance, waste reduction**

2   **efficiency and chemical composition of black soldier fly (*Hermetia illucens*)**

3   **larvae†**

4

5   **RUNNING TITLE: Rearing substrate effects on performance and nutritional**

6   **composition of black soldier fly**

7

8   Marco Meneguz,[a] Achille Schiavone,[bc] Francesco Gai,[c] Andrea Dama,[a] Carola

9   Lussiana,[a] Manuela Renna,[a*] Laura Gasco[ac]

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11  [a]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo

12  P. Braccini 2, 10095 Grugliasco (TO), Italy

13  [b]Department of Veterinary Science, University of Torino, Largo P. Braccini 2, 10095

14  Grugliasco (TO), Italy

15  [c]Institute of the Science of Food Production, National Research Council, Largo P.

16  Braccini 2, 10095 Grugliasco (TO), Italy

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18  * Corresponding author: manuela.renna@unito.it

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30 **ABSTRACT**

31 BACKGROUND: Wastes can be used as rearing substrate by black soldier fly (BSF)

32 larvae, the latter being exploitable as protein source in animal feed. This research

33 aimed to assess the influence of four rearing substrates [Trial 1 (organic wastes): a

34 mixture of vegetable and fruit (VEGFRU) *vs* a mixture of fruits only (FRU); Trial 2

35 (agro-industrial by-products): brewery (BRE) *vs* winery (WIN) by-products] on BSF

36 larvae development, waste reduction efficiency, and nutritional composition.

37 RESULTS: If respectively compared to FRU and WIN, VEGFRU and BRE larvae

38 needed less time to reach the prepupae stage (22.0, 22.2, 20.2 and 8.0 days of

39 trial, respectively) and had higher protein content (229.7, 257.3, 312.9 and 395.7

40 g kg$^{-1}$ DM). The waste reduction index ranged from 2.4 (WIN) to 5.3 g d$^{-1}$ (BRE).

41 BRE larvae showed the lowest saturated and the highest polyunsaturated fatty

42 acids proportions (612.4 and 260.1 g kg$^{-1}$ total fatty acids, respectively).

43 CONCLUSION: Vegetable and fruit wastes and winery by-products can be used as

44 rearing substrates for BSF larvae mass production. Brewery by-products led to very

45 promising larvae performances and nutritional composition. However, given BRE

46 limited availability, low BRE dietary inclusion levels could be used with the purpose

47 of increasing larvae performances.

48

49 **Keywords:** organic waste, agro-industrial by-product, *Hermetia illucens*, animal

50 feed, crude protein, fatty acid profile

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53 **INTRODUCTION**

54 The world population is estimated around 7.3 billion, with a growth rate of about 83

55 million per year. This increase will generate an increment of food demand with a

56 consequent rise in waste and by-products production.[1] Urgent and innovative

57 solutions are needed for the management of the waste streams (WS) that

58 nowadays are estimated around 1.3 billion and 100 million tons per year in the

59 world and in the European Union, respectively.[1,2] Furthermore, the EC Directive No

60 2008/98 unequivocally establishes the order of priority in the choice of WS

61 treatment, the first being their reuse and the last their landfill disposal.

62 Some WS could be valorized through the recovery of the residual bio-elements they

63 contain, with a cost reduction both for the industry (disposal cost) and the

64 environment (pollution).[3] The use of insects in the bioconversion of WS constitutes

65 a new approach and an interesting example of sustainable circular economy. This

66 bioconversion can generate new elements such as proteins and lipids for animal

67 feeds,[4,5,6,7] biodiesel,[8] high value products as chitin[9] or anti-microbial peptides.[10]

68 Processed proteins from seven insect species have recently been approved for

69 aquafeed by the EC Regulation No 2017/893, which also lists the licensed rearing

70 substrates. Among authorized species, black soldier fly (BSF; Diptera:

71 Stratiomydae) is one of the most promising and researches recently aimed to

72 increase knowledge on optimal rearing substrates for larvae and prepupae. In this

73 respect, BSF has shown great flexibility as it can be used to reduce volume and add

74 value to various wastes.[8,11,12] The available literature has highlighted that BSF life

75 cycle and nutritional composition are noticeably influenced by the rearing

76 substrate,[13,14] with the crude protein (CP) content of the larvae ranging from about

77 317 to 630 g kg$^{-1}$ dry matter (DM).[7,15,16]

78 In 2014, around 90 million tons of slaughter and vegetable WS were produced in

79 Europe

80 (EUROSTAT(http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_wasge

81 n&lang=en)). Considering the Italian context, 54% of the total production of waste

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82   and agro-industrial by-products is generated by the manufacturing of vegetable

83   products.[17] About 1.5 million tons of winery by-products and 406 tons of brewery

84   by-products                     are                     produced                     every                     year

85   (EUROSTAT(http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language

86   =en&pcode=tag00034&plugin=1;                               EU                     Report

87   (https://www.brewersofeurope.org/site/media-

88   centre/index.php?doc_id=905&class_id=31&detail=true)).[18]

89   The aim of this research was to evaluate the effects of organic wastes (vegetables

90   and fruits) and agro-industrial by-products (winery and brewery) generated by the

91   Italian food sector as rearing substrates for BSF larvae on their development, waste

92   reduction efficiency and chemical composition.

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94                               **MATERIAL AND METHODS**

95   Two trials were carried out at the Experimental Facility of the Department of

96   Agricultural, Forest and Food Sciences (DISAFA; University of Torino, Torino, Italy).

97

98                               ***Rearing substrates***

99   In Trial 1, two organic wastes were compared:

100  - Vegetable-fruit waste (VEGFRU) obtained from a street market (Torino, Italy) and

101  containing a mixture of vegetables and fruits (celery 43.4%, oranges 28.9% and

102  peppers 27.7%);

103  - Fruit waste (FRU) obtained from a fruit market (Torino, Italy) and containing fruits

104  only (apples 47.8%, oranges 15.5%, apple leftovers 13.8%, strawberries 7.1%,

105  mandarins 4.8%, pears 4.1%, kiwis 3.4%, bananas 1.9% and lemons 1.6%).

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

107  - Winery by-product (WIN) obtained during the wine making process, before the

108  alcohol extraction, from a private distillery (Distilleria Santa Teresa dei Fratelli

109  Marolo S.R.L., Alba (CN), Italy) and containing grape seeds, pulp, skins, stems and

110  leaves;

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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 Tomberlin[19] 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

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140   chamber under controlled environmental conditions (T: 27±0.5°C; RH: 70±5%;

141   24:0 L:D photoperiod).

142   Each replicate was monitored daily to control the quantity of available feed. If

143   needed, as reported by Harnden and Tomberlin,[19] 50 g of substrate per replicate

144   was added in all replicates at the same time.

145   To avoid the effect of handling on the considered dependent variables,[13] weight and

146   length data were collected every four days until the appearance of the first

147   prepupae, thereafter every day for the relative substrate. Thirty larvae were

148   randomly sampled for three consecutive times from each container to measure

149   weight and length. As measurement was not destructive, the larvae were re-

150   introduced into the containers between two consecutive samplings. The sampled

151   larvae were individually cleaned, dried with a paper towel and weighed, and

152   photographed orthogonally (Lumix G1; Panasonic Corporation, Kadoma, Osaka,

153   Japan) with a metric scale (mm). The images were analyzed with ImageJ software

154   package (v. 1.50b) to record larvae length (i.e., from mouthpart to the bottom of

155   the last abdominal segment).

156   For each container, weight and length data collection ended when 30% of the

157   larvae reached the prepupae stage. The prepupae were removed from the

158   containers. The remaining 70% of the larvae were hand-counted, washed, dried

159   with a paper towel and individually weighed and photographed. The total final

160   biomass (larvae + prepupae) and the residual rearing substrate were also weighed.

161   The following parameters were then calculated:

162   – larvae mortality (LM)

163   LM = [initial number of larvae - (final number of larvae + number of prepupae)] /

164   initial number of larvae * 100;

165   - growth rate (GR),[20] readapted for this research substituting prepupa body weight

166   (g) with larva body weight (g)

167   GR = (larva average final body weight (g) - larva initial body weight (g)) / days of

168   trial (d);

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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.

182

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.

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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*.[5] 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*.[22] was used. Neutral detergent fiber (NDF) was

205    analyzed according to Van Soest *et al*.[23] Acid detergent fiber and acid detergent

206    lignin (ADF and ADL) were determined according to method no. 973·18 of AOAC

207    International.[24] The residual nitrogen in ADF (ADFN) was determined according to

208    method no. 984.13 of AOAC International.[24] Chitin (CHI, g kg$^{-1}$ DM) was estimated

209    as: ash free ADF (g kg$^{-1}$) – ADFN * N-factor (g kg$^{-1}$).[9] 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.[25] The results were

212    expressed as g kg$^{-1}$ 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

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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**

238    ***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 ± SD:

246    0.004±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±0.0084 g) compared to the FRU larvae (0.037±0.0058 g). Such

249    trend was maintained at each test day until day 16 (VEGFRU: 0.148±0.0103 g;

250    FRU: 0.120±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±1.32 mm and 5.3±1.17 mm, respectively ($P<0.05$; Figure 1B).

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

9

255  last statistical assessment (day 16). VEGFRU and FRU larvae achieved a final length

256  of 17.7±0.46 mm and 17.8±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±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±0.0063 and 0.017±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±1.36 and 6.4±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±1.84 mm (BRE) and 8.7±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 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.

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284

### *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 *c*9 and C18:2 n6

301 were the most represented unsaturated FA in all treatments.

302

### 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

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312 moisture contents of VEGFRU and BRE, confirming the results obtained by other

313 authors.[26,27]

314 The need for high dietary moisture content could be ascribed to the morphology of

315 the mouthparts of BSF larvae, which resembles the characteristics of scavenger

316 insects.[28,29] This kind of macerating mouth apparatus allows BSF larvae to scrape

317 off the food from the feeding surface. By softening the feed solids, increased

318 dietary moisture content makes easier for the larvae to feed.[30]

319 The results obtained in our trials could be also reflective of possible differences

320 between rearing substrates in terms of the content of nutrients other than CP (e.g.,

321 ether extract, structural and non-structural carbohydrates, amino acids) and/or in

322 terms of nutrient digestibility. In both trials, the EE content of substrates (<90 g

323 $kg^{-1}$ DM) was far below the 200-260 g $kg^{-1}$ found by Nguyen *et al*.[12,13] to have

324 detrimental effects for the survival of BSF larvae and adults. BRE larvae showed

325 very good performances despite the high structural carbohydrates content of the

326 relative rearing substrate (NDF: 447 g $kg^{-1}$ DM; ADF: 225 g $kg^{-1}$ DM). Such a result

327 clearly demonstrates that BSF larvae are also able to efficiently bioconvert wastes

328 and by-products characterized by high fiber content, thanks to the presence, in the

329 digestive tract of the insect, of intestinal bacteria able to degrade cellulose.[31] The

330 amino acid composition of the rearing substrates was not analyzed in our trials, and

331 little literature is available concerning the effects of dietary amino acids on

332 development and nutritional composition of BSF larvae.[32,33] Studying the nutritional

333 composition of BSF prepupae reared on different organic waste substrates,

334 Spranghers *et al*.[32] showed that the amino acid content of the prepupae had narrow

335 ranges, particularly when compared to the noticeable differences found in the

336 amino acid composition of the rearing substrates. Concerning nutrient digestibility,

337 to the best of our knowledge no studies are currently available. Further studies are

338 necessary to deepen these aspects for the optimization of BSF feeding and

339 nutrition.

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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

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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

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397 adaptation to low temperatures was demonstrated by the lowest BSF survival rate

398 at about 16°C.[45] We can argue that the high SFA presence could be ascribed to BSF

399 adaptation to the sub-tropical climate. In particular the high content of lauric acid

400 (melting point: 43.2°C) could preserve BSF larvae from lipid oxidation and allow

401 them to survive at temperatures above 40°C.[41] Consistent with other findings,[7,15,32]

402 C18:1 *c*9 was the main represented MUFA in the larvae, while C18:2 n6 and C18:3

403 n3 were the main represented PUFA n6 and PUFA n3, respectively. The low quantity

404 of recovered n3 PUFA in the larvae could represent a problem if insect meals are

405 intended to be used for animal feed. Indeed, researches highlighted a decrease in

406 nutritional product quality with the inclusion of insect meals in animal diets

407 especially when full-fat meals are used.[5,6,46] Nevertheless, BSF larvae can be

408 enriched in n3-PUFA through the substrate.[33,47] Authors [10,48] reported that C12:0 is

409 a good inhibitor of bacteria strains and could be of great interest in the reduction of

410 the use of antibiotics in animal feeding.[10,49,50] In this context, BSF larvae reared on

411 organic wastes resulted very interesting with up to 574 g kg$^{-1}$ TFA of lauric acid.

412

413 Especially in Southern Europe, the large availability of vegetable and fruit wastes

414 (mainly from markets and supermarkets) may allow the development of a BSF

415 larvae mass production, enabling as well to obtain economic and environmental

416 benefits from the sustainable management of organic wastes. Regarding the

417 considered agro-industrial by-products, the use of winery by-products as rearing

418 substrate for BSF larvae could be conditioned, both from a technological and

419 economical point of view, by the need of preliminarily processing to remove or

420 reduce polyphenols, pesticides and insecticides contents, which could exert a

421 negative influence on the growth performance of the larvae. Remarkable positive

422 results were obtained in terms of overall development time, growth performances

423 and nutritional composition for the larvae reared on brewery by-products, which

424 should therefore be considered promising rearing substrates. However, as brewery

425 by-products are characterized by a more limited availability than vegetables, fruits

15

426 or winery by-products, it could be advisable to use BRE at low dietary inclusion

427 levels with the purpose of increasing BSF larvae performances.

428 Overall, our results show that the performance and chemical composition of BSF

429 larvae are largely affected by the chemical composition of the provided substrate.

430 This clearly demonstrates that insects, like farm animals, have nutritional

431 requirements which have to be met for optimal performance.

432 The performances obtained in our bench top trials may vary when transferred to an

433 industrial scale. For instance, the large volumes of waste used as well as the high

434 larvae concentration could result in environmental oxygen depletion and heat

435 production.[51] Attention should then be placed to the airflow to guarantee

436 appropriate rearing conditions. In addition, to optimize land use, insect breeding

437 should exploit the verticality of the breeding structure. However, this can lead to

438 the stratification of temperature and particular attention must be given to an

439 adequate air circulation to guarantee a homogeneous temperature in all parts of

440 the building. At industrial level, the production system would also require a

441 constant supply of substrates, possibly with a fairly constant chemical composition,

442 as to obtain BSF larvae with relatively constant nutrient profile.

443 Future studies should be designed to assess the nutritional requirements of BSF

444 larvae and to evaluate other agro-industrial by-products, as well as the effect of

445 mixing different organic wastes and agro-industrial by-products, to obtain optimal

446 BSF larvae performances in terms of development, waste reduction efficiency and

447 nutritional composition.

448

449 **ACKNOWLEDGEMENTS**

451

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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 ± SD; n = 6).**

| | Trial 1 (organic wastes) | | | Trial 2 (agro-industrial by-products) | | |
|---|---|---|---|---|---|---|
| | VEGFRU | FRU | *P* | WIN | BRE | *P* |
| 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.

1 **Table 2: Proximate composition (g kg$^{-1}$ dry matter, unless otherwise stated) of organic wastes (vegetables and fruits) and**

2 **agro-industrial by-products (winery and brewery) generated by the Italian food sector and used as rearing substrates by**

3 **black soldier fly larvae.**

|  | Trial 1 (organic wastes) | | Trial 2 (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 |

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).

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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 (organic wastes) | | Trial 2 (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 *c*9 | 5.89 | 5.96 | 4.43 | 1.75 |
| C18:0 | 26.14 | 43.36 | 50.24 | 15.42 |
| C18:1 *c*9 | 65.91 | 208.61 | 185.09 | 103.27 |
| C18:1 *c*11 | 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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3
4 1 **Table 5: Fatty acid composition (g kg$^{-1}$ total fatty acids, unless otherwise stated)** of black soldier fly larvae reared on organic
5
6 2 wastes (vegetables and fruits) and agro-industrial by-products (winery and brewery) generated by the Italian food sector
7
8 3 (mean ± SD; n = 6).
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| | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| | (organic wastes) | | | (agro-industrial by-products) | | |
| | VEGFRU | FRU | P | WIN | BRE | P |
| 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 *c*9 | 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 *c*9 | 85.37±4.075 | 93.19±2.086 | 0.002 | 124.59±4.280 | 92.23±2.414 | 0.004 |
| C18:1 *c*11 | 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.
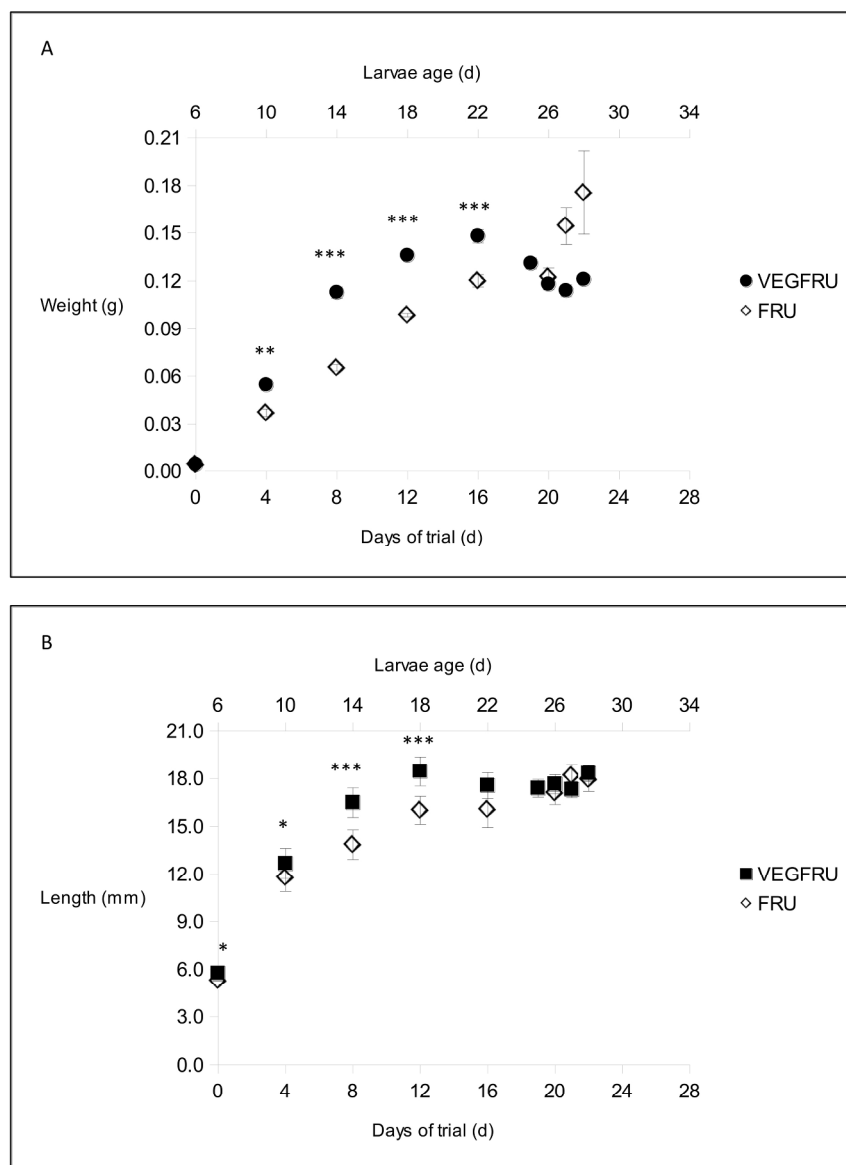
Figure 1. Trial 1: Development (A: weight; B: length) of black soldier fly larvae reared on organic wastes (VEGFRU: 70% vegetable and 30% fruit waste; FRU: 100% fruit waste) generated by the Italian food sector. P-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

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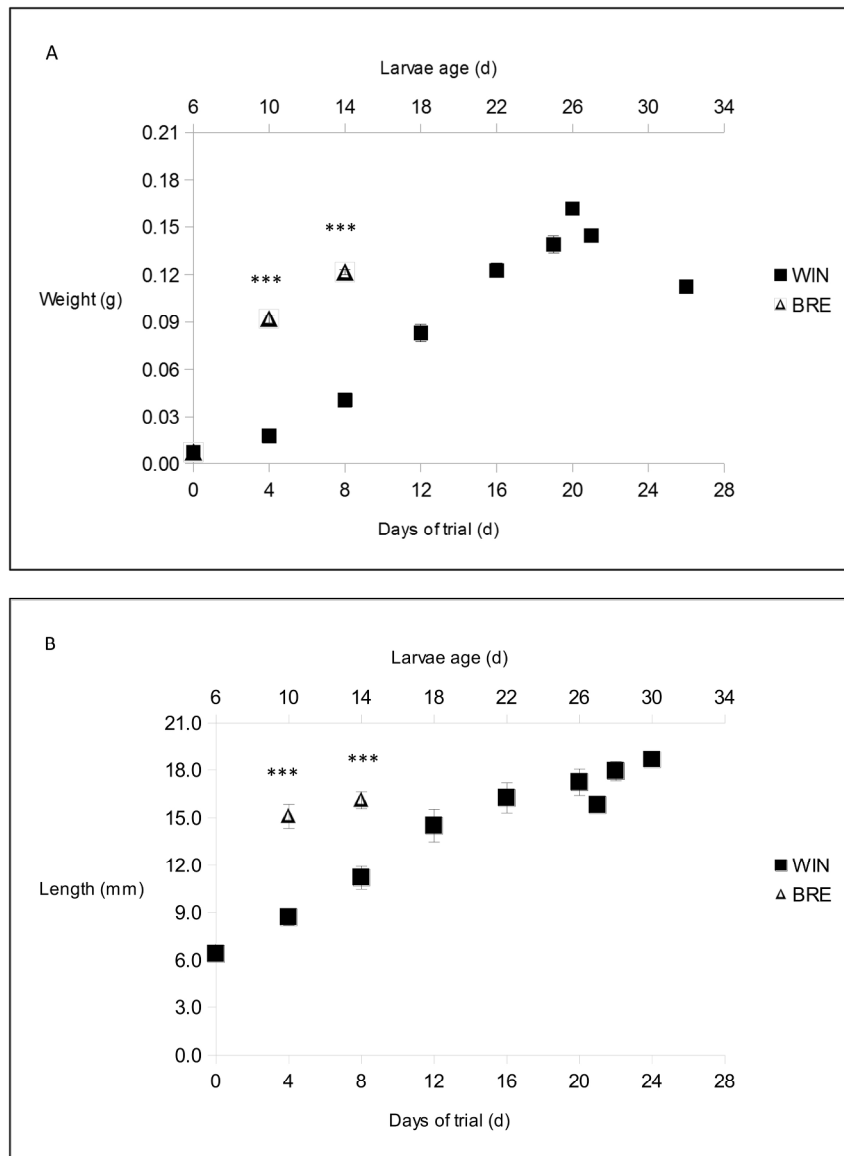50
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Figure 2. Trial 2: Development (A: weight; B: length) of black soldier fly larvae reared on agro-industrial by-products (WIN: winery by-product; BRE: brewery by-product) generated by the Italian food sector. P-value: *P<0.05, **P<0.01, ***P<0.001. Error bars represent the standard error of the mean.

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.