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Growth performance, proximate composition and fatty acid profile of black soldier fly larvae reared on two grape pomace varieties

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ABSTRACT

The black soldier fly (*Hermetia illucens*) is attracting increasing interest for its ability to convert low-value substrates into highly nutritious feed. This study aimed at evaluating grape pomace from two varieties (Becuet – **B**; Moscato – **M**) as rearing substrates for black soldier fly larvae (**BSFL**), focusing on the related effects on larval growth performance, proximate composition, and fatty acid profile. A total of six replicates per treatment, and 1 000 BSFL per replica, were used. Larval development was assessed by larvae weight, which was recorded eight times during the trial: the day after the beginning of the trial, and then on days 5, 8, 13, 15, 20, 22, and 27 (day in which the 30% of BSFL reached the prepupal stage). Production and waste reduction efficiency parameters, namely the growth rate, substrate reduction and substrate reduction index, were calculated. The two grape pomace varieties were analysed for their proximate composition and fatty acid profile; the same analyses were conducted on BSFL (30 larvae per replica) that were collected at the end of the trial (day 27). The growth rate of BSFL showed a higher value when the larvae were reared on B substrate (4.4 and 3.2 mg/day for B and M, respectively; $P < 0.01$). The rearing substrate did not significantly affect the proximate composition of BSFL. The percentage of total lipids (**TL**) in M-fed BSFL was significantly higher than in B ones. Total saturated ($P < 0.001$) and monounsaturated fatty acids ($P < 0.05$) were significantly higher in M-fed BSFL, while an opposite trend was observed for total branched-chain ($P < 0.001$) and total polyunsaturated fatty acids ($P < 0.001$). Interestingly, some conjugated linoleic acid (**CLA**) isomers [i.e., C18:2 *c*9*t*11(+*t*7*c*9+*t*8*c*10) and *t*9*t*11] were detected in low amounts in both rearing substrates (total CLA equal to 0.085 and 0.16 g/100 g TL in B and M substrate, respectively). Some CLA isomers (i.e., C18:2 *c*9*t*11, *t*7*c*9, and *t*10*c*12) were also found in BSFL, reaching a total CLA concentration equal to 2.95 and 0.052 g/100 g of TL in B-fed and M-fed BSFL, respectively. This study demonstrates that winery by-products from different grape varieties can significantly affect the development and lipid composition of BSFL. The CLA biosynthesis potential of BSFL opens newsworthy perspectives for a new valorisation of winery by-products to produce full-fat black soldier fly meal and black soldier fly oil enriched in specific fatty acids of potential health-promoting interest.

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Implications

The black soldier fly is attracting interest for its ability to convert organic waste into high-value feeds. In this study, we assessed the effect of using grape pomace from two varieties as rearing substrate for the larvae. The grape pomace variety influenced the growth and the chemical composition of the larvae. We detected some conjugated linoleic acid isomers in the grape pomace, which then accumulated in the larvae. Winery by-products could be valorised within black soldier fly–rearing media to obtain larvae enriched in fatty acids of potential health-promoting interest.

Introduction

In a world of finite resources, several studies have been carried out in search of new food sources for humans and animals. According to the Food and Agriculture Organisation, in the creation of a circular economy, recycling of food waste is one of the key points to achieve sustainability and avoid wasting resources (FAO, 2021). Insects are recently being considered for this purpose; they are deemed as a safe and eco-sustainable food and feed resource

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not only for their nutritional value but also for their limited rearing requirements, such as small growth land and waste-based rearing substrates, as well as their rapid life cycle (Gasco et al., 2023). One of the insects studied for this purpose is *Hermetia illucens* (Diptera: Stratiomyidae), also known as black soldier fly. This insect is native of the warm temperate zone of America, and it is today spread almost worldwide (Kaya et al., 2021). The black soldier fly larvae (**BSFL**) have been reported to consume and degrade organic materials such as fruit and vegetable waste and manure (Siddiqui et al., 2024).

With a global surface area equal to 7.3×10^6 ha (OIV, 2023), grape is a fruit cultivated all over the world both for direct human consumption and to produce several food products such as juice, jelly, vinegar and, of course, wine. Globally, in year 2022, the grape production was set at about 8.0×10^7 tonnes (OIV, 2023). Just considering Italy, the 2021 grapes production reached 7.5×10^6 tonnes (ISTAT, 2024). The winemaking process generates a series of by-products, of which grape pomace (**GP**) is the most abundant one (Ianni and Martino, 2020; Baroi et al., 2022). The GP represents about 25% of the weight of processed grapes, thus amounting to about 1.8×10^6 tonnes produced in Italy only. The GP is obtained after mechanical press or fermentation, and includes the peels (skin), seeds and some parts of the stems (Sirohi et al., 2020). Due to the different relative percentages of its constituent parts, which in turn depend on the variety and its degree of ripeness, GP has a complex and variable chemical composition (Antonić et al., 2020). The presence of antioxidants, vitamins, and unsaturated fatty acids in GP makes it a feedstuff with a valuable nutritional composition (Baroi et al., 2022).

Recent research has shown how different rearing substrates can impact the chemical composition of BSFL, particularly for what concerns their lipid content and fatty acid profile (Ewald et al., 2020; Suryati et al., 2023). The bioaccumulation capacity of BSFL, and the possibility of modifying their fatty acid profile through the rearing substrate, can open interesting prospects for a new valorisation of GP and to produce insect meals and oils enriched with fatty acids of specific nutritional characteristics. So far, only few research investigated the potential of BSFL to bio-convert GP (Meneguz et al., 2018; Gold et al., 2022; Ribeiro et al., 2022). Therefore, this study aims to evaluate the effects of GP from two different varieties as rearing substrate on the growth performance and chemical composition of BSFL, with a particular focus on the impact on their fatty acid profile.

Material and methods

Rearing substrates and black soldier fly larvae

The pomaces from two different grape varieties were used as rearing substrate for BSFL: Becuet (**B**) (red grape) and Moscato (**M**) (white grape) (MASAF, 2023). They were obtained during the winemaking process from a private distillery located in North-Western Italy (Distilleria Santa Teresa dei Fratelli Marolo S.r.L., Alba, Cuneo, Italy). Immediately after collection, the GP was transported to the labs of the Department of Agricultural, Forest and Food Sciences of the University of Turin, where the GP samples were dried at 60 °C until reaching a constant weight. After drying, the GP samples were sieved with the aim of separating the skins from the seeds. To be used as rearing substrates for BSFL, the dried skins and seeds were individually ground with a laboratory cutting mill to pass a 1 mm screen sieve (Pulverisette 15-Fritsch GmbH, Idar-Oberstein, Germany). Given the material availability, two dry−rearing substrates with 6 kg skins and 1.25 kg grape seeds were prepared for each grape variety. To reach the correct rearing substrate humidity (60%) for BSFL growth, water was then added.

Six-day−old BSFL were obtained from eggs produced at the experimental facility of the Department of Agricultural, Forest and Food Sciences. Twelve containers ($23 \times 30 \times 9$ cm^3) were filled with 1 220 g (40% of DM) of one of the two rearing substrates (six replicates / rearing substrate) and placed into a climatic room (temperature = 28 ± 0.5 °C; relative humidity: 65 ± 0.5%) to warm up. Thereafter, 1 000 6-day−old larvae were poured on the top of the rearing substrate in each container that was placed again in the climatic room. The total number of larvae was estimated by weighing twenty samples of 10 larvae (Kern & Sohn GmbH; Balingen, Germany; readability = 0.001). During the trial, no further substrate was provided to the larvae.

To follow the larvae growth, 10 larvae per replica were randomly sampled, washed and gently dried before being individually weighed at the following times: the day after the beginning of the trial (T1), then at day 5 (T2), day 8 (T3), day 13 (T4), day 15 (T5), day 20 (T6), day 22 (T7), and day 27 (T8); the latter corresponded to the end of the trial. As the measure was not destructive, the larvae were reintroduced into their replica (Bellezza Oddon et al., 2022a). Sampling and weighing lasted until 30% of the larvae reached the prepupa stage (day 27, T8), which was identified thanks to the change in the colour of the exoskeleton from white to black/brown as previously described (Tomberlin et al., 2002). For the larval final weights, the average weight of the leftover 70% larvae after removing the 30% of prepupae was calculated. At the end of the larval growth, 30 larvae / replica (sampled as previously described) were separated from the frass and were weighed. All the larvae were carefully washed and frozen. Subsequently, the larvae were freeze-dried (Freeze Drying Equipments – Criofarma, Turin, Italy) and stored at −80 °C prior to subsequent chemical analysis.

Production and waste reduction efficiency parameters were calculated (on a fresh matter basis) as follows:

- Growth rate (mg/day) = (larva average final BW (mg) − larva average initial BW (mg))/days of trial;
- Substrate reduction (%) = [(rearing substrate (g) − residue (g)) / rearing substrate (g)] × 100;
- Substrate reduction index = substrate reduction / days of trial.

Chemical composition of grape pomace and black soldier fly larvae

The dried GP (mixed proportion of skins and seeds used to prepare the rearing substrates, as previously described) and BSFL were ground using a knife mill (Grindomix GM200, Retsch GmbH, Haan, Germany; final fineness < 0.05 mm). AOAC International (2000) procedures were used to determine DM (method no. 930.15), ash (method no. 942.05), CP (method no. 984.13) and ADF (method no. 973.18) of both dried GP and BSFL. The CP content of BSFL was calculated using the nitrogen−to−protein conversion factor of 4.76 as suggested by Janssen et al. (2017), while for dried GP, the CP content was calculated using the conventional nitrogen−to−protein conversion factor of 6.25. In dried GP, the ether extract (method no. 2003.05) content was determined according to AOAC International (2003). Total lipids (**TL**) in dried GP and BSFL were extracted with a chloroform/methanol solution (2:1, v/v), according to Rodriguez-Estrada et al. (1997), with some modifications. Briefly, 0.4 g of sample was added to 80 mL of a chloroform/methanol solution (1:1, v/v), homogenised for 30 s and placed in an oven for 20 min at 60 °C. After the addition of 400 mL of chloroform, reaching a final ratio of 2:1 (v/v) of chloroform/methanol (Folch et al., 1957), the mixture was homogenised for 1 min and filtered to eliminate the solid residue. The filtered fraction was added to 400 mL of potassium chloride 1 M and was left overnight at 4 °C in a refrigerator. After phase separation, the chloroform phase was dried with a rotavapor, and the lipid extract

was weighed. The NDF content of dried GP was analysed according to Mertens (2002); α-amylase (Merck, Darmstadt, Germany) and sodium sulphite (Merck, Darmstadt, Germany) were added, and the results were corrected for residual ash content. The ADL of dried GP was determined according to method no. 973.18 of AOAC International (2000). Non-structural carbohydrates were calculated as: 100 − (CP + ether extract + ash + NDF).

The proximate composition of dried GP and BSFL was expressed as g /100 g DM.

All analyses were performed in duplicate. Any sample replications with CV values greater than 5% were reanalysed.

Fatty acid composition and phenolic fractions of grape pomace

A sample of 250 mg of dried GP was weighed to analyse the fatty acid composition using a combined direct transesterification and solid-phase extraction as reported by Alves et al. (2008). Fatty acid methyl esters were separated and identified using a GC-FID (GC 2010 plus, Shimadzu, Kyoto, Japan) and a fused silica capillary column (CP-Sil 88, Agilent J&W, Santa Clara, California, United States of America; 100 m × 0.25 mm i.d.; film thickness 0.20 μm) with the same instrumental parameters detailed in Renna et al. (2014). Peaks were identified by comparing retention times to pure standards (Restek Corporation, Bellefonte, Pennsylvania, United States of America) and by comparison with published chromatograms (Alves et al., 2008). Quantification was assessed by using heptadecanoic acid (C17:0) as internal standard. The results were expressed as g /100 g of TL.

The contents of total extractable phenols and different polyphenol fractions (total tannins, non-tannin phenols, condensed tannins, and hydrolysable tannins) in GP were determined as detailed in Iussig et al. (2015).

All analyses were performed in duplicate. Any sample replications with CV values greater than 5% were reanalysed.

Fatty acid profile and conjugated linoleic acid isomers determination of black soldier fly larvae

After TL extraction, the fatty acid methyl esters were prepared by the acid-base-catalysed trans-methylation (Kramer et al., 1997) with some modifications. Briefly, 1 mL of C9:0 and C19:0 (1 mg/mL) internal standards was added to 15 mg of TL. After the addition of 2 mL of sodium methoxide in methanol (0.5 M), the solution was vortexed and left to react for about 10 min at 50 °C, after cooling to room temperature 3 mL of 10% HCl solution in methanol was added and the solution was allowed to react for another 15 min at 50 °C. Once cooled, 6% aqueous potassium carbonate was added in two portions of 2 mL to prevent excessive effervescence, followed by the addition of 2 mL of hexane. The solution was then vortexed, centrifuged, and the organic layer transferred to another tube. The extraction step with hexane was repeated twice. The final solution was dried over anhydrous sodium sulphate and, after centrifugation, the solvent was collected into another tube and evaporated under a stream of nitrogen at room temperature. The residue was then dissolved in 1 mL of hexane.

The fatty acid methyl esters were identified and quantified, according to Mele et al. (2014), using a GC-FID (GC 2000 plus, Shimadzu, Columbia, Maryland, United States of America) and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, The Netherlands; 100 m × 0.25 mm i.d.; film thickness 0.20 μm). Individual fatty acid methyl esters were identified by comparison to a standard mixture of 37 Component fatty acid methyl esters Mix (Supelco, Bellefonte, Pennsylvania, United States of America). The identification of C18:1 isomers was based on commercial standard mixtures (Supelco, Bellefonte, Pennsylvania, United States of America) and published isomeric profiles (Wolff and Bayard, 1995). Conjugated linoleic acid (**CLA**) isomers of TL were separated and quantified by three silver ion HPLC columns (ChromSpher 5 Lipids, Varian, Middelburg, The Netherlands; 250 mm; 4.6 mm i.d.) according to Conte et al. (2019). Quantitative measurements were performed through a calibration curve, using high−purity individual *c*9*t*11 and *t*10*c*12 CLA isomers (Matreya Inc., Pleasant Gap, Pennsylvania, United States of America). The CLA mix standard (Sigma Chemical Co., St. Louis, Missouri, United States of America) and published isomeric profiles (Kramer et al., 2004) were used to help identify the CLA isomers in TL. Individual fatty acids were expressed as g /100 g of TL.

Statistical analysis

The statistical analysis of data was performed using IBM SPSS Statistics v. 27 for Windows. Outliers lying over 1.5 IQRs below the first quartile (Q1) or above the third quartile (Q3) were first detected and removed from the statistical analysis. Larval weights were subjected to a two-way mixed ANOVA. The Shapiro–Wilk test was used to verify whether the dependent variables were normally distributed for each combination of the groups of within-subject (test day, considered as a repeated measure) and between-subject (rearing substrate) factors. Levene's test was used to verify the homogeneity of variances for each combination of the groups of within- and between-subject factors. Mauchly's test was used to verify the assumption of sphericity; if such an assumption was violated, the Greenhouse–Geisser or the Huynh–Feldt correction (in cases of estimates of sphericity lower or higher than 0.75, respectively) was applied to correct the df of the F-distribution. The means for the main effects of test day, rearing substrate and their interaction were compared using the Bonferroni confidence interval adjustment. Differences in terms of larval weights for each test day and larval final weights, as well as production and waste reduction efficiency parameters (growth rate, substrate reduction and substrate reduction index), proximate composition and fatty acid profile of BSFL between rearing substrates were also assessed using independent-sample Student's *t*-tests. No statistical analysis was conducted to compare the chemical composition of the rearing substrates, as each of them was derived from a single commercial batch. Significance was declared at $P \leq 0.05$.

Results

Proximate composition, phenolic fractions and fatty acid profile of grape pomace

The proximate composition of dried GP from B and M varieties, used as rearing substrate for BSFL, is reported in Table 1. The two substrates contained similar contents of CP (on average

Table 1
Proximate composition (g/100 g DM, unless otherwise stated) of dried grape pomace used as rearing substrate for black soldier fly larvae.

Item	Grape pomace variety	
	B	M
DM (g/100 g)	94.06	88.18
Ash	13.27	5.83
CP	11.75	10.11
Ether extract	6.33	5.91
NDF	49.48	37.96
ADF	34.48	34.18
ADL	22.77	19.99
Non-structural carbohydrates	19.17	40.19
Protein: non-structural carbohydrates	1:1.6	1:4.0
Protein + non-structural carbohydrates	30.92	50.30

Abbreviations: B = Becuet; M = Moscato.

10.9 g/100 g DM), and ADF (about 34 g/100 g DM) and mainly differed in ash and non-structural carbohydrates contents, the variations of which were more than double between the substrates (higher ash in B and higher non-structural carbohydrates in M). The NDF content was about 1.3-fold higher in B than M substrate.

The TL content was comparable between the substrates (on average 5.6 on a wet basis; Table 2). Regarding the GP fatty acid profile (Table 2), the B variety showed about 1.1-fold higher amount of total saturated (**SFA**), total monounsaturated (**MUFA**) and total polyunsaturated (**PUFA**) fatty acids. The most represented individual fatty acids for both varieties were linoleic acid (C18:2 n-6; on average 56.16 g/100 g TL), followed by oleic acid (C18:1 *c*9; on average 12.57 g/100 g), both being higher in B than M, and palmitic acid (C16:0; on average 9.43 g/100 g TL) being very similar between the two GP varieties. Among PUFA, α-linolenic acid (C18:3 n-3) was found in relatively low amounts, with slightly higher contents in M than B variety. In both GP varieties, some CLA isomers were detected, namely rumenic acid (C18:2 *c*9*t*11, which coeluted with other two CLA isomers, *t*7*c*9 and *t*8*c*10, with the applied chromatographic conditions) and C18:2 *t*9*t*11. The detected amount of CLA isomers was low (total CLA equal to 0.085 and 0.16 g/100 g TL in B and M, respectively), with M variety showing slightly higher amounts of the sum C18:2 *c*9*t*11 + *t*7*c*9 + *t*8*c*10.

The recorded amount of total extractable phenols in GP was remarkable, being slightly higher in B than M variety (173.7 vs 156.9 g/kg DM, respectively). In both GP varieties, 97% of total extractable phenols were represented by tannins, with a clear prevalence of hydrolysable (163.4 vs 133.6 g/kg DM, respectively) over condensed (5.3 vs 19.3 g/kg DM) forms.

Growth performance and waste reduction efficiency of black soldier fly larvae

The development of BSFL reared on GP from B and M varieties is shown in Fig. 1. The results showed a significant main effect of rearing substrate [F(1,10) = 28.140; $P < 0.001$], with average BSFL weights being significantly higher when the larvae were reared on B rather than M GP variety. The results also showed a significant main effect of test day [F(8,80) = 231.243; $P < 0.001$]. Bonferroni corrected posthoc tests showed the following BSFL weight trend: day 0 < day 1 < day 5 < day 8 < day 13 < day 15 = day 20 < day 22 = day 27. The interaction term (test day × rearing substrate) was also highly significant [F(8,80) = 8.497; $P < 0.001$], showing that the larval weights significantly differed in the test days between rearing substrates. In particular, the independent-sample Student's *t*-tests showed that the larval weights started differing from test day 3 ($P < 0.05$) and remained significantly different till the end of the trial. Regarding the production and waste reduction efficiency parameters, the growth rate showed a higher value for BSFL reared on B substrate (4.4 and 3.2 mg/day for B and M, respectively; $P < 0.01$). On the other hand, the substrate reduction (44.59 vs 43.45% for B and M, respectively) and the substrate reduction index (1.65 vs 1.61 for B and M, respectively) were not significantly affected by the rearing substrate.

Table 2
Total lipids (g/100 g wet basis), fatty acid composition (g/100 g TL) and total fatty acids (g/100 g wet basis) of dried grape pomace used as rearing substrate for black soldier fly larvae.

Item	Grape pomace variety	
	B	M
TL	5.96	5.22
C10:0	0.14	0.10
C12:0	0.17	0.066
C14:0	0.22	0.11
C15:0	0.053	0.046
C16:0	9.22	9.63
C16:1 *c*9	0.31	0.32
C18:0	4.01	2.81
C18:1 *t*9-11	0.044	0.074
C18:1 *c*9	13.66	11.47
C18:1 *c*11	0.76	1.09
C18:2 n-6	59.15	53.16
C20:0	0.62	1.00
C20:1 *c*11	0.18	0.16
C18:3 n-3	1.39	2.31
C18:2 *c*9*t*11 (+*t*7*c*9 + *t*8*c*10)	0.039	0.10
C18:2 *t*9*t*11	0.047	0.064
Total SFA	14.43	13.77
Total MUFA	14.95	13.11
Total PUFA	60.54	55.47
Total CLA	0.085	0.16
Total fatty acids	5.36	4.31

Abbreviations: B = Becuet; M = Moscato; TL = total lipids; *c* = *cis*; *t* = *trans*; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CLA = conjugated linoleic acids.

Proximate composition and fatty acid profile of black soldier fly larvae

None of the proximate constituents of the BSFL was significantly affected by the rearing substrate (Table 3). The TL content of the larvae was significantly higher in M–fed when compared to B-fed BSFL ($P < 0.05$) and the fatty acid profile of the larvae also significantly varied according to the rearing substrate (Table 4). The total straight-chain SFA and some individual SFA (C10:0, C12:0, C14:0 and C16:0) were higher in M-fed than B-fed larvae. Conversely, the total branched-chain fatty acids (**BCFAs**) and the majority of the detected individual BCFA (C15:0 *anteiso*, C16:0 *iso*, C17:0 *iso* and C18:0 *iso*) were significantly higher in B-fed than M-fed larvae. The BSFL reared on the M substrate showed significantly higher contents of total MUFA, C16:1 *c*9 and C18:1 *c*9, while the BSFL reared on the B substrate showed higher contents of C16:1 *t*9, C16:1 *c*7 and C18:1 *c*13. The total PUFA content was higher in B-fed than M-fed larvae ($P < 0.001$). All individual detected PUFAs were significantly different between B-fed and M-fed larvae, except for C20:3 n-6 and C22:3 n-3 which did not differ between the substrates. Some CLA isomers (C18:2 *c*9*t*11, C18:2 *t*7*c*9 and C18:2 *t*10*c*12) were detected in the BSFL. The most abundant detected CLA isomer was rumenic acid, accounting for 93 and 46% of total CLA in B-fed and M-fed BSFL, respectively. The *t*7*c*9 CLA isomer ranked second, accounting for 44% of total CLA in M-fed BSFL, while only for 6% in B-fed BSFL. All detected CLA isomers were significantly higher, with the total CLA concentration being 59-fold higher, in B-fed than M-fed larvae.

Discussion

Proximate composition, phenolic fractions and fatty acid profile of grape pomace

The proximate composition of the GP varieties used in the current trial falls within the wide ranges reported by Antonić et al. (2020), except for the ash content in B variety (13.27 g/100 g DM), which was higher than the maximum value (9.10 g/100 g DM) reviewed by the above-mentioned authors. No data is currently available in published literature concerning the proximate composition of the GP obtained from B variety. However, it is known that the variety (genotype) significantly influences the chemical composition of GP (Ahmed et al., 2020), with other sources of variation being the planting environment, the harvesting conditions, and the applied processing method (Antonić et al., 2020). Our results show that fibre is a predominant compound in

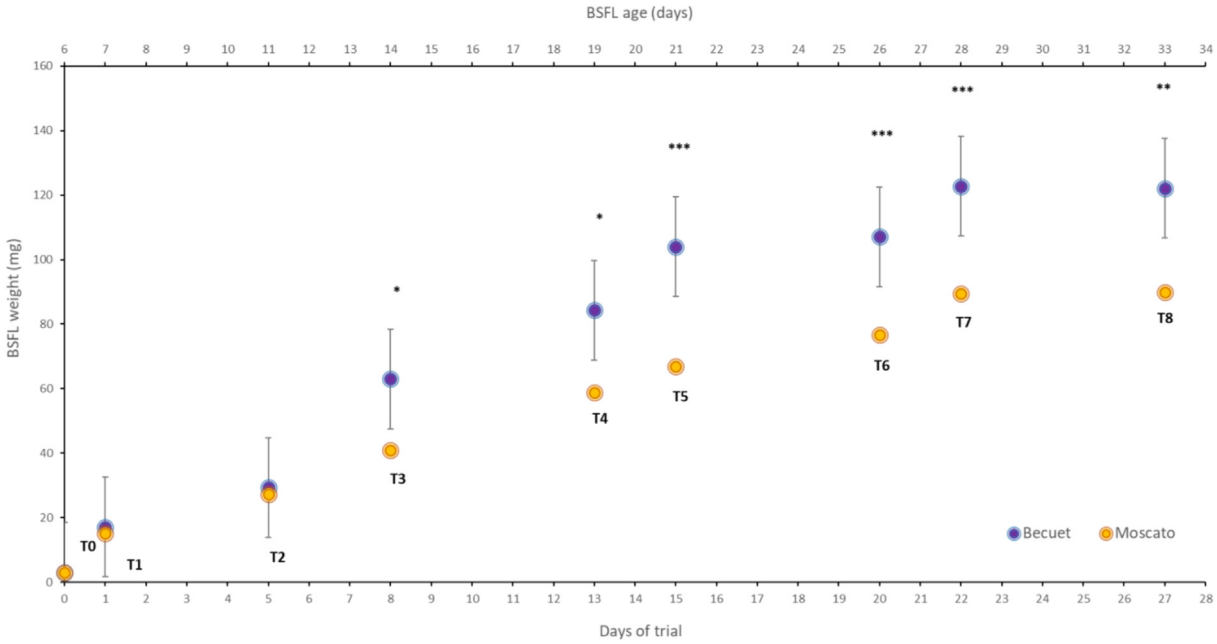

Fig. 1. Weight development of black soldier fly larvae (BSFL) reared on grape pomace from Becuet and Moscato varieties at different time points [days 1 (T1), 5 (T2), 8 (T3), 13 (T4), 15 (T5), 20 (T6), 22 (T7) and 27 (T8) after the beginning of the trial]. Error bars represent SEM. *: $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 3
Proximate composition (g/100 g DM, unless otherwise stated) of black soldier fly larvae reared on grape pomace from Becuet (B) and Moscato (M) varieties (n = 6).

Item	B-fed BSFL	M-fed BSFL	SED	*P*-value
DM (g/100 g)	31.28	30.85	0.831	0.615
Ash	13.89	13.37	0.377	0.196
CP	35.84	35.94	0.326	0.758
TL	16.84	20.17	0.826	0.035
ADF	10.75	10.79	0.249	0.869

Abbreviations: TL = total lipids.

GP (Table 1). The B variety showed numerically higher NDF values when compared to the M variety, which is consistent with the significantly higher dietary fibre contents found in published literature for red GP when compared to white GP (Antonić et al., 2020). The latter authors also reported that most of dietary fibre in GP comprises insoluble components, such as hemicellulose and cellulose. As ADF and ADL values were similar between the B and M varieties, it is likely that in our GP samples, the main difference in fibre composition was imputable to the hemicellulose content. Another important variation found when comparing the B and M GP used in our study regards the non-structural carbohydrates content, which comprises starches and sugars and was about 2-fold higher in the M variety. Deng et al. (2011) found that soluble sugars are the largest constituents in white GP, while in red GP, the related amounts are usually low (Antonić et al., 2020), thus corroborating the results obtained in our study.

Various phenolic compounds have been detected in GP, including catechins, flavonols, phenolic acids, alcohols and, only in red grape pomaces, anthocyanins. During grape processing, such compounds remain in GP due to their incomplete extraction, conferring GP valuable antioxidant properties (Ahmed et al., 2020; Antonić et al., 2020). Total extractable phenols values in the GP varieties used in our study fell within the ranges recently reported by Guaita et al. (2023) for the other seven varieties sampled from local wineries of Piedmont (Northwest Italy), the same geographical region of origin of B and M varieties used in our study.

The TL content of the two GP varieties used as substrates for BSFL rearing in the current study was quite similar (Table 2), as a consequence of their comparable proportion of seeds (rich in oil) and skin. As far as the fatty acid profile of GP is concerned, our results confirm previous findings. In fact, other authors found a prevalence of PUFA in GP, with linoleic acid being the most abundant individual fatty acid, followed by oleic, palmitic, and stearic acids (Yi et al., 2009; Kolláthová et al., 2020; Nakov et al., 2022). Small quantities of other individual fatty acids characterised by variable carbon chain length, unsaturation degree and double bond configuration have also been reported in GP (Yi et al., 2009). Even if no statistical analysis was performed to compare the fatty acid profile of B and M varieties in the current study, some inter-variety variability can be observed regarding both individual detected fatty acids and groups of fatty acids (Table 2). Available literature data show, in fact, that the fatty acid profile of GP is significantly influenced by grape variety and cultivation area (Yi et al., 2009; Ahmed et al., 2020; Kolláthová et al., 2020). Interestingly, our results show that GP also contains small quantities of some CLA isomers. Recently, Nakov et al. (2022) reported the presence of 0.22 g/100 g oil of CLA in a red variety of GP; however, these authors did not specify which CLA isomers were detected and did not hypothesise any reasons for the presence of CLA in GP. Conjugated linoleic acids are polyunsaturated fatty acids mainly found in ruminant-derived food products, such as dairy and meat products (Renna et al., 2010). Various CLA isomers exist in nature, all of them being characterised by conjugated double bonds at different geometric (cis/trans) and positional location along the aliphatic chain. Interest in CLA derives from the health-promoting effects, such as anti-carcinogenic, anti-obesogenic and anti-atherosclerotic properties, shown by some CLA isomers (den Hartigh, 2019). The presence of CLA isomers in milk and meat is

Table 4
Total lipids (g/100 g wet basis), fatty acid composition (g/100 g TL) and total fatty acids (g/100 g wet basis) of black soldier fly larvae reared on grape pomace from Becuet (B) and Moscato (M) varieties (n = 6).

Item	B-fed BSFL	M-fed BSFL	SED	*P*-value
TL	16.84	20.17	0.826	0.035
C10:0	0.33	0.50	0.043	0.003
C12:0	12.94	18.05	1.185	0.002
C14:0	2.17	3.63	0.168	0.000
C15:0	0.23	0.23	0.027	0.890
C16:0	9.01	11.44	0.512	0.003
C17:0	0.29	0.34	0.042	0.311
C18:0	2.03	2.69	0.197	0.057
C20:0	0.11	0.11	0.024	0.963
C22:0	0.076	0.071	0.021	0.795
C23:0	0.16	0.029	0.04	0.008
C24:0	<0.001	0.013	0.009	0.197
Total straight-chain SFA	27.59	37.09	1.589	0.000
C15:0 *iso*	0.20	0.14	0.037	0.175
C15:0 *anteiso*	0.29	0.058	0.024	0.000
C16:0 *iso*	0.14	0.084	0.023	0.044
C17:0 *iso*	0.59	0.52	0.029	0.030
C18:0 *iso*	0.86	0.60	0.088	0.015
Total BCFA	2.07	1.40	0.129	0.000
C16:1 *t*9	0.15	0.013	0.027	0.001
C16:1 *c*7	0.16	0.015	0.015	0.000
C16:1 *c*9	0.99	1.28	0.099	0.013
C17:1 *c*9	0.11	0.12	0.022	0.635
C18:1 *t*9	0.014	<0.001	0.007	0.106
C18:1 *t*10	n.d.	n.d.	–	–
C18:1 *t*11	0.21	0.014	0.086	0.069
C18:1 *c*9	11.41	14.03	0.603	0.001
C18:1 *c*11	0.53	0.54	0.047	0.778
C18:1 *c*13	0.31	<0.001	0.013	0.000
C24:1 *c*15	0.14	0.18	0.029	0.143
Total MUFA	14.04	16.28	0.739	0.013
C18:2 n-6	30.66	24.75	1.512	0.003
C18:3 n-3	0.40	0.57	0.032	0.000
C18:4 n-3	0.14	0.019	0.028	0.002
C20:2 n-6	0.14	0.25	0.050	0.044
C20:3 n-6	0.055	0.0040	0.031	0.166
C20:4 n-6	0.17	0.042	0.039	0.018
C22:2 *c*13*c*16	0.0051	0.057	0.015	0.006
C20:5 n-3	0.21	0.31	0.038	0.019
C22:3 n-3	0.15	0.19	0.026	0.166
C18:2 *t*9*t*11	n.d.	n.d.	–	–
C18:2 *t*10*c*12	0.026	0.010	0.006	0.007
C18:2 *c*9*t*11	2.74	0.024	0.691	0.011
C18:2 *t*8*c*10	n.d.	n.d.	–	–
C18:2 *t*7*c*9	0.17	0.023	0.036	0.005
PUFA n-6/n-3	35.06	23.31	2.787	0.002
Total CLA	2.95	0.052	0.727	0.010
Total PUFA	34.87	26.25	1.313	0.000
Total PUFA without CLA	31.92	26.20	1.476	0.003
Total fatty acids	13.25	16.34	1.228	0.031

Abbreviations: TL = total lipids; SFA = saturated fatty acids; BCFA = branched-chain fatty acids; *t* = *trans*; *c* = *cis*; n.d. = not determined; MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acids; PUFA = polyunsaturated fatty acids.

partly the consequence of the biohydrogenation process occurring inside the rumen, operated by rumen bacteria that express linoleate isomerase activity (e.g., *Butyrivibrio fibrisolvens*), at the expense of the linoleic acid ingested by the ruminant with the diet (Enjalbert et al., 2017). Linoleate isomerase is an enzyme able to convert linoleic acid into CLA (Nasrollahzadeh et al., 2023). Its activity is influenced by linoleic acid concentration, pH, temperature, and microbial growth stage; moreover, it is strain−dependent (Gorissen et al., 2015). Renna et al. (2023) and Yi et al. (2009) also reported the presence of low amounts of both non-conjugated (*t*9*t*12, *c*9*t*12 and *t*9*c*12) and conjugated (*c*9*t*11 and *t*9*t*11) trans-octadecadienoic acids in the pomaces of three different red grape varieties. Renna et al. (2023) hypothesised that the presence of trans-octadecadienoic acids in GP, including CLA isomers, would be the consequence of the microbial metabolism of linoleic acid during winemaking. Although no microbiological analyses have been conducted on B and M GP in the current trial, the bacteria involved in the winemaking process are well known (Ageyeva et al., 2021). The latter authors reported the presence of lactic acid bacteria (e.g., *Lactobacillus* spp. and *Pediococcus* spp.) in GP of both red and white varieties. The above-mentioned genera of lactic acid bacteria are involved in the malolactic fermentation that occurs, during or after the alcoholic fermentation, mainly in red wines, but also in some white wines and base sparkling wines (Gil-Sánchez et al., 2019; Virdis et al., 2021). Strains of food-derived lactobacilli have been shown to possess high linoleate isomerase activity and *L. plantarum*, a malolactic starter inoculated during the winemaking process (Brizuela et al., 2019), has been identified as one of the most efficient CLA producers (Yang et al., 2014), being able to produce both *c*9*t*11 and *t*9*t*11 isomers (Kishino et al., 2003), the same we detected in B and M GP. Therefore, the presence of CLA *c*9*t*11 and *t*9*t*11 in our GP samples is most probably imputable to the linoleate isomerase activity of lactic acid bacteria usually found in GP. As a final consideration, it is worth mentioning that

the presence of CLA *t*9*t*11 in GP could also be the consequence of an artefact of fatty acids analysis. In fact, for the fatty acid analysis of matrices where CLA isomers are expected to be found (e.g., milk, meat, and rumen digesta), a low-temperature (50 °C) base-catalysed methylation is usually applied, as acid catalysis combined with high temperatures may determine undesirable geometric isomerisations of CLA *c*9*t*11 to CLA *t*9*t*11 (Liu et al., 2018). Analysing milk samples, the latter authors reported significant losses of CLA *c*9*t*11 when using an acid-catalysed transesterification (6% sulfuric acid in methanol at 80 °C for 60 min) when compared to a base-catalysed (0.2 M potassium hydroxide in methanol at 50 °C for 20 min) one, while no significant differences were found between the base-catalysed method and a milder acid catalysis (6% sulfuric acid in methanol at 60 °C for 2 h). When analysing feed lipids, temperatures higher than 50 °C are usually applied, because no CLA isomers, and therefore no risk of isomerisations, are expected. In the current trial, for fatty acid analysis in GP, we applied an acid-catalysed transesterification using hydrogen chloride in methanol at 70 °C for 2 h, and we cannot exclude that isomerisations may have occurred. It is also worth mentioning that other authors who previously reported the presence of CLA *t*9*t*11 in GP (Yi et al., 2009) also applied a transesterification method (hydrogen chloride in methanol at 80 °C for 1 h) which could have led to artefacts of analysis. Further investigations should therefore confirm or disprove the presence of CLA *t*9*t*11 in by-products of the winemaking industry.

Growth performance and waste reduction efficiency of black soldier fly larvae

Several studies have reported an influence of the chemical composition of the rearing substrate on the development time, growth performance and waste reduction efficiency of BSFL (Barragan-Fonseca et al., 2021; Pliantiangtam et al., 2021; Singh et al., 2022). On both the rearing substrates used in our trial, 30% of the larvae developed into the prepupa stage in 27 days, when they were 33 days old. Applying similar rearing conditions as those used in the current trial, Meneguz et al. (2018) reported that BSFL reared on a winery by-product containing grape seeds, pulp, skins, stems, and leaves took on average 22 days to reach the prepupa stage, corresponding to a BSFL age of 28 days. In a meta-analysis used to evaluate the effects of different organic substrates on the growth performance of BSFL, Fitriana et al. (2022) reported that, when using food waste as a rearing substrate, the average rearing time of BSFL was slightly lower (24 days) than that observed in our study, but noticeable variability was also observed according to the type of food waste used, with a SD equal to ± 13.2 days.

Heavier weights were recorded for the B-fed than for the M-fed BSFL since day 8 of the trial (which corresponded to 14-day−old larvae; Fig. 1). Eggink et al. (2023) recently found that the dietary protein to non-fibre carbohydrate ratio significantly affected the growth performance of BSFL. In particular, evaluating ratios equal to 1:1, 1:1.5, 1:2, 1:3, 1:5 and 1:9, these authors achieved the best performance of BSFL when using substrates characterised by dietary protein to non-fibre carbohydrate ratios equal to 1:2 and 1:3, while ratios outside this range (as it occurred in our trial, being values equal to 1:1.6 and 1:4.0 for B and M substrates, respectively) resulted in reduced larval growth performance. Previously, without differentiating between structural and non-structural carbohydrates, Barragan-Fonseca et al. (2021) showed that BSFL performance was more affected by the sum of dietary protein and carbohydrate concentration rather than by the protein−to−carbohydrate ratio. These authors found that the best BSFL performance was achieved at protein + carbohydrate values of 25 and 50% and protein: carbohydrate ratios equal to 1:2 and 1:4. In our study, the protein + carbohydrate values were equal to 30% (B) and 50% (M), but for the calculation, the non-structural carbohydrates and not the total carbohydrates content was used (dietary protein to non-fibre carbohydrate in Table 1). Finally, analysing different dietary fat sources and levels, Li et al. (2022) found that the palmitic acid content of the rearing substrate was a major factor in affecting the final BW of BSFL. However, such a finding does not seem to be confirmed by our data, as similar total lipids and palmitic acid contents characterised the two GP varieties, suggesting that other factors are also involved.

The growth rate values obtained in the current trial are comparable to those obtained by Meneguz et al. (2018) when rearing BSFL using winery by-products of different variety and material (seeds, skin, stems, pulp, leaves) proportion (6 ± 0.9 mg/day). Performing a meta-analysis on the effects of organic substrates of different origin and composition on BSFL performance and nutrient composition, great variation in growth rate values was reported by Fitriana et al. (2022). Indeed, when considering BSFL reared on food waste, these authors reported values of 189 ± 255.3 mg/day, while values as low as 31 ± 83.1 mg/day were indicated for "other substrates" likely including single organic waste. Even if no data on fibre or lignin content of the substrates was reported in the meta-analysis conducted by Fitriana et al. (2022), we could argue that differences between our data and values reported for food waste by these authors could be imputable to the lower lignin content of the food waste when compared to the lignin content of the two GP varieties used in our study (ADL: about 32 and 20–23 g/100 g DM, respectively). Indeed, although some studies have shown that BSFL appear as midgut fermenters and, thanks to their microbial community, are able to degrade the cellulose, hemicellulose, and lignin present in the substrate (Seyedalmoosavi et al., 2022), the digestion may be more difficult if compared to food waste usually characterised by lower fibre content, resulting in long development times and/or low growth rate.

Finally, in our trial, the substrate reduction and the substrate reduction index were not affected by the GP variety used. Both indexes showed values that were lower when compared to values obtainable using a standard (Gainesville) diet (49.4% and 4.94, respectively) or the average obtainable using various types of food waste (69.4% ± 15.05 and 2.1 ± 1.57) as reported by Pliantiangtam et al. (2021) and Fitriana et al. (2022), respectively.

The poor BSFL performances was an expected outcome in the current trial, as it is known that single substrates are not tailored for optimal larval performance. Indeed, BSFL, as any other animal, have specific nutritional requirements in terms of protein (Bellezza Oddon et al., 2022a) and lipids (Bellezza Oddon et al., 2022b) that should be covered to optimise growth and other performance parameters. It is therefore difficult to cover these requirements using a single substrate, while compound diets formulated by mixing different waste or by-products can easily reach this aim (Bellezza Oddon et al., 2024). In this trial, the CP levels of the two substrates were 11.75 and 10.11% for B and M, respectively. These levels are lower than the range (14–16% DM) suggested as optimal by Bellezza Oddon et al. (2022a). Substrates characterised by a high abundance of readily accessible carbons, but low nitrogen content, are not conducive to larval development and can reduce the overall process efficiency (Suryati et al., 2023). As previously mentioned, the chemical composition of GP greatly varies according to grape variety (Antonić et al., 2020), and this can explain the variability observed in the performance of GP-fed BSFL in published literature. This aspect also highlights the importance of clearly stating the variety of GP when used in studies evaluating BSFL performance, which has been a missing information in published literature so far (Meneguz et al., 2018; Ribeiro et al., 2022).

Proximate composition of black soldier fly larvae

In the current trial, no major differences were found in the proximate composition of the BSFL (Table 3) when reared on GP of B or M varieties. However, TLs (Table 4) were significantly higher in M-fed than B-fed larvae. The BSFL reared on B and M had a protein content slightly lower than the range values (from 37 to 63% DM) reported in literature (Barragan-Fonseca et al., 2017; Chia et al., 2020). This can be partly the consequence of different nitrogen–to–protein conversion factors used by different authors for CP calculation. In the current trial, we used a nitrogen to protein conversion factor equal to 4.76, as suggested by Janssen et al. (2017), while data reported by Barragan-Fonseca et al. (2017) derived from the use of the conventional nitrogen–to–protein conversion factor of 6.25. Galassi et al. (2021) reported that when substrates are imbalanced, the optimal incorporation of CP from the substrate is not achieved. In this present study, both B and M GP substrates were characterised by a low protein content, supporting this hypothesis.

As far as the TL content is concerned, the observed differences could be the consequence of the much greater non-structural carbohydrate content of GP of M variety (Table 1). In fact, a positive correlation between the BSFL fat content and the non-structural carbohydrate level of the substrate was reported by Spranghers et al. (2017). Finally, considering ash, it has been shown that its content is proportional to the development time of the larvae, as this value is particularly affected by the development of the exoskeleton (Fitriana et al., 2022), due to the ability of BSFL to accumulate amorphous calcium carbonate in the early stages and calcite in the older stages, increasing the amount of calcium carbonate along the life cycle (Rebora et al., 2023). This explains why the ash content in the BSFL reared on B and M substrates had very similar values despite the different ash content of the GP. In fact, the growing period was 27 days for both substrates, and therefore, the larvae were analysed at the same age and stage of development.

Fatty acid profile of black soldier fly larvae

Available published literature data have shown that the nutritional composition of the rearing substrate can exert a significant effect on the lipid profile of BSFL (Ewald et al., 2020; Georgescu et al., 2022; Li et al., 2022). As a confirmation, also in the current trial, the GP variety used as rearing substrate significantly affected the majority of detected individual fatty acids and groups of fatty acids (i.e., straight-chain SFA, odd-chain fatty acids and BCFA, MUFA, PUFA and CLA) in the larvae.

Straight-chain saturated fatty acids

As expected, BSFL reared on both GP varieties contained high levels of total straight-chain SFA, with a prevalence of lauric (C12:0) and palmitic (C16:0) acids (Meneguz et al., 2018; Hoc et al., 2020). High C12:0 levels are a peculiarity of BSFL, when compared to other insect species in which the detected C12:0 amounts are considerably lower (Renna et al., 2022). As hypothesised by Meneguz et al. (2018), the high SFA, and particularly the high C12:0 levels, in BSFL could be the consequence of an adaptation to the hot climate typical of subtropical areas. It is known that, in BSFL, some fatty acids are directly bioaccumulated from the rearing substrate, while other fatty acids are biosynthesised by the larvae (Hoc et al., 2020). The high C12:0 amounts detected in BSFL in the current study are not a direct consequence of the fatty acid profile of the rearing media; in fact, both GP varieties contained very low amounts of C12:0 (< 0.2 g/100 g TL; Table 2). Hoc et al. (2020) reported that various insect species are able to biosynthesise *de novo* individual SFA with a chain length from 12 to 18 carbon atoms, using dietary carbohydrates (Oonincx et al., 2020; Liu et al., 2023). The same authors hypothesised that such ability is also probably found in the black soldier fly species, as two genes coding for key enzymes (acetyl-CoA carboxylase and fatty acid synthase) involved in *de novo* synthesis of SFA have been characterised in this insect species (Giannetto et al., 2020). Ewald et al. (2020) reported a significant influence of the larva weight on the fatty acid composition of BSFL, with heavier larvae containing higher total SFA and C12:0, and lower total PUFA percentages. Previously, also Liu et al. (2019) found that C12:0 accumulated in black soldier fly during larval development. In our trial, B-fed and M-fed BSFL reached the prepupa stage at the same time, with B-fed larvae being significantly heavier than M-fed ones, but the latter were able to accumulate significantly higher C12:0 amounts. Overall, the significantly higher total and individual (C12:0, C14:0, C16:0, and C18:0) straight-chain SFA amounts found in M–fed rather than B-fed BSFL could be the consequence of the differences in the content and type of carbohydrates found in GP of M and B varieties (Table 1). Given the results obtained in our trial, we hypothesise that straight-chain SFAs are mainly biosynthesised by BSFL starting from non-structural rather than structural carbohydrates; further trials will be necessary to confirm or deny this hypothesis. In addition, the slightly higher total extractable phenols content found in GP of B variety may also have played a role for the observed differences in the straight-chain SFA content between B-fed and M-fed BSFL. In fact, polyphenols have been reported to reduce the expression of lipogenic enzymes, among which acetyl-CoA carboxylase and fatty acid synthase (Ma et al., 2020; Fang et al., 2022; Cui et al., 2024).

Odd- and branched-chain fatty acids

In BSFL, we also detected the presence of odd-chain and branched-chain fatty acids. As fatty acid synthase is a multienzyme complex that catalyses a sequence of reactions in which a fatty acid is lengthened by two carbon atoms at a time, the presence of odd-chain fatty acids (e.g., C15:0 and C17:0) in BSFL may be of microbial origin and/or may derive from α-oxidation of even-chain fatty acids (C16:0 and C18:0, respectively) (Vlaeminck et al., 2006).

The BCFA are mostly saturated fatty acids with an additional methyl group at n-2 carbon (configuration *iso*) or n-3 carbon (configuration *anteiso*) from the methyl end of the aliphatic carboxylic chain (Gozdzik et al., 2023). These compounds are mainly found in milk, meat, and fermented foods (He et al., 2023), but their presence has been reported also in living organisms including ruminants, poultry, and rabbits (Dabbou et al., 2017; Kim et al., 2020; Renna et al., 2022). Interest in BCFA mainly relies on their potential relevance to human health and disease, as they have been reported to exert a role in regulating lipid metabolism, exerting anti-inflammatory and anti-cancer properties, and promoting growth and development (He et al., 2023). These compounds are of microbial origin, being major components of bacterial membranes across several genera and species (Vlaeminck et al., 2006; Gozdzik et al., 2023). They have been reported to be particularly abundant in *Bacillus* spp. and *Lactobacillus* spp., where they comprise up to 95% of total detected fatty acids (Ran-Ressler et al., 2014). The BCFA are formed starting from amino acids, such as leucine, isoleucine, and valine, thanks to the activity of branched-chain amino acid aminotransferase, branched-chain α-keto-acid decarboxylase and branched chain fatty acid synthetase, as summarised by Vlaeminck et al. (2006). In our trial, given the absence of BCFA in GP, we hypothesise that BCFA were detected in BSFL because of the presence of *Lactobacillus* spp. in the gut of the larvae, as reported by various authors (Jiang et al., 2019; Wynants et al., 2019). From the current trial, it is not possible to understand if the higher BCFA content found for B-fed rather than M-fed BSFL is the consequence of a higher presence of the above-mentioned

amino acids in the gut of B-fed BSFL, an up- or down-regulation of enzymes involved in BCFA synthesis, or rather to a greater accumulation of *Lactobacillus* species in BSFL gut from the rearing medium.

Monounsaturated fatty acids

The most abundant individual MUFA detected in BSFL in our trial was oleic acid, which confirms previous findings (Ewald et al., 2020; Li et al., 2022; Tognocchi et al., 2023). Hoc et al. (2020) demonstrated that C18:1 *c*9 is both synthesised *de novo* by the larvae and bioaccumulated from the rearing substrate. Both GP varieties used as rearing substrates in our study contained appreciable amounts of C18:1 *c*9, with B variety being slightly richer than M variety (Table 2). However, M-fed BSFL showed a significantly higher content of C18:1 *c*9 when compared to B-fed BSFL. The presence of stearoyl Co-A desaturase enzyme, also named as Δ9-desaturase, in BSFL has been hypothesised, similarly to what already demonstrated for other insect species belonging to the Diptera order (Hoc et al., 2020). This enzyme is able to add a *cis*-9 double bond to both diet-derived and *de novo* synthesised saturated and unsaturated fatty acids with a chain length from 12 to 19 carbon atoms (Benito-Vicente et al., 2021). Preferred substrates for stearoyl Co-A desaturase are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively (Benito-Vicente et al., 2021). The higher *de novo* synthesis of C16:0 and C18:0 in M-fed BSFL, as previously discussed, may therefore be the reason for the observed significantly higher C16:1 *c*9 and C18:1 *c*9 presence in M-fed BSFL when compared to B-fed BSFL; the same can be observed when considering C17:0 and C17:1 *c*9. Moreover, it has been demonstrated that epigallocatechin gallate, a polyphenolic compound present in grape pomace (Sinrod et al., 2023), can exert a down-regulation effect of stearoyl Co-A desaturase expression in rodents (Wolfram et al., 2005), which may further explain the obtained results. It is very interesting to highlight that, besides the most abundant C18:1 *c*9 and C16:1 *c*9, other individual MUFA have been detected in low amounts in BSFL in the current study. Some of them (i.e., *cis* and trans-octadecenoic acids) are intermediate products usually formed during the biohydrogenation process operated under anaerobic conditions (e.g., inside the rumen of ruminant animals) by some bacteria at the expense of unsaturated fatty acids, which has stearic acid as end product (Dewanckele et al., 2020). A complete microbiological characterisation (up to species level) for BSFL gut is still lacking in available literature; therefore, it is not possible here to clarify if the detection of octadecenoic isomers other than oleic acid in BSFL can be related to the presence of bacteria (e.g., *Butyrivibrio* spp.) usually involved in the biohydrogenation process (Hoc et al., 2020). However, in our study, some of these octadecenoic acids were already present in the GP used as a rearing substrate (Table 2) and therefore, BSFL may have also bioaccumulated them from the rearing medium, with probable subsequent isomerisations (C18:1 *c*13 was absent and present in GP and BSFL, respectively) operated by some bacteria present in BSFL gut.

Polyunsaturated fatty acids

Linoleic acid was the most abundant PUFA in BSFL, as already observed when using rearing substrates rich in omega-6 fatty acids (Georgescu et al., 2022). The significantly higher C18:2 n-6 levels found in B-fed rather than M−fed BSFL seem to reflect the differences in the C18:2 n-6 amounts found in the two GP varieties used as rearing substrates. However, it cannot be excluded that part of C18:1 *c*9 could have been converted to C18:2 n-6, thanks to the presence of a Δ12 desaturase, as reported by Hoc et al. (2020). On the contrary, in our study, individual omega-3 fatty acids, such as C18:3 n-3, C18:4 n-3, C20:5 n-3, and C22:6 n-3, were only detected in low amounts in BSFL. This is consistent with published research showing that to enrich BSFL with n-3 PUFA, it is necessary to use substrates containing ingredients such as linseed oil, seaweed or fish discards, which are naturally enriched in C18:3 n-3 or other n-3 PUFA of higher carbon chain length and unsaturation degree (e.g., C20:5 n-3, C22:5 n-3 and C22:6 n-3) (Liland et al., 2017; Georgescu et al., 2022; Rodrigues et al., 2022). As previously pointed out by other authors (Oonincx et al., 2020), also in our study, the BSFL showed therefore a suboptimal n-6/n-3 PUFA ratio.

Conjugated linoleic acids

Similarly to what observed and previously discussed for GP, also BSFL contained CLA isomers (Table 4). Some authors already reported the presence of CLA in BSFL (Hoc et al., 2020; Tognocchi et al., 2023). Accepting the reasonable hypothesis of the presence of the stearoyl Co-A desaturase enzyme in BSFL, rumenic acid, which is the most abundant CLA isomer usually found in nature (Lee, 2008), could have been *de novo* biosynthesised using trans-vaccenic acid (C18:1 *t*11) as precursor, as demonstrated to occur in the mammary gland and adipose tissues of ruminant animals, as well as in humans (Niwińska, 2010). In this sense, the significantly higher levels of CLA *c*9*t*11 found in B-fed when compared to M−fed BSFL reflect the observed tendency towards higher C18:1 *t*11 levels in B-fed when compared to M−fed larvae. However, the same cannot be hypothesised for another CLA isomer (C18:2 *t*7*c*9), we detected in BSFL, as the biosynthesis of CLA *t*7*c*9 is predominantly mediated by the stearoyl Co-A desaturase enzyme (Corl et al., 2002) and no C18:1 *t*7 was found in BSFL (Table 4) or in GP (Table 2). Both CLA *c*9*t*11 and *t*7*c*9 may have been bioaccumulated in BSFL starting from the rearing media, as these CLA isomers were already found in GP, as previously discussed. However, when considering the bioaccumulation hypothesis, it should also be noted that the CLA content was slightly higher in the GP of M variety when compared to the B variety and therefore we could have expected a higher CLA content in M-fed than B-fed larvae, but the opposite was found, moreover with a conspicuous difference in the CLA amount between B-fed and M-fed larvae. In addition, the presence in BSFL of CLA isomers not found in GP (e.g., C18:2 *t*10*c*12) and the noticeable C18:2 *c*9*t*11 content (especially in B-fed BSFL) despite the low amounts found in GP of both varieties, lead us formulating additional hypotheses. Some authors reported the presence of *Lactobacillus* spp. in the gut of BSFL reared on different substrates (Jiang et al., 2019; Wynants et al., 2019). While analysing the correlation existing between intestinal microbial abundance and proteolytic enzyme activity in BSFL gut, Yu et al. (2023) were able to identify the presence of *L. plantarum* and *L. brevis.* As already mentioned, several lactic acid bacteria are capable of producing CLA using linoleic acid as precursor. In particular, CLA *c*9*t*11 is produced by a great number of *Lactobacillus* species, among which *L. plantarum, L. brevis, L. casei, L. rhamnosus, L. acidophilus, L. delbrueckii, L. reuteri,* and *L. curvatus* (Yang et al., 2014; Nasrollahzadeh et al., 2023). Considering the high linoleate isomerase activity found in numerous *Lactobacillus* strains (Yang et al., 2014), the greater C18:2 n-6 content in GP of B variety could be one of the reasons for the observed significantly higher CLA *c*9*t*11 levels found in B-fed when compared to M-fed BSFL. Another concomitant explanation could be the higher presence of *Lactobacilli* in the gut of B-fed BSFL, as a consequence of a higher presence of such bacteria in the GP of B variety (being a red grape variety). In fact, it has been recently shown that substrate-associated bacteria can dominate the BSFL gut microbiota (Schreven et al., 2022). Some *Lactobacillus* species, such as, among others, *L. plantarum*, are also able to synthesise CLA *t*10*c*12, again using linoleic acid as precursor (Yang et al., 2014; Sosa-Castañeda et al., 2015). This could explain the presence of this CLA isomer in the BSFL, even though it was not found in GP. Such a hypothesis seems also to be consistent with the significantly higher CLA *t*10*c*12 levels found in B-fed when compared to M-fed

larvae, given the greater C18:2 n-6 content in GP of B variety. However, no CLA *t*9*t*11 was found in BSFL, despite the presence of this CLA isomer in GP and despite the ability of linoleate isomerase to convert linoleic acid into CLA *t*9*t*11 (Yang et al., 2014). The latter result seems to support the previously mentioned hypothesis of the occurrence of undesirable isomerisations of CLA *c*9*t*11 to CLA *t*9*t*11 during the methylation of GP lipids.

Overall considering, besides the *de novo* synthesis mediated by stearoyl Co-A desaturase with trans-vaccenic acid as precursor, we may assume that, in BSFL, CLA *c*9*t*11 is also formed from the isomerisation of linoleic acid mediated by linoleate isomerases of bacterial origin. However, if this phenomenon was unrelated to a biohydrogenation process, we should have found appreciable amounts of CLA *c*9*t*11 in both B-fed and M-fed larvae, probably at different concentrations because the amount of linoleic acid in GP of B and M varieties was different (Table 2). However, in this trial, the difference in CLA *c*9*t*11 content in B-fed and M-fed larvae was unexpectedly high (137-fold higher content in B-fed larvae). As previously mentioned, Hoc et al. (2020) hypothesised the occurrence of the biohydrogenation process in the gastrointestinal tract of BSFL. The major biohydrogenation pathway of linoleic acid involves a first isomerisation to CLA *c*9*t*11, the latter being further saturated to form trans-vaccenic acid and, finally, stearic acid as the end product of the whole process (Dewanckele et al., 2020). From the results obtained in the current trial, it seems reasonable to hypothesise that the isomerisation of linoleic acid to CLA *c*9*t*11 is the start of a biohydrogenation process. Interestingly, we noticed that the amount of linoleic acid in GP of B variety was 1.1-fold greater than that found in GP of M variety, and such proportion remained practically unaltered in BSFL (1.2-fold greater linoleic acid content in B-fed than M-fed larvae). On the contrary, the amount of stearic acid was 1.4-fold higher in GP of B than M variety, but a reverse result was found in BSFL, with M-fed larvae showing a tendency towards significantly higher stearic acid content than B-fed larvae. We may therefore assume that linoleic acid was taken from the BSFL and subjected to a first isomerisation to CLA *c*9*t*11 with the same efficiency. In this sense, the significantly higher amount of CLA *c*9*t*11, the tendency towards a significantly higher amount of C18:1 *t*11 and the tendency towards a significantly lower amount of C18:0 found in B-fed than M-fed larvae could be the consequence of inhibition of the steps of the biohydrogenation pathway that lead to the formation of C18:1 *t*11 starting from CLA *c*9*t*11 and to the formation of C18:0 starting from C18:1 *t*11. Researchers focusing on ruminant nutrition have reported that polyphenols are able to inhibit the growth and development of rumen bacteria able to convert CLA *c*9*t*11 to C18:1 *t*11 and able to convert C18:1 *t*11 to C18:0 (Vasta et al., 2019). Assuming as reasonable the hypothesis of the occurrence of biohydrogenation in the gastrointestinal tract of BSFL, the slightly higher total extractable phenols (especially tannins) found in the GP of B variety when compared to the M variety could explain the observed variation in the contents of CLA *c*9*t*11, C18:1 *t*11 and C18:0 in B-fed and M-fed larvae. The biohydrogenation hypothesis would also explain why CLA *t*9*t*11, even if found in GP of both varieties, was not found in BSFL, as this isomer could have been simply hydrogenated to form C18:1 *t*9 and/or C18:1 *t*11 first, and lastly C18:0. Finally, the presence of CLA *t*10*c*12 in BSFL could also be explained if the biohydrogenation hypothesis would be correct. In fact, ruminant nutritionists have demonstrated that certain diets administered to ruminants (e.g., diets rich in rapidly fermentable carbohydrates, diets low in physically effective fibre, and/or diets supplemented with long-chain PUFA) can determine a shift of the "normal" biohydrogenation pathway of linoleic acid, leading to the formation of CLA *t*10*c*12 instead of CLA *c*9*t*11 during the first isomerisation step. In ruminant nutrition, the occurrence of this alternative biohydrogenation pathway has been studied in detail, as it is considered undesirable given the fact that CLA *t*10*c*12 and its hydrogenation product (C18:1 *t*10) have an inhibitory effect on milk fat synthesis, causing the so–called "diet-induced milk fat depression" (Dewanckele et al., 2020). In the current study, the most abundant detected CLA isomer in BSFL was by far CLA *c*9*t*11 (up to 2.74 g/100 g TL), while only small amounts of CLA *t*10*c*12 were detected (0.026 and 0.010 g/100 g TL in B-fed and M-fed larvae), with C18:1 *t*10 not being detected at all. This suggests that no major shifts of the "normal" biohydrogenation pathway of linoleic acid occurred. The biohydrogenation process occurring at the expense of unsaturated fatty acids within the rumen is mainly operated by bacteria as a detoxification mechanism (Dewanckele et al., 2020). Biohydrogenation is a process occurring in anaerobic environments (e.g., within the rumen); therefore, anaerobic bacteria are involved. The presence of anaerobic microbiota in BSFL seems to be possible. In fact, in BSFL fed on a mixture of empty fruit bunches and palm kernel meal pretreated by fermentation with the fungi *Bjerkandera adusta*, Klüber et al. (2022) observed a shift of the BSFL microbial gut towards a predominantly anaerobic community, reporting the presence of obligately anaerobic cellulolytic bacteria (i.e., *Ruminococcaceae* and *Lachnospiraceae*) which have been reported to be probably involved in the trans-11 ("normal") biohydrogenation pathway (Dewanckele et al., 2020). Interestingly, we noted that BSFL samples that contained high amounts of CLA also contained high amounts of trans-octadecenoic isomers, while three samples of M-fed BSFL in which no CLA isomers were detected, were devoid of trans-octadecenoic isomers. Such results seem to corroborate the hypothesis that biohydrogenation occurs in BSFL.

Conclusion

This study demonstrates that winery by-products from different grape varieties can significantly affect the development and lipid composition of BSFL. The growth performance of BSFL we obtained while rearing them on GP was lower if compared to that obtainable using other food waste or nutritionally balanced diets, as reported in published literature. However, using GP as rearing substrate could be of interest for the improvement of the fatty acid profile of BSFL, especially when considering fatty acids of potential health-promoting interest, such as CLA *c*9*t*11. In this regard, as outlined by the results obtained in our trial, noticeable variations should be expected based on the GP variety under consideration; this aspect should be further investigated using winery by-products from other red and white grape varieties. The possibility to include CLA-enriched full-fat BSFL meal or BSFL oil in the diet of monogastric animals, as recently authorised within the European Union (EU Regulations 2017/893 and 2021/1372), opens new perspectives to enhance the quality of animal-derived food products for human consumption. As mono-waste streams represent sub-optimal feeding conditions for the development and bioconversion ability of BSFL, future research should be directed to formulate rearing substrates containing GP mixed with other organic waste and agro-industrial by-products, with the aim of enhancing the efficiency of BSFL growth performance, at the same time improving the fatty acid profile of the BSFL, in a way that is exploitable at an industrial perspective. The addition of lactic acid bacteria strains to the rearing medium should also be investigated to favour CLA synthesis and bioaccumulation in BSFL. More information on the BSFL gut microbiome would be required to fully understand the role of bacteria in the synthesis of fatty acids characterised by potential health benefits in BSFL.

Ethics approval

Not applicable.

Data and model availability statement

None of the data or models are deposited in an official repository. The data presented in this article will be available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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References

Ageyeva, N.M., Tikhonova, A.N., Burtsev, B.V., Biryukova, S.A., Globa, E.V., 2021. Grape pomace treatment methods and their effects on storage. Foods and Raw Materials 9, 215–223. https://doi.org/10.21603/2308-4057-2021-2-215-223.
Ahmed, I.A.M., Özcan, M.M., Juhaimi, F.A., Babiker, E.F.E., Ghafoor, K., Banjanin, T., Osman, M.A., Gassem, M.A., Alqah, H.A.S., 2020. Chemical composition, bioactive compounds, mineral contents, and fatty acid composition of pomace powder of different grape varieties. Journal of Food Processing and Preservation 44, e14539.
Alves, S.P., Cabrita, A.R.J., Fonseca, A.J.M., Bessa, R.J.B., 2008. Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. Journal of Chromatography A 1209, 212–219. https://doi.org/10.1016/j.chroma.2008.08.114.
Antonić, B., Jančíková, S., Dordević, D., Tremlová, B., 2020. Grape pomace valorization: a systematic review and meta-analysis. Foods 9, 1627. https://doi.org/10.3390/foods9111627.
Association of Official Analytical Chemists (AOAC), 2000. Official methods of analysis, volume 1, 16th edition. AOAC, Gaithersburg, MD, USA.
Association of Official Analytical Chemists (AOAC), 2003. Official methods of analysis, volume 1, 17th edition. AOAC, Gaithersburg, MD, USA.
Baroi, A.M., Popitiu, M., Fierascu, I., Sărdărescu, I.D., Fierascu, R.C., 2022. Grapevine wastes: a rich source of antioxidants and other biologically active compounds. Antioxidants 11, 393. https://doi.org/10.3390/antiox11020393.
Barragan-Fonseca, K.B., Dicke, M., van Loon, J.J., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed–a review. Journal of Insects as Food and Feed 3, 105–120. https://doi.org/10.3920/JIFF2016.0055.
Barragan-Fonseca, K.B., Gort, G., Dicke, M., van Loon, J.J.A., 2021. Nutritional plasticity of the black soldier fly (*Hermetia illucens*) in response to artificial diets varying in protein and carbohydrate concentrations. Journal of Insects as Food and Feed 7, 51–61. https://doi.org/10.3920/JIFF2020.0034.
Bellezza Oddon, S., Biasato, I., Gasco, L., 2022a. Isoenergetic-practical and semi-purified diets for protein requirement determination in *Hermetia illucens* larvae: consequences on life history traits. Journal of Animal Science and Biotechnology 13, 1–10. https://doi.org/10.1186/s40104-021-00659-y.
Bellezza Oddon, S., Biasato, I., Resconi, A., Gasco, L., 2022b. Determination of lipid requirements in black soldier fly through semi-purified diets. Scientific Reports 12, 10922. https://doi.org/10.1038/s41598-022-14290-y.
Bellezza Oddon, S., Biasato, I., Resconi, A., Gasco, L., 2024. Black soldier fly life history traits can be influenced by isonutrient-waste-based diets. Italian Journal of Animal Science 23, 331–341. https://doi.org/10.1080/1828051X.2024.2316687.
Benito-Vicente, A., Jebari-Benslaiman, S., Galicia-Garcia, U., Larrea-Sebal, A., Uribe, K.B., Martin, C., 2021. Molecular mechanisms of lipotoxicity-induced pancreatic β-cell dysfunction. International Review of Cell and Molecular Biology 359, 357–402. https://doi.org/10.1016/bs.ircmb.2021.02.013.
Brizuela, N., Tymczyszyn, E.E., Semorile, L.C., La Hens, D.V., Delfederico, L., Hollmann, A., Bravo-Ferrada, B., 2019. *Lactobacillus plantarum* as a malolactic starter culture in winemaking: a new (old) player? Electronic Journal of Biotechnology 38, 10–18. https://doi.org/10.1016/j.ejbt.2018.12.002.
Chia, S.Y., Tanga, C.M., Osuga, I.M., Cheseto, X., Ekesi, S., Dicke, M., van Loon, J.J.A., 2020. Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products. Entomologia Experimentalis et Applicata 168, 472–481. https://doi.org/10.1111/eea.12940.
Conte, G., Serra, A., Casarosa, L., Ciucci, F., Cappucci, A., Bulleri, E., Corrales-Retana, L., Buccioni, A., Mele, M., 2019. Effect of linseed supplementation on total longissimus muscle lipid composition and shelf-life of beef from young Maremmana bulls. Frontiers in Veterinary Science 5, 326. https://doi.org/10.3389/fvets.2018.00326.
Corl, B.A., Baumgard, L.H., Griinari, J.M., Delmonte, P., Morehouse, K.M., Yurawecz, M.P., Bauman, D.E., 2002. *Trans*-7, *cis*-9 CLA is synthesized endogenously by Δ^9-desaturase in dairy cows. Lipids 37, 681–688. https://doi.org/10.1007/s11745-002-0949-4.
Cui, W., Xu, B., Chen, F., Shen, W., Wan, F., Cheng, A., 2024. Effects of grape peel phenolics on lipid accumulation in sodium palmitate-treated HepG2 cells. Journal of Functional Foods 112, 105923. https://doi.org/10.1016/j.jff.2023.105923.
Dabbou, S., Gai, F., Renna, M., Rotolo, L., Dabbou, S., Lussiana, C., Kovitvadhi, A., Brugiapaglia, A., De Marco, M., Helal, A.N., Zoccarato, I., Gasco, L., 2017. Inclusion of bilberry pomace in rabbit diets: effects on carcass characteristics and meat quality. Meat Science 124, 77–83. https://doi.org/10.1016/j.meatsci.2016.10.013.
den Hartigh, L.J., 2019. Conjugated linoleic acid effects on cancer, obesity, and atherosclerosis: a review of pre-clinical and human trials with current perspectives. Nutrients 11, 370. https://doi.org/10.3390/nu11020370.
Deng, Q., Penner, M.H., Zhao, Y., 2011. Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Research International 44, 2712–2720. https://doi.org/10.1016/j.foodres.2011.05.026.
Dewanckele, L., Toral, P.G., Vlaeminck, B., Fievez, V., 2020. Role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: an update. Journal of Dairy Science 103, 7655–7681. https://doi.org/10.3168/jds.2019-17662.
Eggink, K.M., Donoso, I.G., Dalsgaard, J., 2023. Optimal dietary protein to carbohydrate ratio for black soldier fly (Hermetia illucens) larvae. Journal of Insects as Food and Feed 9, 789–798. https://doi.org/10.3920/JIFF2022.0102.
Enjalbert, F., Combes, S., Zened, A., Meynadier, A., 2017. Rumen microbiota and dietary fat: a mutual shaping. Journal of Applied Microbiology 123, 782–797. https://doi.org/10.1111/jam.13501.
Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – Possibilities and limitations for modification through diet. Waste Management 102, 40–47. https://doi.org/10.1016/j.wasman.2019.10.014.
Fang, J., Zeng, L., He, Y., Liu, X., Zhang, T., Wang, Q., 2022. Effects of dietary tannic acid on obesity and gut microbiota in C57BL/6J mice fed with high-fat diet. Foods 11, 3325. https://doi.org/10.3390/foods11213325.

Fitriana, E.L., Laconi, E.B., Astuti, D.A., Jayanegara, A., 2022. Effects of various organic substrates on growth performance and nutrient composition of black soldier fly larvae: a meta-analysis. Bioresource Technology Reports 18, 101061. https://doi.org/10.1016/j.biteb.2022.101061.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 226, 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5.

Food and Agriculture Organization (FAO), 2021. UNEP Food Waste Index, Report 2021. United Nations Environment Programme, United Nations Avenue, Gigiri, Nairobi, Kenya. Retrieved on 18 January 2024 from https://wedocs.unep.org/bitstream/handle/20.500.11822/35280/FoodWaste.pdf.

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S., Colombini, S., 2021. Impact of agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the black soldier fly (Diptera: Stratiomyidae) larvae. Journal of Insect Science 21, 8. https://doi.org/10.1093/jisesa/ieaa148.

Gasco, L., Renna, M., Bellezza Oddon, S., Rezaei Far, A., Naser El Deen, S., Veldkamp, T., 2023. Insect meals in a circular economy and applications in monogastric diets. Animal Frontiers 13, 81–90. https://doi.org/10.1093/af/vfad016.

Georgescu, B., Boaru, A.M., Muntean, L., Sima, N., Struți, D.I., Păpuc, T.A., Georgescu, C., 2022. Modulating the fatty acid profiles of *Hermetia illucens* larvae fats by dietary enrichment with different oilseeds: a sustainable way for future use in feed and food. Insects 13, 801. https://doi.org/10.3390/insects13090801.

Giannetto, A., Oliva, S., Lanes, C.F.C., de Araújo Pedron, F., Savastano, D., Baviera, C., Parrino, V., Lo Paro, G., Spanò, N.C., Cappello, T., Maisano, M., Mauceri, A., Fasulo, S., 2020. *Hermetia illucens* (Diptera: Stratiomydae) larvae and prepupae: biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. Journal of Biotechnology 307, 44–54. https://doi.org/10.1016/j.jbiotec.2019.10.015.

Gil-Sánchez, I., Suáldea, B.B., Moreno-Arribas, M.V., 2019. Malolactic fermentation. In: Morata, A. (Ed.), Red Wine Technology. Academic Press, Elsevier, Amsterdam, The Netherlands, pp. 85–98. https://doi.org/10.1016/B978-0-12-814399-5.00006-2.

Gold, M., Fowles, T., Fernandez-Bayo, J.D., Miner, L.P., Zurbrügg, C., Nansen, C., Bischel, H.N., Mathys, A., 2022. Effects of rearing system and microbial inoculation on black soldier fly larvae growth and microbiota when reared on agri-food by-products. Journal of Insects as Food and Feed 8, 113–127. https://doi.org/10.3920/JIFF2021.0038.

Gorissen, L., Leroy, F., De Vuyst, L., De Smet, S., Raes, K., 2015. Bacterial production of conjugated linoleic and linolenic acid in foods: a technological challenge. Food Science and Nutrition 55, 1561–1574. https://doi.org/10.1080/10408398.2012.706243.

Gozdzik, P., Magkos, F., Sledzinski, T., Mika, A., 2023. Monomethyl branched-chain fatty acids: health effects and biological mechanisms. Progress in Lipid Research 90, 101226. https://doi.org/10.1016/j.plipres.2023.101226.

Guaita, M., Motta, S., Messina, S., Casini, F., Bosso, A., 2023. Polyphenolic profile and antioxidant activity of green extracts from grape pomace skins and seeds of Italian Cultivars. Foods 12, 3880. https://doi.org/10.3390/foods12203880.

He, Y., Xu, M., Liu, Y., Xu, Y.J., 2023. Association of branched-chain fatty acids with metabolic syndrome: a systematic review and meta-analysis of observational studies. Food & Function 14, 6312–6319. https://doi.org/10.1039/D3FO01320K.

Hoc, B., Genva, M., Fauconnier, M.L., Lognay, G., Francis, F., Caparros Megido, R., 2020. About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae. Scientific Reports 10, 1–8. https://doi.org/10.1038/s41598-020-68784-8.

Ianni, A., Martino, G., 2020. Dietary grape pomace supplementation in dairy cows: effect on nutritional quality of milk and its derived dairy products. Foods 9, 168. https://doi.org/10.3390/foods9020168.

International Organisation of Vine and Wine (OIV), 2023. State of the world vine and wine sector in 2022. Retrieved on 18 January 2024 from https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf.

Italian National Institute of Statistics (ISTAT), 2024. Crops: grapes, wine, olives, oil. Retrieved on 18 January 2024 from http://dati.istat.it/Index.aspx?QueryId=33706&lang=en#.

Iussig, G., Renna, M., Gorlier, A., Lonati, M., Lussiana, C., Battaglini, L.M., Lombardi, G., 2015. Browsing ratio, species intake, and milk fatty acid composition of goats foraging on alpine open grassland and grazable forestland. Small Ruminant Research 132, 12–24. https://doi.org/10.1016/j.smallrumres.2015.09.013.

Janssen, R.H., Vincken, J.-P., Van den Broek, L.A.M., Fogliano, V., Lakemond, C.M.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. Journal of Agricultural and Food Chemistry 65, 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471.

Jiang, C.L., Jin, W.Z., Tao, X.H., Zhang, Q., Zhu, J., Feng, S.Y., Xu, X.H., Li, H.Y., Wang, Z.H., Zhang, Z.J., 2019. Black soldier fly larvae (*Hermetia illucens*) strengthen the metabolic function of food waste biodegradation by gut microbiome. Microbial Biotechnology 12, 528–543. https://doi.org/10.1111/1751-7915.13393.

Kaya, C., Generalovic, T.N., Ståhls, G., Hauser, M., Samayoa, A.C., Nunes-Silva, C.G., Roxburgh, H., Wohlfahrt, J., Ewusie, E.A., Kenis, M., Hanboonsong, Y., Orozco, J., Carrejo, N., Nakamura, S., Gasco, L., Rojo, S., Tanga, C.M., Meier, R., Rhode, C., Picard, C.J., Jiggins, C.D., Leiber, F., Tomberlin, J.K., Hasselmann, M., Blanckenhorn, W.U., Kapun, M., Sandrock, C., 2021. Global population genetic structure and demographic trajectories of the black soldier fly. *Hermetia Illucens*. BMC Biology 19, 94. https://doi.org/10.1186/s12915-021-01029-w.

Kim, Y.B., Kim, D.H., Jeong, S.B., Lee, J.W., Kim, T.H., Lee, H.G., Lee, K.W., 2020. Black soldier fly larvae oil as an alternative fat source in broiler nutrition. Poultry Science 99, 3133–3143. https://doi.org/10.1016/j.psj.2020.01.018.

Kishino, S., Ogawa, J., Ando, A., Iwashita, T., Fujita, T., Kawashima, H., Shimizu, S., 2003. Structural analysis of conjugated linoleic acid produced by *Lactobacillus plantarum*, and factors affecting isomer production. Biosciences, Biotechnology and Biochemistry 67, 179–182. https://doi.org/10.1271/bbb.67.179.

Klüber, P., Tegtmeier, D., Hurka, S., Pfeiffer, J., Vilcinskas, A., Rühl, M., Zorn, H., 2022. Diet fermentation leads to microbial adaptation in black soldier fly (*Hermetia illucens*; Linnaeus, 1758) larvae reared on palm oil side streams. Sustainability 14, 5626. https://doi.org/10.3390/su14095626.

Kolláthová, R., Hanušovský, O., Gálik, B., Bíro, D., Šimko, M., Juráček, M., Gierus, M., 2020. Fatty acid profile analysis of grape by-products from Slovakia and Austria. Acta Fytotechnica et Zootechnica 23, 78–84. https://doi.org/10.15414/afz.2020.23.02.78-84.

Kramer, J.K.G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.M., Yurawecz, M.P., 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 32, 1219–1228. https://doi.org/10.1007/s11745-997-0156-3.

Kramer, J.K.G., Cruz-Hernandez, C., Deng, Z.Y., Zhou, J.Q., Jahreis, G., Dugan, M.E.R., 2004. Analysis of conjugated linoleic acid and *trans* 18:1 isomers in synthetic and animal products. The American Journal of Clinical Nutrition 79, 1137S–1145S. https://doi.org/10.1093/ajcn/79.6.1137S.

Lee, Y., 2008. Isomer specificity of conjugated linoleic acid (CLA): 9*E*, 11*E*-CLA. Nutrition Research and Practice 2, 326–330. https://doi.org/10.4162/nrp.2008.2.4.326.

Li, X., Dong, Y., Sun, Q., Tan, X., You, C., Huang, Y., Zhou, M., 2022. Growth and fatty acid composition of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) larvae are influenced by dietary fat sources and levels. Animals 12, 486. https://doi.org/10.3390/ani12040486.

Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E., Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PloS ONE 12, e0183188.

Liu, Z., Ezernieks, V., Rochfort, S., Cocks, B., 2018. Comparison of methylation methods for fatty acid analysis of milk fat. Food Chemistry 261, 210–215. https://doi.org/10.1016/j.foodchem.2018.04.053.

Liu, Y., Liu, J., He, J., Lu, H., Sun, S., Ji, F., Dong, X., Bao, Y., Xu, J., He, G., Xu, W., 2023. Chronological and carbohydrate-dependent transformation of fatty acids in the larvae of black soldier fly following food waste treatment. Molecules 28, 1903. https://doi.org/10.3390/molecules28041903.

Liu, C., Wang, C., Yao, H., 2019. Comprehensive resource utilization of waste using the black soldier fly (Hermetia illucens (L.)) (Diptera: Stratiomyidae). Animals 9, 349. https://doi.org/10.3390/ani9060349.

Ma, J., Zheng, Y., Tang, W., Yan, W., Nie, H., Fang, J., Liu, G., 2020. Dietary polyphenols in lipid metabolism: a role of gut microbiome. Animal Nutrition 6, 404–409. https://doi.org/10.1016/j.aninu.2020.08.002.

Mele, M., Serra, A., Pauselli, M., Luciano, G., Lanza, M., Pennisi, P., Conte, G., Taticchi, A., Esposto, S., Morbidino, L., 2014. The use of stoned olive cake and rolled linseed in the diet of intensively reared lambs: effect on the intramuscular fatty-acid composition. Animal 8, 152–162. https://doi.org/10.1017/S1751731113001924.

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. Journal of the Science of Food and Agriculture 98, 5776–5784. https://doi.org/10.1002/jsfa.9127.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 85, 1217–1240.

Ministero dell'Agricoltura, della Sovranità Alimentare e delle Foreste (MASAF), 2023. National Catalogue of Grape Varieties. [Catalogo nazionale delle varietà di vite]. Retrieved on 4 March 2024 from http://catalogoviti.politicheagricole.it.

Nakov, G., Ivanova, S., Lazova-Borisova, I., Temkov, M., 2022. Identification of fatty acids in grape and tomato pomace–sustainable valorisation of agricultural waste. Scientific Papers: Management, Economic Engineering in Agriculture and Rural Development 22, 431–436.

Nasrollahzadeh, A., Tavani, S.M., Arjeh, E., Jafari, S.M., 2023. Production of conjugated linoleic acid by lactic acid bacteria; important factors and optimum conditions. Food Chemistry: X 20, 100942. https://doi.org/10.1016/j.fochx.2023.100942.

Niwińska, B., 2010. Endogenous synthesis of rumenic acid in humans and cattle. Journal of Animal and Feed Sciences 19, 171–182. https://doi.org/10.22358/jafs/66279/2010.

Oonincx, D.G., Laurent, S., Veenenbos, M.E., van Loon, J.J., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Insect Science 27, 500–509. https://doi.org/10.1111/1744-7917.12669.

Pliantiangtam, N., Chundang, P., Kovitvadhi, A., 2021. Growth performance, waste reduction efficiency and nutritional composition of black soldier fly (*Hermetia illucens*) larvae and prepupae reared on coconut endosperm and soybean curd residue with or without supplementation. Insects 12, 682. https://doi.org/10.3390/insects12080682.

Ran-Ressler, R.R., Bae, S., Lawrence, P., Wang, D.H., Brenna, J.T., 2014. Branched-chain fatty acid content of foods and estimated intake in the USA. British Journal of Nutrition 112, 565–572. https://doi.org/10.1017/S0007114514001081.

Rebora, M., Salerno, G., Piersanti, S., Saitta, V., Morelli Venturi, D., Li, C., Gorb, S., 2023. The armoured cuticle of the black soldier fly *Hermetia illucens*. Scientific Reports 13, 22101. https://doi.org/10.1038/s41598-023-49549-5.

Renna, M., Collomb, M., Münger, A., Wyss, U., 2010. Influence of low-level supplementation of grazing dairy cows with cereals or sugar beet pulp on the concentrations of CLA isomers in milk. Journal of the Science of Food and Agriculture 90, 1256–1267. https://doi.org/10.1002/jsfa.3968.

Renna, M., Gasmi-Boubaker, A., Lussiana, C., Battaglini, L.M., Belfayez, K., Fortina, R., 2014. Fatty acid composition of the seed oils of selected *Vicia* L. taxa from Tunisia. Italian Journal of Animal Science 13, 2. https://doi.org/10.4081/ijas.2014.3193.

Renna, M., Coppa, M., Lussiana, C., Le Morvan, A., Gasco, L., Maxin, G., 2022. Full-fat insect meals in ruminant nutrition: in vitro rumen fermentation characteristics and lipid biohydrogenation. Journal of Animal Science and Biotechnology 13, 138. https://doi.org/10.1186/s40104-022-00792-2.

Renna, M., Martínez-Marín, A.L., Lussiana, C., Colonna, L., Mimosi, A., Cornale, P., 2023. Caprine milk fatty acid responses to dietary dried grape pomace. Italian Journal of Animal Science 22, 1186–1194. https://doi.org/10.1080/1828051X.2023.2276259.

Ribeiro, N., Costa, R., Ameixa, O.M.C.C., 2022. The influence of non-optimal rearing conditions and substrates on the performance of the black soldier fly (*Hermetia illucens*). Insects 13, 639. https://doi.org/10.3390/insects13070639.

Rodrigues, D.P., Ameixa, O.M., Vázquez, J.A., Calado, R., 2022. Improving the lipid profile of black soldier fly (*Hermetia illucens*) larvae for marine aquafeeds: current state of knowledge. Sustainability 14, 6472. https://doi.org/10.3390/su14116472.

Rodriguez-Estrada, M.T., Penazzi, G., Caboni, M.T., Bertacco, G., Lercker, G., 1997. Effect of different cooking methods on some lipid and protein components of hamburgers. Meat Science 45, 365–375. https://doi.org/10.1016/S0309-1740(96)00123-4.

Schreven, S.J., de Vries, H., Hermes, G.D., Zeni, G., Smidt, H., Dicke, M., Van Loon, J.J., 2022. Black soldier fly larvae influence internal and substrate bacterial community composition depending on substrate type and larval density. Applied and Environmental Microbiology 88, e00084–e00122. https://doi.org/10.1128/aem.00084-22.

Seyedalmoosavi, M.M., Mielenz, M., Veldkamp, T., Daş, G., Metges, C.C., 2022. Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (*Hermetia illucens*) larvae compared to monogastric livestock species: a review. Journal of Animal Science and Biotechnology 13, 31. https://doi.org/10.1186/s40104-022-00682-7.

Siddiqui, S.A., Süfer, Ö., Koç, G.Ç., Lutuf, H., Rahayu, T., Castro Muñoz, R., Fernando, I., 2024. Enhancing the bioconversion rate and end products of black soldier fly (BSF) treatment – a comprehensive review. Environment, Development and Sustainability, https://doi.org/10.1007/s10668-023-04306-6, Published online by Springer 04 January 2024.

Singh, A., Marathe, D., Raghunathan, K., Kumari, K., 2022. Effect of different organic substrates on selected life history traits and nutritional composition of black soldier fly (*Hermetia illucens*). Environmental Entomology 51, 182–189. https://doi.org/10.1093/ee/nvab135.

Sinrod, A.J.G., Shah, I.M., Surek, E., Barile, D., 2023. Uncovering the promising role of grape pomace as a modulator of the gut microbiome: an in-depth review. Heliyon 9, e20499.

Sirohi, R., Tarafdar, A., Singh, S., Negi, T., Gaur, V.K., Gnansounou, E., Bharathiraja, B., 2020. Green processing and biotechnological potential of grape pomace: current trends and opportunities for sustainable biorefinery. Bioresource Technology 314, 123771. https://doi.org/10.1016/j.biortech.2020.123771.

Sosa-Castañeda, J., Hernández-Mendoza, A., Astiazarán-García, H., Garcia, H.S., Estrada-Montoya, M.C., González-Córdova, A.F., Vallejo-Cordoba, B., 2015. Screening of *Lactobacillus* strains for their ability to produce conjugated linoleic acid in milk and to adhere to the intestinal tract. Journal of Dairy Science 98, 6651–6659. https://doi.org/10.3168/jds.2014-8515.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., De Smet, S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture 97, 2594–2600. https://doi.org/10.1002/jsfa.8081.

Suryati, T., Julaeha, E., Farabi, K., Ambarsari, H., Hidayat, A.T., 2023. Lauric acid from the black soldier fly (*Hermetia illucens*) and its potential applications. Sustainability 15, 10383. https://doi.org/10.3390/su151310383.

Tognocchi, M., Conte, G., Rossi, E., Perioli, R., Mantino, A., Serra, A., Mele, M., 2023. Characterization of polar and non-polar lipids of *Hermetia illucens* and *Tenebrio molitor* meals as animal feed ingredients. Animal Feed Science and Technology 295, 115524. https://doi.org/10.1016/j.anifeedsci.2022.115524.

Tomberlin, J.K., Sheppard, D.C., Joyce, J.A., 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95, 379–386. https://doi.org/10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2.

Vasta, V., Daghio, M., Cappucci, A., Buccioni, A., Serra, A., Viti, C., Mele, M., 2019. Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: experimental evidence and methodological approaches. Journal of Dairy Science 102, 3781–3804. https://doi.org/10.3168/jds.2018-14985.

Virdis, C., Sumby, K., Bartowsky, E., Jiranek, V., 2021. Lactic acid bacteria in wine: technological advances and evaluation of their functional role. Frontiers in Microbiology 11, 612118. https://doi.org/10.3389/fmicb.2020.612118.

Vlaeminck, B., Fievez, V., Cabrita, A.R.J., Fonseca, A.J.M., Dewhurst, R.J., 2006. Factors affecting odd-and branched-chain fatty acids in milk: a review. Animal Feed Science and Technology 131, 389–417. https://doi.org/10.1016/j.anifeedsci.2006.06.017.

Wolff, R.L., Bayard, C.C., 1995. Fatty acid composition of some pine seed oils. Journal of the American Oil Chemists' Society 72, 1043–1046. https://doi.org/10.1007/BF02660719.

Wolfram, S., Raederstorff, D., Wang, Y., Teixeira, S.R., Elste, V., Weber, P., 2005. Teavigo™ (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. Annals of Nutrition and Metabolism 49, 54–63. https://doi.org/10.1159/000084178.

Wynants, E., Frooninckx, L., Crauwels, S., Verreth, C., De Smet, J., Sandrock, C., Wohlfahrt, J., Van Schelt, J., Depraetere, S., Lievens, B., Van Miert, S., Claes, J., Van Campenhout, L., 2019. Assessing the microbiota of black soldier fly larvae (*Hermetia illucens*) reared on organic waste streams on four different locations at laboratory and large scale. Microbial Ecology 77, 913–930. https://doi.org/10.1007/s00248-018-1286-x.

Yang, B., Chen, H., Gu, Z., Tian, F., Ross, R.P., Stanton, C., Chen, Y.Q., Chen, W., Zhang, H., 2014. Synthesis of conjugated linoleic acid by the linoleate isomerase complex in food-derived lactobacilli. Journal of Applied Microbiology 117, 430–439. https://doi.org/10.1111/jam.12524.

Yi, C., Shi, J., Kramer, J., Xue, S., Jiang, Y., Zhang, M., Ma, Y., Pohorly, J., 2009. Fatty acid composition and phenolic antioxidants of winemaking pomace powder. Food Chemistry 114, 570–576. https://doi.org/10.1016/j.foodchem.2008.09.103.

Yu, Y., Zhang, J., Zhu, F., Fan, M., Zheng, J., Cai, M., Zheng, L., Huang, F., Yu, Z., Zhang, J., 2023. Enhanced protein degradation by black soldier fly larvae (*Hermetia illucens* L.) and its gut microbes. Frontiers in Microbiology 13, 1095025. https://doi.org/10.3389/fmicb.2022.1095025.