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ORIGINAL ARTICLE

Enhancement of fruit byproducts through bioconversion by *Hermetia illucens* (Diptera: Stratiomyidae)

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Abstract Bioconversion is a biological process by which organic materials are converted into products with higher biological and commercial value. During its larval stage the black soldier fly *Hermetia illucens* is extremely voracious and can feed on a wide variety of organic materials. To study the impact of different fruit byproducts on the insect's growth, final larval biomass, substrate reduction, bioconversion parameters, and larval nutritional composition, 10 000 black soldier fly larvae (BSFL) were reared on 7.0 kg of one of three substrates (strawberry, tangerine, or orange) or on a standard diet as a control. The results highlight that BSFL can successfully feed and grow on each of these diets, though their development time, growth rate, and final biomass were differently impacted by the substrates, with strawberry being the most suitable. The lipid and protein contents of BSFL were similar among larvae fed on different substrates; however, major differences were detected in ash, micronutrient, fiber, fatty acid, and amino acid contents. Overall, the results indicate that fruit waste management through the BSFL bioconversion process represents a commercially promising resource for regional and national agrifood companies. Our study offers new perspectives for sustainable and environmentally friendly industrial development by which fruit byproducts or waste might be disposed of or unconventionally enhanced to create secondary products of high biological and economic value, including BSFL biomass as animal feed or, in perspective, as alternative protein source for human nutrition.

Key words bioconversion; black soldier fly; byproducts; circular economy; feed

Introduction

Rapid population growth and urbanization are increasingly raising concerns about food supplies (protein sources in particular) and agricultural waste management. The global population is anticipated to grow to almost 8.5 billion by 2030, 9.7 billion by 2050, and 10.9 billion by 2100 (UN, 2019). Consequently, the global demand for animal proteins is expected to increase by 9.1% between 2020 and 2027 (Ismail *et al.*, 2020). Intensified production of animal-based protein will have a negative environmental impact, including elevated gas emissions; increased exploitation of nonrenewable resources, such as water and land; soil acidification, erosion, and compaction; and overgrazing

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and nitrification (Grossi *et al.*, 2019). Recently, insects have been identified as an alternative source of protein for both animal feed and human nutrition, with farmed insects entailing a lower environmental impact as compared with traditional livestock such as cows, sheep, pigs, and chickens (Oonincx & de Boer 2012; Van der Spiegel *et al.*, 2013; Kim *et al.*, 2019; MacLeod *et al.*, 2019; Rodríguez-Miranda *et al.*, 2019). Insect farming requires less water and emits lower levels of greenhouse gases and NH_3 compared with traditional livestock farming. Another advantage of insects as a protein source is linked to a high percentage of edibility and digestibility of the insect body (Kinyuru *et al.*, 2010); indeed, entomophagy has occurred for thousands of years, with upward of 2000 species of insects included in the traditional diets of at least 2 billion people, especially in Asia, Africa, and South America (Jongema, 2017). Moreover, many insects feed naturally on organic waste, simultaneously reducing its mass and gaining nutrients (Fowles & Nansen, 2020) through bioconversion processes (Van Huis, 2013; Smetana *et al.*, 2016; Henchion *et al.*, 2017). The black soldier fly *Hermetia illucens* (L.) is one of the most important and most studied bioconverter insects in the world (Wang & Shelomi, 2017). Black soldier fly larvae (BSFL) can feed on a range of decomposing organic matter of plant and animal origin (Bava *et al.*, 2019; da Silva & Hesselberg, 2020), including fruit and vegetable waste from the agrifood chain (Nguyen *et al.*, 2015; Supriyatna *et al.*, 2016; Jucker *et al.*, 2017; Barbi *et al.*, 2020; Scala *et al.*, 2020; Koffi *et al.*, 2021; Romano *et al.*, 2022), distillers grains (Webster *et al.*, 2016; Chia *et al.*, 2018; Bava *et al.*, 2019; Grossule *et al.*, 2020; Jucker *et al.*, 2020a; Scala *et al.*, 2020), and manure (Newton *et al.*, 2005; Zhou *et al.*, 2013; Bortolini *et al.*, 2020; Franco *et al.*, 2022a). In this regard particular attention should be paid to agroindustrial waste, a substrate allowed as feed for insects, which, in turn, can then be used for pet food, aquaculture, poultry and pig feeds (Commission Regulations [EU] 2017/893 and 2021/1372). The latest reports of FAO, IFAD, UNICEF, WFP, and WHO (2019, 2022) estimate that more than 20% of the global vegetable and fruit production (15% in Europe alone) is lost postharvest. This estimate increases when the manufacturing stage (production and packaging) is considered, with almost 30% of the fruit and vegetable production in Europe being lost (Ganesh *et al.*, 2022). Indeed, fruit producers must comply with specific standards for the fruit to be marketable (e.g., no altered organoleptic characteristics, specific requirements around dimensions and color, and suitable ripeness vs. states of decomposition). When these standards are not respected, the fruit must be considered as waste or byproduct.

BSFL can effectively feed on various byproducts from the agrifood chain, although the efficiency of bioconversion is strictly related to the environmental conditions and characteristics of the substrate (Tomberlin *et al.*, 2009; Bekker *et al.*, 2021); indeed, the larvae can reduce such substrate by up to 80% while converting it into larval biomass within timeframes that depend on the rearing conditions (Diener *et al.*, 2011; Zhou *et al.*, 2013; Barragan-Fonseca *et al.*, 2017; Jucker *et al.*, 2020b). At the end of the larval development stage, equivalent to the end of the bioconversion process, products of high biological and commercial value are obtained: in particular the larval biomass, composed of proteins (ranging from 37% to 63% of their dry weight), lipids (saturated and unsaturated fatty acids, which can exceed 40% of larval dry matter), minerals and fibers. All parameters related to the growth of BSFL such as time to reach the pupal stage, weight gain, and nutritional composition of the larvae, and the reduction of the organic waste are strictly linked to the substrate and environmental conditions (Nguyen *et al.*, 2013; Tschirner & Simon, 2015; Rehman *et al.*, 2017; Julita *et al.*, 2019; Fadhillah & Bagastyo, 2020; Nur'aini & Prawanto, 2021). Besides the larval biomass, which is rich in macronutrients and micronutrients (usable in feeds and prospectively in human food), frass biomass and other bioactive compounds of high biological and economic value can be obtained at the end of the bioconversion process, including lipids, chitin, and antimicrobial peptides (Moretta *et al.*, 2020, 2021; Manniello *et al.*, 2021; Franco *et al.*, 2021, 2022b; Triunfo *et al.*, 2021, 2022; Di Somma *et al.*, 2022; Guarnieri *et al.*, 2022). This study investigated the ability of BSFL to feed on specific fruit byproducts. Three different byproducts were separately tested, and larval growth, substrate reduction, bioconversion parameters, and nutritional composition of the larvae in terms of ash, mineral, protein, amino acid, lipid, fatty acid, and fiber contents were evaluated. We hope that these assessments contribute to validating the use of BSFL to dispose of fruit processing byproducts.

Materials and methods

Insect rearing

Black soldier fly eggs were provided by Xflies s.r.l. (Potenza, Italy). After egg hatching, four groups of 10 000 neonates were reared on the standard Gainesville diet, provided by the animal feed factory Mangimi Losasso s.r.l.—Balvano (Potenza, Italy), consisting of 20% corn, 30% alfalfa meal, and 50% wheat bran

Table 1 Nutritional value of each substrate, as published at the USDA, the U.S. Department of Agriculture (https://fdc.nal.usda.gov/) and standard diet, provided by the animal feed factory.

Substrate	Protein	Lipid	Ash	Fiber
Standard diet	12.16%	3.5%	3.94%	11.02%
Strawberry	0.06%	0.03%	0.04%	0.18%
Tangerine	0.11%	0.05%	0.05%	0.26%
Orange	0.12%	0.02%	0.08%	0.32%

Note: Data are reported as percentage of dry matter.

(Hogsette, 1992) at 82% moisture, for 4 days, as described in Scala *et al.* (2020). On the fifth day, each group of 10 000 larvae, the frass, and the leftover Gainesville diet were transferred into plastic boxes containing 7.00 kg of one of the three substrates or the standard diet. The selected three fruit diets were byproducts of strawberry, tangerine, and orange production, provided by the cooperative Apofruit Italia—Montalbano Jonico (Potenza, Italy). The cooperative deals with the collection, storage, packaging, and marketing of fresh fruits that are supplied by the co-op members themselves. Each year, the storehouse at Montalbano Jonico deals with ∼20 000 tons of fruit, of which 10%–15% (2000–3000 tons) are byproducts that cannot be marketed.

Table 1 lists the reported nutritional value of each substrate, as published by the United States Department of Agriculture (https://fdc.nal.usda.gov), and that of the standard diet, provided by the animal feed factory.

Plastic boxes with 7.00 kg of each substrate were placed in an environmental test chamber with a temperature of 27.0 ± 1.0°C and 70.0% relative humidity.

Determinations of dry matter and water content of the substrates, larvae, and frass

The water content of the diets was calculated at the beginning of the experiment, whereas the water content of the BSFL and frass were assessed at the end of the experiment. An amount equal to 10 g of each organic diet/larvae/frass in replicates was weighed and then placed into an aluminum dish and dried for 24 h at 55°C in a Gallenkamp Hotbox Oven (London). Dry matter and water content were determined according to the following formulas:

$$\text{Dry matter}\ \ (\%) = \left(\frac{\text{Final weight}\ \ (\text{g})}{\text{Inital weight}\ \ (\text{g})}\right) \times 100. \quad (1)$$

$$\text{Water content}\ \ (\%) = 100\ \ \text{Dry matter}\ \ (\%). \quad (2)$$

Growth curves, growth rate, and index of growth by time

Each day, six groups of 10 larvae from each box were randomly selected, weighed on an analytical balance (Sartorius AG, Göttingen, Germany), and returned to their respective boxes. Each experiment was considered concluded when a decrease in larval weight was registered, revealing that the larvae had stopped feeding. The mean larval weight recorded on the date that the larvae stopped gaining weight defined the final larval weight. The maximum larval weight was recorded the day before the end of the trail. Larval growth rate was then calculated following the formula of Leong *et al.* (2016):

$$\text{Growth rate}\ \ \left(\frac{\text{g}}{\text{d}}\right) = \frac{(10\ \text{larvae average final weight}\ \ (\text{g}) - 10\ \text{larvae average initial weight}\,(\text{g}))}{\text{days of experimental trials}\ (\text{d})}. \quad (3)$$

The index of the larval growth by time, hereafter "average growth rate," was calculated as follows (Scala *et al.*, 2020):

$$\text{Average growth rate}\ \left(\frac{\text{g}}{\text{d}}\right) = \frac{\text{Total biomass}\ (\text{g})}{\text{days of experimental time}\ (\text{d})}. \quad (4)$$

At the end of the trials, the BSFL were separated and collected manually after sieving the frass. Frass refers to excretory products and undigested food. The larvae and frass were weighed separately.

Ash and mineral contents of the larvae

To determine the ash content (i.e., mineral and metal composition), 10 g of larvae from each experimental trial was dried for 48 h at 80°C in a Gallenkamp Hotbox Oven (London), followed by incineration at 550°C for 3 h in a Muffle Furnace (Gefran 1001, Provaglio d'Iseo, Italy). Ash content was calculated as follows:

$$\text{Ash content } (\%) = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}} \times 100 \qquad (5)$$

The mineral profile was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES), as described by Addeo *et al.* (2021). Approximately 0.5 g of each sample was weighed, added with 5.0 mL of 65% HNO_3 and 2.0 mL of 30% H_2O_2, and then digested in a microwave system (25 min at 190°C). After cooling at 32°C, the digested samples were transferred into flasks and diluted to 50 mL by adding Milli-Q water. Concentrations of trace elements were determined by ICP-OES testing using a PerkinElmer Optima 2100 DV device coupled with a CETAC U5000AT Ultrasonic Nebulizer. The calibration curve and two blanks were run during each set of analyses to check the purity of the chemicals. Reference materials (BCR-422 [cod muscle], Institute for Reference Materials and Measurements, Belgium; DORM-5 [fish protein], National Research Council, Canada) were also included for quality control. All the values of the reference materials were within certified limits. Instrumental detection limits are expressed as wet weight (w.w.) and determined following the protocol of PerkinElmer ICP application study no. 57 (Barnard *et al.*, 1993).

Protein and amino acid contents of larvae

To determine protein content, an amount of 10 g of larvae from each experimental trial was dried for 24 h at 55°C in a Gallenkamp Hotbox Oven (London). The samples of larvae were analyzed for crude protein content using the Kjeldahl method, according to AOAC (2004). The nitrogen-to-crude protein conversion factor used in this trial was 4.76, following Janssen *et al.* (2017).

Free amino acids were analyzed following the method of Troise *et al.* (2018). Samples were weighed (50 mg) and 2 mL of a mix of acetonitrile/water (50 : 50 v/v, 0.1% formic acid) was added. The samples were vortexed for 1 min and sonicated at 4°C for 10 min. This step was followed by centrifugation for 5 min at 5000× *g* and 4°C, and then the upper phase was filtered using 0.22-μm PVDF filters (EMD Millipore Corp., Billerica, MA). To determine the amino acid contents of the protein, acid hydrolysis of the sample was performed; 50 μL of a solution of butylhydroxytoluene (2% in methanol) and 2 mL of 6N HCl were added to 10 mg of lyophilized and ground sample. The mix was incubated for 24 h at 110°C. Subsequently, the sample was vortexed and filtered on a 0.22-μm PVDF filter. Both samples (free amino acids and amino acids bonded in proteins) were analyzed using Orbitrap mass spectrometry (UHPLC-HRMS). Chromatographic separation of amino acids was achieved at 40°C using a bioZen Glycan LC column (2.6 μm, 100 × 2.1 mm; Phenomenex, Italy). Mobile phases used 10 mmol/L ammonium formate in water (solvent A), 10 mmol/L ammonium formate in acetonitrile (solvent B), and the following linear gradients of solvent B (min/%B): (0.01/100), (2/95), (7/50), (8/50), (8.1/100), and (12/100); the flow rate was set to 500 μL/min and the injection volume was 5 μL. The ultrahigh-performance liquid chromatograph (UHPLC) system (Thermo Fisher Scientific, Waltham, MA), equipped with a Dionex Ultimate 3000 quaternary pump, was interfaced to an Exactive Orbitrap HRMS system (Thermo Fisher Scientific, Bremen, Germany) and the analytes were detected through a heated electrospray ionization (ESI) source operating in positive mode. Full mass spectrometry experiments were carried out with the following settings: microscans, 3; AGC target, 1e6; maximum injection time, 200 ms; and mass resolution, 70 000 FWHM at *m*/*z* 200. The instrument was set to spray voltage 3.5 kV; capillary temperature 310°C; sheath gas 30 (arbitrary units); auxiliary gas 10 (arbitrary units); *m*/*z* range 70–300, in data acquisition profile mode. The accuracy of mass spectrometry analysis was ensured by calibrating the detector using the commercial calibration solutions that were provided by the manufacturer by setting mass tolerance at 5 ppm. Xcalibur software v. 3.1.66.10 (Thermo Fisher Scientific) was used to perform data analysis and processing.

Lipid and fatty acid contents of larvae

Lipid content was measured by taking 10 g of larvae from each experimental trial and drying them for 24 h at 55°C in a Gallenkamp Hotbox Oven (London). Total fat was extracted following the method of Folch *et al.* (1957) and fatty acid transmethylation effectuated by a base-catalyzed procedure reported by Christie (1982) and modified by Chouinard *et al.* (1999). The methyl esters were quantified using a FOCUS gas chromatograph (Thermo Scientific Co., Walthman, MA) equipped with a fused silica SP®-2380 capillary column (100 m length × 0.25 mm inner diameter × 0.2 μm film

thickness) (Supelco Inc., Bellefonte, PA) using an AS 3000 II autosampler. The carrier gas (helium) was set at the constant pressure of 180 kPA, splitting flow of 50 mL/min, injection volume of 1 μL, in accordance with Zicarelli *et al.* (2016). Fatty acid peaks were identified by comparing the retention times of commercial standard containing 37 methyl esters of fatty acids (Sigma-Aldrich Inc., St Louis, MO). The retention times of the conjugated linoleic acid (CLA) isomers were controlled by the elution of commercial standards (Larodan AB, Solna, Sweden) of these fatty acids. The area of each individual fatty acid identified in the sample was quantified by percentage calculation on the total area of the eluted peaks.

Fiber content of larvae

To determine the fiber content, an amount of 10 g of larvae from each experimental trial was dried for 24 h at 55°C in a Precision Scientific Gallenkamp Hotbox Oven. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the method of Van Soest *et al.* (1991) using an Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Sodium sulfite was used in the NDF procedure, and both fractions were expressed as exclusive of residual ash. Acid detergent lignin (ADL) was determined following the procedure of Robertson and Van Soest (1981).

As ADF determines the fiber content in terms of chitin and catecholamines, whereas ADL represents the exclusive contribution of catecholamines, it was possible to determine the chitin content of each sample, applying the following formula (Triunfo *et al.*, 2022):

$$\text{Chitin content } (\%) = \text{ ADF } (\%) - \text{ADL } (\%). \quad (6)$$

Substrate reduction

The reduction of the starting substrate (the quantity of bioconverted substrate) was calculated applying the following equation (Tschirner & Simon, 2015):

$$\text{Substrate reduction } (\%) = \left(\frac{\text{Initial substrate (g)} - \text{Frass (g)}}{\text{Initial substrate (g)}} \right) \times 100. \quad (7)$$

Waste reduction index and calculation of efficiency of conversion of digested feed

Different parameters were calculated for each experimental assay: the waste reduction index (WRI), which indicates the ability of the larvae to reduce the amount of given food; and the efficiency of conversion of digested feed (ECD), which indicates the efficiency of the larvae in converting the feed intake to larval biomass.

WRI was calculated as follows (modified from Leong *et al.*, 2016):

$$\text{WRI } = \frac{\left(\frac{\text{Initial substrate} - \text{frass}}{\text{Initial substrate}} \right)}{\text{days of experimental trial}} \times 100. \quad (8)$$

ECD was calculated as follows (Leong *et al.*, 2016):

$$\text{ECD } = \frac{\text{Final larval biomass}}{\text{Initial substrate} - \text{frass}}. \quad (9)$$

Statistical analysis

All experiments were performed in triplicates and results were expressed as mean ± standard error and standard error of the mean. Data were analyzed with one-way ANOVA and Bonferroni *post hoc* tests. All statistical analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, California; www.graphpad.com).

Results

Water content of the substrates

At the beginning of the bioconversion experiment, the water content of each substrate was verified. Water content of the standard diet (82.86% ± 0.84%) differed from that of the substrates from the fruit chain (strawberry: 88.63% ± 0.78%, tangerine: 85.84% ± 1.23%, and orange: 87.13% ± 0.49%).

Growth parameters of larvae

The control larvae completed the larval stage in 13 days after eggs hatching, with a growth rate and an average growth rate higher than larvae reared on the other substrates (maximum weight: 155.20 ± 1.91 mg; final weight: 134.72 ± 10.63 mg) (Fig. 1; Table 2). Larvae fed on different substrates from the fruit chain regularly increased their weight over the course of the experiment, reaching the mature larval stage with delay in respect to the control larvae: 18 days for larvae fed on strawberry (maximum weight: 196.69 ± 2.76 mg; final weight: 188.32 ± 2.32 mg) and on tangerine (maximum weight: 157.44 ± 2.86 mg; final weight 142.53 ± 4.12 mg), and 19 days for larvae fed on orange (maximum weight: 151.84 ± 6.08 mg; final weight 142.95 ±

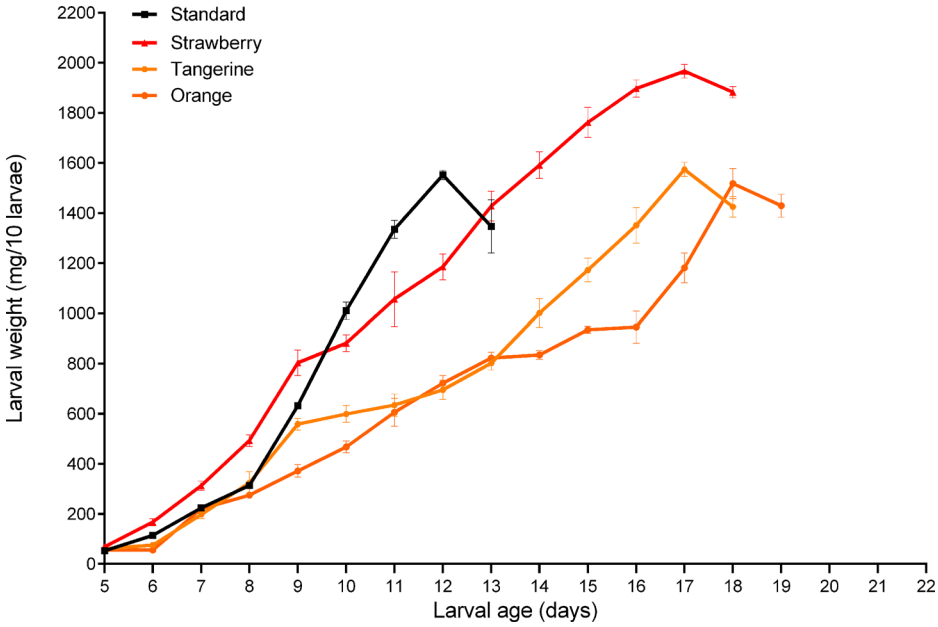

Fig. 1 Growth curves of BSFL fed on standard diet, strawberry, tangerine and orange. Each day, six groups of 10 larvae from each box were randomly selected, weighed on an analytical balance and returned to their respective boxes. Data are presented as mean ± SE of 3 independent biological replicates.

Table 2 Larval dry matter (%), growth rate (mg/day), average growth rate (kg/day), and larval total biomass (kg) of BSFL fed on standard diet, strawberry, tangerine, and orange.

	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
Larval dry matter (%)	33.69^{a}	28.31^{ab}	27.95^{ab}	27.35^{b}	2.01	0.0189
Growth rate (mg/day)	143.78^{a}	129.57^{ab}	97.43^{bc}	91.56^{c}	0.016	0.0019
Average growth rate (kg/day)	0.104^{a}	0.111^{a}	0.077^{b}	0.111^{a}	0.013	< 0.0001
Larval total biomass (kg)	1.29^{b}	1.53^{a}	1.07^{bc}	0.95^{c}	0.15	0.0001

Note: Dry mass of BSFL was measured at the end of the experiment applying the equation (1) (see Materials and methods). Growth rate was calculated applying the equation (3) (see Materials and methods), considering the initial and final larval weight and the days of bioassay and showed the daily increase of larval weight. Average growth rate was calculated applying the equation (4) (see Materials and methods), considering the final larval total biomass and the days of bioassay. Total biomass of BSFL was measured at the end of the experiment, manually dividing larvae from frass. Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups. SEM = standard error of the mean.

4.62 mg) (Fig. 1). Larvae fed on the standard diet and on strawberry showed the highest growth rate and the highest average growth rate (Table 2), that returns a better evaluation of the best diet, the one on which BSFL can convert the highest biomass in the shortest time. Statistical differences were recorded between the standard and strawberry substrates and the other substrates. Larval total biomass differed among the different substrates (Table 2). Larvae fed on orange had the lowest larval total weight, and larvae fed on strawberry had the highest larval total weight, which differed significantly from the other samples (Table 2). Larval dry mass calculated at the end of the experiment was between 27.35% and 33.69% (Table 2).

Nutritional composition of larvae

Larvae fed on the standard diet contained the highest ash content (12.89% ± 0.26%), while the lowest ash content occurred in larvae fed on tangerine (8.92% ± 0.16%)

Table 3 Mineral content (mg/100g and mg/kg) of BSFL fed on standard diet, strawberry, tangerine, and orange.

Mineral (mg/100 g)	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
Calcium	3244.33[a]	1321.68[b]	2998.67[c]	1752.33[d]	535.49	<0.0001
Potassium	374.67[b]	388.00[b]	440.00[a]	332.33[b]	27.38	0.0011
Phosphorus	795.00[a]	790.3[a]	778.67[a]	770.67[a]	15.06	0.632
Magnesium	113.5[a]	96.37[b]	96.73[b]	97.35[b]	5.39	0.0052
Sodium	116.00[a]	97.12[b]	89.76[b]	113.77[a]	7.78	0.0013
Iron	3.09[a]	1.69[b]	3.30[a]	2.23[c]	0.43	<0.0001
Zinc	23.37[a]	20.66[b]	23.33[a]	21.25[ab]	0.98	0.012
Copper (mg/kg)	3.10[a]	2.85[a]	2.99[a]	2.75[a]	0.14	0.2779
Manganese (mg/kg)	47.20[a]	44.58[a]	48.70[a]	47.56[a]	1.45	0.1451

Note: Mineral content was determined by Inductively Coupled Plasma Optical Emission Spectroscopy. Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups. SEM = standard error of the mean.

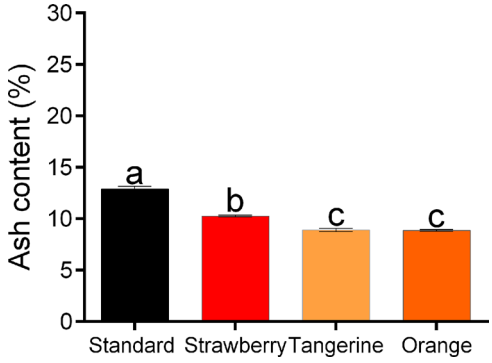

Fig. 2 Ash content (%) of BSFL fed on standard diet, strawberry, tangerine and orange. Ash content of BSFL was measured at the end of the experiment, after incineration at 550°C for 3 h in a Muffle Furnace. Data are presented as mean ± SE of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups (P value < 0.0001).

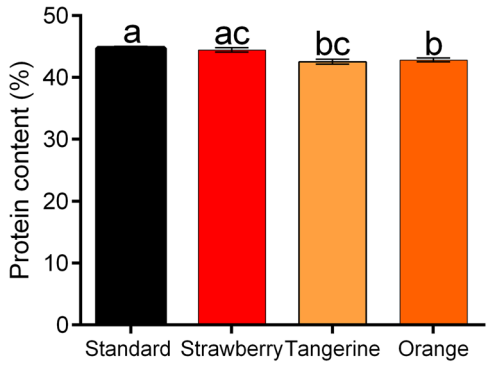

Fig. 3 Protein content (%) of BSFL fed on standard diet, strawberry, tangerine and orange. Protein content of BSFL was measured at the end of the experiment, using the Kjeldahl method. Data are presented as mean ± SE of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups (P value = 0.0029).

and orange (8.87% ± 0.09%) (Fig. 2). The most abundant macroelement among the analyzed substances was calcium, while the most deficient were sodium and magnesium. Differences in macroelements were detected in almost all fruit samples compared to the standard diet, except for phosphorus, copper, and manganese (Table 3). No traces of selenium, cadmium, lead, and chromium were detected.

Protein content was approximately 42%–44%, with statistically significant differences among samples (Fig. 3). In all the analyzed samples, the less abundant amino acids were tryptophan, methionine, glutamine, and asparagine, while the most abundant were tyrosine, proline, histidine, threonine, and the couple leucine/isoleucine (Table 4). Although differences in percentages of amino acids were detected among the analyzed samples, essential amino acids were more abundant than nonessential ones (Table 4).

Lipid content was approximately 15%–19%, without statistically significant differences between samples (Fig. 4). In all the analyzed samples, the most abundant fatty acids were lauric, palmitic, oleic, and linoleic acids, followed by myristic and heptadecanoic acids. No statistical differences among samples were detected in caprylic, capric, myristic, palmitic, heptadecanoic, stearic, petroselinic, linoleic, and behenic acids. Overall, all samples showed higher total saturated fatty acids composition compared to the unsaturated ones (Table 5).

Table 4 Amino acid composition (%) of BSFL fed on standard diet, strawberry, tangerine, and orange.

Amino acid (%)	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
Tryptophan	0.60^{b}	0.38^{c}	0.33^{c}	1.52^{a}	0.32	<0.0001
Phenylalanine	6.69^{c}	9.69^{a}	8.76^{b}	6.51^{c}	0.90	<0.0001
Leucine + isoleucine	12.44^{c}	13.95^{b}	18.19^{a}	13.72^{bc}	1.45	<0.0001
Methionine	0.32^{b}	0.21^{c}	1.16^{a}	0.84^{d}	0.26	<0.0001
Proline	7.41^{b}	8.84^{ab}	9.90^{a}	8.63^{ab}	0.65	0.0026
Valine	4.65^{b}	5.95^{a}	6.50^{a}	5.86^{a}	0.46	<0.0001
Tyrosine	14.01^{a}	12.83^{ab}	12.86^{ab}	11.31^{b}	0.77	0.0129
Alanine	7.48^{b}	7.30^{b}	9.49^{a}	7.65^{b}	0.62	0.0008
Threonine	6.81^{ab}	6.72^{b}	7.49^{a}	6.16^{b}	0.34	0.0015
Glycine	3.38^{b}	3.49^{b}	3.60^{b}	4.02^{a}	0.17	0.0018
Glutamine	0.002^{a}	0.002^{a}	0.003^{b}	0.007^{b}	0.001	<0.0001
Asparagine	0.002^{b}	0.0008^{c}	0.0005^{d}	0.003^{a}	0.0006	<0.0001
Serine	2.72^{ab}	2.89^{a}	2.67^{b}	2.85^{ab}	0.07	0.0243
Arginine	6.37^{b}	5.01^{c}	2.85^{d}	6.99^{a}	1.05	<0.0001
Histidine	11.71^{a}	8.86^{b}	5.10^{c}	10.55^{a}	1.67	<0.0001
Lysine	7.63^{a}	6.80^{b}	3.66^{c}	6.51^{a}	0.99	<0.0001
Glutamic acid	4.46^{b}	4.26^{b}	4.61^{b}	5.13^{a}	0.22	0.0003
Aspartic acid	3.32^{a}	2.82^{b}	2.82^{b}	1.76^{c}	0.36	<0.0001
Essential AA	50.86^{a}	52.55^{a}	51.19^{a}	51.67^{a}	0.0383	<0.0001
Not essential AA	49.14^{a}	47.45^{a}	48.81^{a}	48.33^{a}	1.78	<0.0001

Note: Specific amino acid composition was detected by UHPLC-HRMS Orbitrap analysis. Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups. SEM = standard error of the mean.

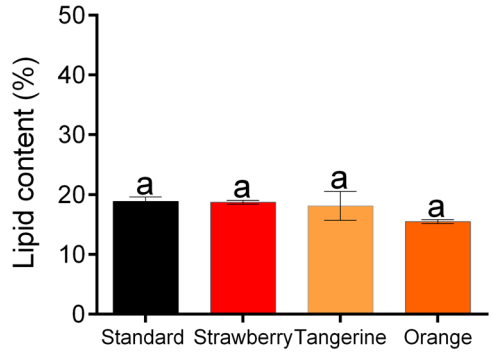

Fig. 4 Lipid content (%) of BSFL fed on standard diet, strawberry, tangerine and orange. Lipid content of BSFL was measured at the end of the experiment, using the Folch extraction method. Data are presented as mean ± SE of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups (*P* value = 0.228).

Differences in the fiber content of larvae were detected between those fed on strawberry and the other samples (Table 6). In contrast, no significant differences in chitin content were detected among all the analyzed samples (Table 6).

Substrate reduction and bioconversion parameters

At the end of the bioassay, larvae and frass were manually separated and weighed. The weight of the frass differed slightly among treatments, with the lowest dry matter for frass derived from larvae fed on strawberry (Table 7). In agreement with frass weight, the percentage of substrate reduction slightly differed among substrates (Table 7). The WRI and the ECD, parameters strictly related to bioconversion, were calculated for each experimental condition. The WRI and ECD values differed slightly among larvae reared on different substrates, except for larvae reared on the standard diet, which had the highest value of WRI (8.23 ± 0.13 on wet basis; 5.25 ± 0.50 on dry basis) (Table 7), and larvae reared on the strawberry substrate diet had the highest value of ECD (0.30 ± 0.001 on wet basis; 1.14 ± 0.24 on dry basis) (Table 7).

Discussion

This study is one of the few in which fruit byproducts have been used separately as substrates for BSFL. We investigated how different diets affected larval

Table 5 Fatty acid composition (%) of BSFL fed on standard diet, strawberry, tangerine, and orange.

Fatty acid (%)	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
C4:0	3.94[a]	2.33[a]	10.69[b]	3.51[a]	2.25	0.0001
C6:0	2.50[a]	0.18[b]	0.45[b]	0	0.68	0.0002
C8:0	0.72[a]	0.19[b]	0.34[b]	0.15[b]	0.15	0.6541
C10:0	0.72[a]	0.68[a]	0.54[a]	0.67[a]	0.11	0.6541
C12:0	32.34[b]	35.88[c]	31.55[b]	42.28[a]	2.81	<0.0001
C14:0	4.67[a]	4.89[a]	7.11[a]	5.44[a]	0.72	0.1269
C14:1	0.45[a]	0.34[ab]	0.19[b]	0.19[b]	0.08	0.0021
C15:0	0.14[a]	0.34[ab]	0.46[b]	0.31[ab]	0.09	0.0368
C16:0	8.07[a]	7.54[a]	9.15[a]	9.43[a]	0.67	0.0681
C16:1	0.45[a]	0.34[a]	0.15[b]	0	0.11	0.0006
C17:0	4.71[a]	6.08[a]	4.97[a]	4.88[a]	0.51	0.1364
C17:1	0	0	0.29[a]	0.34[a]	0.11	0.4673
C18:0	2.36[a]	2.32[a]	2.14[a]	2.11[a]	0.32	0.9322
C18:1 CIS6	0.25[a]	0.42[a]	0.26[a]	0.23[a]	0.07	0.0857
C18:1 trans 11 (TVA)	0.46[ac]	0.65[a]	0.31[bc]	0.19[b]	0.12	0.0002
C18:1 CIS9	20.61[a]	19.30[a]	16.36[b]	15.96[b]	1.39	0.0008
C18:1 CIS11	0	0	0.21[a]	0.14[a]	0.06	0.0712
C18:2 CIS N6 (LA)	11.23[a]	9.07[a]	10.76[a]	9.86[a]	0.82	0.14740
C18:2 c9-t11 (CLA)	0	2.31[a]	1 .76[a]	1.42[a]	0.60	0.0925
C18:3 N3 (ALA)	3.10[a]	2.33[ab]	0.91[b]	1.24[b]	0.64	0.0028
C20:1	0.27[ab]	0.30[a]	0.13[b]	0.30[a]	0.06	0.193
C20:2 N6	0	0.84	0	0	-	-
C20:4 N6 (AA)	0.36[ab]	0.46[a]	0.30[b]	0.26[b]	0.09	0.0052
C22:0	0.62[a]	0.55[a]	0.42[a]	0.75[a]	0.13	0.2943
C22:1	1.69[a]	0.37[b]	0.50[b]	0.23[b]	0.41	0.006
C22:2 N6	0	0.24	0	0	-	-
C22:4 N6	0	0.30	0	0	-	-
C24:0	0.85[a]	0.38[b]	0.56[ab]	0	0.21	0.0158
Saturated	61.64[b]	61.36[b]	68.38[a]	69.54[a]	2.62	0.0003
Monounsaturated	23.87[a]	21.71[a]	18.39[b]	17.59[b]	1.77	0.0004
Polyunsaturated	14.58[a]	16.71[a]	13.73[a]	12.78[a]	1.23	0.0500
Omega 6	11.47[a]	10.92[a]	11.06[a]	10.13[a]	0.66	0.5116
Omega 3	3.10[a]	2.33[ab]	0.9[b]	1.24[b]	0.64	0.0029

Note: Fatty acid composition was evaluated by gas chromatography. Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test for all the data, except for C17:1 and C18:1 CIS11 in which a *t*-test was applied. Different letters indicate significant differences among groups. SEM = standard error of the mean.

performances and their nutritional composition. BSF is one of the most important bioconverter insect and numerous studies have been carried out to better understand not only the bioconversion process but also the influence of the feed substrate. Here, three different substrates with low nutritional value in terms of protein and lipids were tested for BSFL. Larvae reared on each of the tested diets developed successfully, although many parameters connected to larval growth, larval nutritional composition, and to the bioconversion processes are influenced by diet. The development of the larval stage occurred faster in larvae fed on the standard diet (13 days after egg hatching) and slower in larvae reared on fruit byproducts (18–19 days after egg hatching). BSFL fed on the standard diet, which has a balanced composition and a complete nutritional profile, showed the best growth performance, with the best weight gain and the shortest development time. Larvae fed solely on fruit byproducts are

Table 6 Fiber and chitin content (%) of BSFL fed on standard diet, strawberry, tangerine, and orange.

	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
NDF (%)	22.70[b]	28.84[a]	21.23[b]	22.95[b]	2.04	0.0004
ADF (%)	20.01[ab]	23.33[a]	18.02[b]	20.77[ab]	1.51	0.0206
ADL (%)	8.71[ab]	10.30[a]	4.89[b]	8.09[ab]	1.62	0.0348
Chitin (%)	11.03[a]	13.03[a]	13.13[a]	12.68[a]	0.84	0.3420

Note: Fiber content was evaluated according to Van Soest *et al.* (1991) and Van Soest and Robertson (1981), chitin content was calculated applying the equation (6) (see Materials and methods). Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups. SEM = standard error of the mean. NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin.

Table 7 Frass dry matter (%), Frass total weight (kg) derived from BSFL fed on standard diet, strawberry, tangerine, orange; substrate (standard diet, strawberry, tangerine, orange) reduction on wet basis after BSFL feeding; WRI and ECD on wet and dry basis after BSFL feeding on standard diet, strawberry, tangerine and orange.

	Standard	Strawberry	Tangerine	Orange	SEM	*P* value
Frass dry matter (%)	38.20[a]	21.96[b]	31.00[c]	32.90[ab]	3.99	< 0.0001
Frass total weight (kg)	1.81[a]	1.77[a]	1.88[a]	1.88[a]	0.08	0.6479
Substrate reduction (%)	74.10	74.76[a]	73.29[a]	73.10[a]	1.07	0.6733
WRI (wet basis)	8.23[a]	5.34[b]	5.24[bc]	4.88[c]	0.89	< 0.0001
WRI (dry basis)	5.25[a]	3.91[ab]	3.09[ab]	2.17[b]	0.87	0.0122
ECD (wet basis)	0.25[b]	0.30[a]	0.21[bc]	0.19[c]	0.03	0.0001
ECD (dry basis)	0.89[a]	1.14[a]	0.83[a]	1.11[a]	0.24	0.7438

Note: Dry matter (%) of frass was measured at the end of the experiment, applying the equation (1). Total biomass of frass was measured at the end of the experiment, manually dividing frass from larvae. Substrate reduction was calculated applying the equation (7) (see Materials and methods). WRI was calculated applying the equation (8) (see Materials and methods). ECD was calculated applying the equation (9) (see Materials and methods). Data are presented as mean of 3 independent biological replicates. Statistical analysis was performed with one-way ANOVA (analysis of variance) and Bonferroni *post hoc* test. Different letters indicate significant differences among groups. SEM = standard error of the mean.

likely impacted by the low nutrient amount (Jucker *et al.*, 2017; Meneguz *et al.*, 2018). There are many reported examples of a prolonged feeding phase in larvae fed on fruit waste or other substrate with low protein content in comparison to larvae fed on substrates rich in proteins (Nguyen *et al.*, 2013; Oonincx *et al.*, 2015; Cammack & Tomberlin, 2017). However, the possibility to rear BSFL on a "simplified" diet, based exclusively on fruits or vegetables, including byproducts from the agrifood chain, despite the prolonged feeding time, is a good opportunity to valorize them, obtaining high-value products (e.g., larvae rich in proteins and lipids). To make this process economically sustainable, the rearing of BSFL should be located near agrifood companies that produce large quantities of waste, thereby reducing the environmental impact of transporting the byproducts to BSF farms (Salomone *et al.*, 2017; Bava *et al.*, 2019).

Many laboratory-scale studies have reported that BSFL can feed on byproducts from the agrifood chain; to the best of our knowledge, our study is one of the first carried out on a semi-industrial scale to evaluate the possibility of disposing of large amounts of fruit byproducts by using them as substrate for BSFL. Under mass-rearing conditions, BSFL generally show better growth performance: a shorter development time, greater final larval weight, and higher larval growth rate (Nguyen *et al.*, 2013; Jucker *et al.*, 2017; Cappellozza *et al.*, 2019). For example, groups of 200 larvae fed on a mix of fruit and vegetables (zucchini, apple, potato, green beans, carrot, pepper, orange, celery, kiwi, plum, eggplant) and on a mix of apple, pear, and orange took almost 50 days to reach the final instar (Jucker *et al.*, 2017; Cappellozza *et al.*, 2019), a lengthy developmental time as compared with that of larvae in our experiments. Moreover,

small groups of larvae that were fed on fruit or vegetable waste (25 and 500 specimens, respectively) showed lower weight gain (6.5 mg/day and 7 mg/day, respectively) when compared with our samples, in which the lowest growth rate was 91.56 mg/day recorded for larvae fed on orange (Meneguz *et al.*, 2018; Magee *et al.*, 2021). In addition, the final average weight of larvae fed on tangerine and orange in our study (142 mg) was similar to the final larval weight of 240 larvae fed on 2 kg of a mix of watermelon, orange, and pineapple (Ewusie *et al.*, 2018), even though the administered feed for each larva is major in the benchtop experiment that in ours (8 g/larva vs. 0.7 g/larva, respectively). This result also confirms better performances of BSFL reared on a semi-industrial scale. Comparing the results for larvae fed under the same conditions (i.e., same number of larvae and equal amount of feed) in our experiment and in Scala *et al.* (2020), in which larvae were reared on apple, banana, and a mix of them, we noticed a 3- or 4-day delay in our developmental time but a better average growth rate, a greater total larval biomass, and better substrate reduction, confirming that the nature of the substrate strongly influences BSFL performances.

Among the analyzed fruit diets, the strawberry-based diet was the best in terms of larval weight gain and time of growth. The better performances of BSFL reared on strawberry could be related to specific compounds, including volatile organic compounds (VOCs) emitted by this substrate, which could make it more attractive or more phagostimulant for the growing larvae, in contrast to the VOCs emitted by the other substrates, as we previously demonstrated with other byproducts that are able to induce excellent growth performances (Scieuzo *et al.*, 2021).

The highest growth rate and the highest average growth rate were achieved by rearing BSFL on the standard diet or on strawberry substrate, with a balance between the development time and the achieved weight. Indeed, control larvae developed in faster time but with a lower total biomass in comparison with larvae fed on strawberry, which reached a heavier weight but in longer time. Moreover, if we normalize the obtained results, namely considering the larval dry matter biomass starting from an equal quantity of dry matter substrate, we obtain a clearly superior result of bioconversion yield for larvae fed on strawberry in terms of larval biomass per kg of bioconverted substrate: 0.55 $\pm$ 0.06 kg for larvae fed on strawberry, versus 0.36 $\pm$ 0.02 kg, 0.31 $\pm$ 0.04 kg, and 0.30 $\pm$ 0.02 kg for larvae fed the standard diet, tangerine, and orange, respectively.

The high percentage of substrate reduction (73%–75%) differed only slightly among substrates and demonstrated a great potential by BSFL in the consumption and decomposition of fruit waste (Nguyen *et al.*, 2013; Nguyen *et al.*, 2015). Several studies have confirmed the efficiency of substrate reduction: 60%–70% for fruit substrate mix (e.g., a mix of apple, strawberry, mandarins, pear, kiwi, banana and lemon: Meneguz *et al.*, 2018); 60%–65% for fruit and vegetable mix (celery, orange, pepper or pear, banana, tomato, leafy green vegetables: Giannetto *et al.*, 2019); 42%–61% for single fruit substrate (banana, avocado or guava: Mentari *et al.*, 2020). Thus, although substrates from the agrifood chain can be demonstrably reduced, it is important to evaluate the potential of BSFL on each single substrate because the larvae cannot efficiently reduce all of them. For instance, substrates with excessive moisture content or high fiber content can negatively influence BSF performances (Manurung *et al.*, 2016; Supriyatna *et al.*, 2016; Mentari *et al.*, 2020). Moreover, the feeding ratio and a continuous/batch feeding strategy can also influence reduction of the substrate (Dzepe *et al.*, 2021).

Values of WRI and ECD can differ according to substrate. Except for the standard diet, the WRI values did not differ among larvae reared on the fruit substrates, indeed both the percentage of substrate reduction and the larval development time were similar between the different experimental conditions. In contrast, significant differences were detected between ECD values, with the highest value observed for larvae fed on strawberry. High values of WRI and ECD indicate that larvae are efficient at reducing waste while accumulating nutrients. The literature reports WRI values between 2.8 and 8 for fruit substrates, and ECD values between 0.03 and 0.18 for agrifood substrates (Cappellozza *et al.*, 2019; Giannetto *et al.*, 2019; Mentari *et al.*, 2020; Wahyuni & Fadhlil, 2022). Meneguz *et al.* (2018) and Leong *et al.* (2016) calculated these indices on wet basis; comparing their results with ours, also in this case, our study obtained better performances. Low WRI and ECD values were recorded with substrates with low water content (Yusup *et al.*, 2020), confirming that the substrate moisture content plays an important role in the potential of BSFL to feed on a given substrate and their food absorption (Putra *et al.*, 2020). The moisture content of our substrates, ranging from 82% in the standard diet to 89% in the strawberry substrate, probably assured the right degree of humidity for BSFL development. Our results, with the WRI values in ranges reported in the literature and high ECD values compared to literature, showed the great bioconversion potential of BSFL fed on these specific fruit substrates.

Our study also confirms that the final nutritional composition of larvae depends on the growing substrate

composition. The ash content of larvae (ranging from 8.87% to 10.27%) was higher than the ash content of larvae reared on other fruits or on a mix of vegetable/fruit waste (ranging from 5% to 8%) (Meneguz *et al.*, 2018; Cappellozza *et al.*, 2019; Giannetto *et al.*, 2019). Our values are also higher than that found in prepupae fed on fruit waste: from 1.68% to 5.77% in the case of larvae and prepupae fed on exotic fruits tested singularly (i.e., pineapple, kiwi, apple, melon, peach: Barbi *et al.*, 2020). Also, the mineral content was strictly linked to the substrate: the percentages of calcium, potassium, sodium, iron, and zinc differed among the larvae fed on different fruit byproducts analyzed in this work. Comparing our findings with those for larvae fed on other fruit waste and substrates of a different origin, although many differences were recorded (summarized in Table 8), the larvae of our study had major concentrations of phosphorus. Previous studies reported that the most abundant mineral in BSFL is calcium, with higher values than in other insects or in fishmeal (Wang & Shelomi, 2017). Because of their physiological functions (e.g., neuro-signaling activity, involvement in metabolic reaction, bone, and muscle development), calcium and phosphorus are particularly important in animal nutrition (Sun *et al.*, 2020).

Protein content differed slightly among the substrates. This is an important data, as the comparison between the standard diet and the fruit substrates highlights that, although the original protein content is low in these substrates, larvae can convert them into high-value proteins. Similar values were found in larvae and prepupae fed on fruit and vegetable waste (Spranghers *et al.*, 2017; Cappellozza *et al.*, 2019; Chun *et al.*, 2019; Giannetto *et al.*, 2019), though our results showed a greater protein content than those reported by Jucker *et al.* (2017) (10% protein content in prepupae fed on a mix of apple, pear and orange), Nguyen *et al.* (2015) (13% in prepupae fed on fruit and vegetable waste), Meneguz *et al.* (2018) (20%–30% in larvae fed on a mix of apple, strawberry, mandarin, pear, kiwi, banana and lemon), Scala *et al.* (2020) (31%–36% in larvae fed on apple, banana, and a mix of them), and Magee *et al.* (2021) (36% in larvae fed on mixed fruit and vegetable waste).

The comparison of the specific amino acid content of larvae fed on fruit waste with that of larvae fed on other substrates, including other fruit waste, presents a complex picture indicating that the substrates significantly affect amino acid content, thus the choice of byproducts on which to feed BSFL is critical for obtaining larvae with a specific amino acid composition (Lalander *et al.*, 2019) (Table 9). It is also possible to notice that the larvae fed on strawberry, tangerine, and orange had minor concen-

Table 8 Comparison of mineral content of larvae fed on the analyzed substrates from the fruit chain with mineral content of larvae fed on other substrates.

Mineral	Chicken feed (Liu *et al.*, 2017)	Chicken manure (Shumo *et al.*, 2019)	Kitchen waste (Shumo *et al.*, 2019)	Spent grain (Shumo *et al.*, 2019)	Supermarket waste (Smets *et al.*, 2020)	Fruit and vegetable waste (Cappellozza *et al.*, 2019)
Calcium	+/=	–	–	–	+	–
Potassium	NA	+	+	+/=	+	NA
Phosphorus	–	–	–	–	–	NA
Magnesium	NA	+	+	+	+	–
Sodium	–/=	+	+	+	+	NA
Iron	+	+	+	+	+	–/=
Zinc	+	–	–	–	–	–
Copper	NA	+	+	+	–	–
Manganese	NA	+	+	+	+	+

Note: – indicates lower values compared with our results; + indicates higher values compared with our results; = indicates similar values compared with our results. NA = not available.

Table 9 Comparison of amino acid content of larvae fed on the analyzed substrates from the fruit chain with amino acid content of larvae fed on other substrates.

Amino acid	Chicken feed (Liu *et al.*, 2017)	Chicken manure (Shumo *et al.*, 2019)	Kitchen waste (Shumo *et al.*, 2019)	Spent grain (Shumo *et al.*, 2019)	Supermarket waste (Smets *et al.*, 2020)	Fruit and vegetable waste (Cappellozza *et al.*, 2019)	Fruit and vegetable waste (Ravi *et al.*, 2020)	Fruit and vegetable waste (Lalander *et al.*, 2019)	Fruit and vegetable waste (Magee *et al.*, 2021)
Tryptophan	NA	NA	NA	NA	+	–/+	+	+	NA
Phenylalanine	–	–	+	+	–	–	–	–	–
Leucine + Isoleucine	–	+	+	+	–	–	–	+ /=	+ /=
Methionine	+	+	+	+	+	+	+	+	+
Proline	–	– /=	+	+	–	–	– /=	–	–
Valine	–	+	+	+	+	–/=	=	+	+
Tyrosine	–	–/+	+	+	–	–	–	–	–
Alanine	–	NA	NA	NA	–/+	+ /=	– /=	–	–/+
Threonine	–	NA	NA	NA	+	–	–	–	–
Glycine	+	NA	NA	NA	+	+	+	+	–
Glutamine	NA	–	+	+	NA	NA	NA	NA	NA
Asparagine	+	NA	NA	NA	NA	NA	NA	NA	NA
Serine	+	NA	NA	NA	+	+	+	+	+
Arginine	–/+	+ /=	+	+	–/+	+	–/+	–/+	–/+
Histidine	– /=	+	+	+	–	–	–	–	–
Lysine	–/+	+	+	+	+	–/+	–/=	+ /=	– /=
Glutamic acid	+	–	+	+	+	+	+	+	+
Aspartic acid	NA	NA	NA	NA	+	+	+	+	+

Note: – indicates lower values compared with our results; + indicates higher values compared with our results; = indicates similar values compared with our results. NA = not available.

trations of methionine, serine, and aspartic and glutamic acid compared with the other substrate groups. In general, differences in amino acid composition are recorded for larvae reared on different substrates, especially in the concentrations of lysine, leucine, valine, alanine, and glutamic and aspartic acid. In contrast, in all research, including the present study, the less abundant amino acids are cysteine, methionine, and tryptophan (Liu *et al.*, 2017; Caligiani *et al.*, 2018; Ravi *et al.*, 2020; Smets *et al.*, 2020; Wang *et al.*, 2020). The most required amino acids for normal growth in fish are tryptophan, threonine, lysine, valine, phenylalanine, isoleucine, arginine, leucine, histidine, methionine, and cysteine. In BSFL the total percentage of these amino acids is generally approximately 50% of the total amino acid amount, making BSFL an appropriate substitute for fishmeal in aquaculture (Müller *et al.*, 2017). As concerns pigs, they cannot synthesize histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine that are essential for their development, so they must be added to the diet (Rezaei *et al.*, 2013). For this reason, BSFL can also be an ideal candidate in pig rearing. A different approach should be considered for poultry, in which methionine and lysine are often two of the most limiting amino acids for their growth (Farkhoy *et al.*, 2012). To obtain the ideal amino acid pattern, the usage of BFSL in poultry feed should be supported by other sources of methionine.

As concerns lipid content, our results are similar to those obtained by Jucker *et al.* (2017) for prepupae fed on fruit waste. The literature data present a wide range of lipid values for larvae fed on fruit and vegetable waste, confirming the strong influence of the substrate: from 47.9% to 27% for larvae reared on fruit waste (Meneguz *et al.*, 2018; Cappellozza *et al.*, 2019; Chun *et al.*, 2019; Giannetto *et al.*, 2019; Scala *et al.*, 2020; Magee *et al.*, 2021), to very low values (from 9.6% to 5.01%) for prepupae reared on single-source fruit waste (Barbi *et al.*, 2020; Borel *et al.*, 2021), to almost totally absence in prepupae reared on vegetable waste (i.e., lettuce, string green beans, and cabbage: Nguyen *et al.*, 2015; Jucker *et al.*, 2017). In our work, although lipid content was not significantly different among the fruit substrates, the specific fatty acid composition varied according to the starting substrate. Table 10 lists the fatty acid values of our samples compared with those of larvae fed on other fruit waste, as well as other kinds of substrates. In all reported cases, saturated fatty acids are predominant, and the most abundant fatty acids are lauric, palmitic, and oleic, followed by myristic, linoleic and stearic (Franco *et al.*, 2021). Omega 3 and omega 6 is normally low in BSFL, and the omega 6/3 ratio (relevant for human and animal health) is not always optimal (Kouba & Mourot, 2011); a good ratio should be less than 5 (Kouba & Mourot, 2011), as in the larvae fed on the standard diet and the strawberry substrate.

The fiber content detected in all our samples was lower than that in larvae fed on a fruit and vegetable mix Giannetto *et al.* (2019) (33%); while the fiber content in larvae fed on tangerine and orange was similar to that on larvae fed on fruit waste in Meneguz *et al.* (2018) (19.7%). Although the substrate appeared not to affect the chitin content of larvae fed with our substrates, a higher chitin content was recorded by Giannetto *et al.* (2019) (16%) and lower chitin content was recorded by Cappellozza *et al.* (2019) (4.02%) and Meneguz *et al.* (2018) (from 2.7% to 7.5%), whose experiments also registered differences in chitin among larvae fed on different substrates. Collecting BSFL at the end of the feeding phase, rather than in the pupal stage, allows not only to obtain a heavier biomass, but also a better digestibility of the larvae: indeed, the higher chitin content in the prepupae, could negatively affect the nutritional digestibility of feed for several fish and poultry species (Henry *et al.*, 2015; Marono *et al.*, 2017). However, the inclusion of chitin in animal feed may have a positive effect on the immune system, and, together with its antimicrobial properties, may also reduce the use of antibiotics (Bovera *et al.*, 2016; Guarnieri *et al.*, 2022).

Overall, these results about the nutritional content of BSFL demonstrate and highlight that not only the original substrate strongly influences the final larval biomass composition, but also that different life stages have different nutritional properties. Consequently, the possibility of feeding larvae on different organic substrates makes it possible to obtain a final product (larval or prepupal biomass) highly diversified for different applications related to the needs of the market. As the usage of insects as an alternative, environmentally friendly sustainable source of nourishment for a wide range of animals and for humans is increasing, further studies on different fruit and/or vegetable byproducts are needed to increase our knowledge of which substrate provides larvae with the best characteristics for market needs and to understand which substrate types can be effectively disposed of with this innovative, ecological process. Aside from the bioconversion capacity, the suitability of all potential single substrates should be evaluated in relation to BSFL performances, for the sake of industrial use with low risk. Waste management via BSFL bioconversion represents an innovative and economically feasible resource for regional and national agrifood companies; this approach offers new possibilities for sustainable and environmentally friendly industrial development in

Table 10 Comparison of the most concentrated fatty acid of larvae fed on the analyzed substrates from the fruit chain with fatty acid of larvae fed on other substrates.

Fatty acid	Fruit and vegetable waste (Magee *et al.*, 2021)	Wheat bran (Gao *et al.*, 2019)	Brewery byproducts (Meneguz *et al.*, 2018)	Fruit waste (Meneguz *et al.*, 2018)	Fruit and vegetable waste (Giannetto *et al.*, 2019)	Chicken feed (Liu *et al.*, 2017)	Supermarket waste (Smets *et al.*, 2020)
C4:0	NA	NA	NA	NA	–	NA	NA
C6:0	NA	NA	NA	NA	–	–	NA
C8:0	–	NA	NA	NA	–	NA	NA
C10:0	– /=	+	NA	NA	–	+	NA
C12:0	–/+	–/+	–	+	–	+	+
C14:0	+	–/+	–/+	+	–	+	+
C14:1	+	NA	NA	NA	+	– /=	NA
C15:0	–	NA	NA	NA	+	NA	NA
C16:0	+	+	+	+	–	– /=	+
C16:1	+	–	+	+	+	+	+
C17:0	NA	–	NA	NA	+	NA	NA
C17:1	NA	NA	NA	NA	+	NA	NA
C18:0	–	–	–	–	–	–	–
C18:1 CIS9	+	– /=	–	–	–	–	–
C18:1 CIS11	NA	NA	+	+ /=	NA	NA	NA
C18:2 CIS N6 (LA)	–	+	+	–	–	–	–
C18:3 N3 (ALA)	– /=	–/+	– /=	–	+	–	–
C20:0	–	NA	NA	NA	–	NA	NA
C20:1	–	NA	NA	NA	+	NA	NA
C20:4 N6 (AA)	–	NA	NA	NA	NA	NA	NA
C22:0	–	NA	NA	NA	–	NA	NA
C22:2	–	NA	NA	NA	NA	NA	NA

Note: – indicates lower values compared with our results; + indicates higher values compared with our results; = indicates similar values compared with our results. NA = not available.

which agricultural byproducts can be disposed of and enhanced in an unconventional way.

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Disclosure

All authors declare no conflicts of interest. Eric Schmitt is employed by Protix B.V., which commercially produces black soldier fly.

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