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RESEARCH ARTICLE

Effect of seasonality and pretreatment of the organic fraction of municipal solid waste for black soldier fly larvae production

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Abstract

This study investigated the potential of using the organic fraction of municipal solid waste (OFMSW) as a rearing substrate for black soldier fly larvae (BSF). Samples of OFSMW were collected in Italy in four different seasons of the year and fed untreated or pulped to the larvae. The larvae successfully grew on OFMSW with minimal impact from either season or pretreatment (pulped vs untreated). Notably, all treatments achieved high larval survival rates (above 89.2%) and a development time within 11 days. Season and pretreatment influenced the chemical composition of the OFMSW (e.g. higher carbohydrates and organic matter in untreated samples). Compared to BSF grown on pulped samples, larvae grown on untreated samples had higher lipid (34.4 vs 28.5% DM), organic matter (90.7 vs 83.6% DM) and apparent metabolizable energy (22.0 vs 15.3 MJ/kg DM) contents. *In vitro* protein digestibility (%) varied by season, reaching the highest values in autumn (78.6%); moreover, the crude protein digestibily of BSF reared on untreated samples was lower than that on pulped samples (68.8 vs 73.3%). The chitin content was not affected by pretreatment or season (on average 5.45% on DM). Overall, the study demonstrates the feasibility of the use of OFMSW for BSF larvae production. Nevertheless, observed variability in larval nutrient composition highlights the need for process optimization to ensure consistent larval characteristics.

Keywords

Hermetia illucens – *in vitro* digestibility – larval biomass – protein production – waste valorization

1 Introduction

The global demand for protein supply for livestock farming and aquaculture is increasing to satisfy the request of the world population growth; livestock production, in particular, provides 33% of the total protein and 17% of the total calories of human diet (Cheng *et al.*, 2022). Every year in the world 73 billion chickens (122 million tons), 1.4 billion pigs (120 million tons), and 332 million cattle (72 million tons) are slaughtered; aquaculture production provided 88 million tons of fishery products (FAO, 2023).

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In the EU the compound feed production for livestock farming in 2021 reached 150 million tons; considering a production of 23.4 and 12.2 million tons of pig and poultry meat, pig and poultry feed production represents about two-thirds of the industrial compound feed production (FEFAC, 2022). The diet of monogastric animals is based on protein feed, mainly soybean meal, which have an important environmental impact. Looking for alternative and sustainable protein sources, insects have been identified as a valuable substitute as shown by the increasing market in recent years (IPIFF, 2021). Moreover, authorization by the EU for their use in aquaculture and the recent authorization of insect-derived proteins in the farming of swine and poultry (Commission Regulation, 2021) contributed to an increase in the mass-rearing insect sector (Pippinato *et al.*, 2020). Numerous studies highlighted that insects can be incorporated into livestock diets without showing negative effects on animal performance and product quality (Biasato *et al.*, 2022; Dabbou *et al.*, 2018; Lu *et al.*, 2022). Among different insect species, *Hermetia illucens* L. (Diptera Stratiomyidae), commonly known as the black soldier fly (BSF), is particularly noteworthy. Saprophagous larvae are extremely voracious and can be reared on a broad range of waste and by-products including vegetable and restaurant waste, agro-industry by-products (Galassi *et al.*, 2021; Jucker *et al.*, 2016) and crop straw (Liu *et al.*, 2019; Manurung *et al.*, 2016). Larvae are able to convert in a short period of time a large quantity of organic matters into biomass, particularly high in proteins and lipids (Franco *et al.*, 2021; Giannetto *et al.*, 2020; Shelomi, 2020). Thus, several low-value wastes can be valorized through their conversion into higher protein and lipid biomass products. Depending on the composition of the growing waste, BSF larvae can reduce the quantity of the substrate up to more than 60% contributing to manage wastes (Surendra *et al.*, 2020). Finally, insect rearing residues can be valorized through their use as organic fertilizer in agricultural practices (Poveda, 2021; Schmitt and de Vries, 2020).

Identifying substrates for mass production of insects is the aim of much researches. In fact, large production of insects requires the use of low-cost foodstuffs, such as by-products, to be sustainable both economically and environmentally (Smetana *et al.*, 2016). A widely available substrate which could be employed for this purpose is represented by the municipal solid waste (MSW); the interest for this matrix is that its production is high (about 1.3 billion ton/year), and it is expected to increase in the next years (about 2.3 billion ton by 2025) (Tyagi *et al.*, 2018). Organic waste represents about 50% of the total municipal waste and generally is a mixture of wastes from pruning residues from parks, and kitchen waste, so its composition can vary widely (Tyagi *et al.*, 2018; Zamri *et al.*, 2021). In their review, Campuzano and González-Martínez (2016) showed that some parameters varied little among the organic fraction of the municipal solid waste (OFMSW) considered (e.g. moisture, carbon, hydrogen, starch and VS/TS ratio), while others showed a higher variability (phosphorus, sulphur, hemicellulose, nitrogen, free sugars, lignin and crude fibre). These variations can probably be attributed not only to the country, the waste management system and the harvesting system, but also to climate, food type and seasonality (Pecorini *et al.*, 2020).

Today, OFMSW produced in Italy is estimated at about 6 Mtons/y (ISPRA, 2017). This large quantity of waste still represents a source of nutrients that can be valorized and contribute to the bioenergy potential; OFMSW mainly contains carbohydrates (30-69%), lipids (10-40%) and proteins (5-17%). Their percentages can vary due to numerous factors, thus influencing the bioenergy potential (Campuzano and Gonzàlez-Martìnez, 2016; Pleissner and Lin, 2013). Anaerobic digestion has been identified as the most promising technology for the treatment of OFSMW recovering energy and nutrients that can be re-used as biogas production and compost for agriculture, particularly rich in nitrogen (Campuzano and Gonzàlez-Martìnez, 2016; Zamri *et al.*, 2021). However, the management of OFMSW with anaerobic digestion does not achieve complete economic viability without financial subsidies, due to the low value of its end products, such as compost and biogas (Horschig *et al.*, 2018).

Bioconversion of OFMSW with BSF larvae can lead to high value-added products (Amrul *et al.*, 2022; Surendra *et al.*, 2020), but, as the composition of OFMSW varies according to the season (Campuzano and Gonzàlez-Martìnez, 2016), this could lead to differences in larval growth and final larval biomass composition. Moreover, also OFMSW mechanical pretreatments, such as the pulping process considered in this work, can influence insect growing performance and bioconversion efficiency. The pretreatment of the substrate, already used for different types of waste such as manure, solid urban waste and agricultural residues (Carrère *et al.*, 2016), allows to increase the surface area and bulk density of waste, thus promoting the activity of degradation by microorganisms, in particular of lignocellulosic fibers. In the case of BSF larvae, some authors (Peguero *et al.*, 2024; Yakti *et al.*, 2023) showed that mechanical pretreatment of the rearing substrate can lead to improved growth performance and bioconversion efficiency, although it depends on the type of substrate. Indeed, the grinding of the substrate can influence the degradation activity of microorganisms, but it can also affect the oxygen availability in the substrate itself (Yakti *et al.*, 2023).

Overall, for a better use of insects in livestock diets it is important to evaluate their nutritive value and digestibility. The nutritional value of diets could be determined by i*n vivo* digestibility trials, however time and cost negatively affected the application of this technique; *in vitro* digestibility trials (reproducible, simple, and cheap technique), instead, allow to estimate the digestibility of different feeds using the same experimental condition (Yegani *et al.*, 2013).

In the present study, the suitability of OFMSW as growing substrates for BSF larvae was assessed. In particular, the influence of OFMSW, collected in different seasons of the year and used untreated or pulped, was evaluated on both the growth performance and the biotransformation ability of the larvae.

In addition, chemical composition and *in vitro* feed digestibility of larvae were assessed to better understand the relationship between OFMSW and the quality of produced larvae.

2 Materials and methods

Substrates

As described in Papa *et al.* (2022), OFMSW samples were collected in Lombardy Region (Northern Italy) from two full-scale anaerobic digestion treatment plants, in four different periods of the year, i.e. summer, autumn, winter and spring. The OFMSW was pretreated with a pulper and processed into a pulpable puree. In particular, the OFMSW was collected from the feeding pipeline and sujected to a mechanical pulping by a pulper prior to anaerobic digestion to reach homogenization and consistent particle size distribution. Figure 1 reports the substrates before and after the mechanical pretreatment. Samples of untreated OFMSW (U) and samples pretreated with biopulper (P) were collected and then placed into 250 g zip-lock plastic bags and stored in the freezer at –20 °C until used for experimental trials and analysis. In Table 1 is reported the mean composition of the collected wastes.

Insect colony and larval production

Black soldier fly larvae used in the study are bred in the rearing system at the Department of Food, Environmental and Nutritional Science (DeFENS) of the University of Milan, Italy (Jucker *et al.*, 2017, 2020).

Four replicates were set up for each experimental substrate collected as previously described, using 500 five-day-old larvae per replicate. Larvae were manually counted with the help of tweezers and counter and put into a plastic container (21 cm × 27 cm × 14 cm) covered with a fine mesh to guarantee air exchange while avoiding the larvae from escaping. Initial larval biomass weight in each replicate was recorded. Larval growth was performed in a climate chamber at constant temperature condition (26 ± 1 °C), constant relative humidity (60 ± 5%) and constant photoperiod (12 h:12 h light:dark). The substrates were supplied to the larvae using a feeding rate of 124 mg $larvae^{-1}d^{-1}$ based on preliminary evaluations, avoiding starvation. Three times per week, three samples constituted by a subset of 20 larvae randomly collected from each replicate, were weighted on an analytical balance (SartoriusCP64, Göttingen, Germany). Following Bosch *et al.* (2020), when 5% of the larvae turned from cream to darker colour corresponding to the switch in the pre-pupal stage, the experimental trials were stopped. At that time, larvae were separated from the residue, counted, washed with tap water and gently dried with absorbent paper. The total weight of the final larval biomass was registered; then the larvae were frozen at –18 °C and kept for further analyses. The final residue (larval frass + spent waste) was also weighted (fresh weight); then it was dried at 105 °C for 24 h to determine dry mass and stored at –18 °C for subsequent chemical analyses.

To evaluate the growing performance, the following parameters were considered: larval survival (%), developmental time (d), and final larval dry weight (g). The larval survival was obtained dividing the number of larvae at collection time (5% prepupae) by 500 (the number of larvae at the start of the trial) *100. Developmental time (d) was considered from the start of the trial (d_0 = start of the trial) and corresponded to five-days-old larvae.

Finally, the bioconversion ability of the larvae was evaluated by calculating the following parameters (on a dry weight basis): substrate reduction (SR) (Equation 1), the efficiency of conversion of ingested feed (ECI) (Equation 2), the waste reduction index (WRI) (Equation 3) as in Diener *et al.* (2009) and the nitrogen conversion ratio (NCR) as in Bosh *et al.* (2019):

$$SR = (W - R)/W * 100 \tag{1}$$

$$ECI = B/(W - R) \tag{2}$$

FIGURE 1 Samples of OFMSW untreated (left, Untreated, U) and after pretreatment with biopulper (right, pulped, P).

TABLE 1 Chemical composition of the rearing substrates before the rearing of BSF

		DM (% FW)	Ash (% DM)	NDF	ADL	NFC	EE	CP
Pretreatment	U	28.2	12.1[b]	34.8[a]	4.81	59.0[a]	14.1	14.9
	P	27.9	18.8[a]	29.5[b]	5.80	54.0[b]	12.6	14.6
SE		1.17	1.36	9.84	4.17	8.55	7.21	9.84
P		0.856	0.011	0.007	0.138	0.005	0.202	0.863
Season	Summer	26.9	15.5	36.1[a]	3.70[b]	58.0[a]	11.3[b]	15.3
	Autumn	28.5	13.5	30.4[bc]	7.98[a]	60.4[a]	11.8[b]	14.3
	Winter	28.6	20.3	33.8[ab]	4.60[b]	50.0[b]	14.5[ab]	15.3
	Spring	28.3	12.5	28.3[c]	4.95[b]	56.7[a]	15.7[a]	14.1
SE		1.65	1.93	1.39	0.59	1.21	1.02	1.39
P		0.869	0.093	0.022	0.007	0.002	0.050	0.898

DM = dry matter, NDF = neutral detergent fiber, ADL = acid detergent lignin, NFC = non-fibrous carbohydrates, EE = ether extract, CP = crude protein.
U = untreated organic fraction of municipal solid waste, P = pulped organic fraction of municipal solid waste.
SE = Standard error.
[a,b,c] Means in the same column with different superscript letters differ ($P < 0.05$).

$$\text{WRI} = ((\text{W} - \text{R})/\text{W})/\text{days of trial (d)} \times 100 \qquad (3)$$

$$\text{NCR} = \text{B} - \text{N}/\text{N-diet}$$

where, W is the total amount of diet provided and R is the residue of the diet; B is the total larval +prepupal biomass (g) and B-N is the nitrogen content (g) of the collected larval biomass over the experiment and N- diet is the dietary nitrogen supplied over the experiment (g).

Substrates and larval chemical analyses

The substrates and the larvae were analysed for chemical composition using the following methods: dry matter content (DM) and ash were determined using 945.15 and 942.05 AOAC methods (AOAC International, 1995). The ether extract content (EE) was determined using 920.29 (AOAC International, 1995). Dumas method was used to determine crude protein (CP) (N × 6.12) content using MAX N exceed (Elementar Analysensystem GmbH, Langenselbold, Germany). The fatty acid (FA) composition of the larvae was done using a modified Bligh and Dyer method (Bligh and Dyer, 1959) as reported by Su *et al.* (2022). The analyses were performed using a polar capillary column ZB-FAME (Zebron, Phenomenex, Castel Maggiore, Italy) of 30 m ×

0.25 mm (ID) and a film thickness of 0.20 μm and He as carrier gas.

The chitin content in the larvae was also calculated according to Finke (2007) considering the ADF (acid detergent fibre) and the value of protein linked to acid detergent fibre (ADIP) as follows (Equation 4):

$$\text{Chitin}(\%\text{DM}) = \text{ash free ADF}(\%) - \text{ADIP}(\%) \tag{4}$$

The ADF content corrected for insoluble ash was also determined (Van Soest *et al.* 1991). The NDF (Neutral Detergent Fiber; Mertens, 2002), corrected for insoluble ash and with the addition of α-amylas (aNDFom), and ADL (acid detergent lignin) (Van Soest *et al.*, 1991) of substrates were established using the Ankom 200 fibre apparatus (Ankom Technology Corp., Fairport, NY, USA). The NFC (non-fibrous carbohydrates) were calculated as follow (Equation 5):

$$\text{NFC} = 100 - (\text{EE} + \text{CP} + \text{NDF} + \text{Ash}) \tag{5}$$

Gross energy (GE) of larvae was evaluated using an adiabatic bomb calorimeter (IKA 4000; IKA Werke GmbH & Co. KG, Staufen, Germany).

***Digestibility* in vitro**

The apparent crude protein digestibility for monogastric (DIG CP) was determined used the enzymatic method proposed by Boisen and Fernández (1997), which consistes of three different phases (gastric and small and large intestine digestion). More details about the analysis are reported in Galassi *et al.* (2021).

The energy digestibility was determined *in vitro* according to Yegani *et al.* (2013). The *in vitro* digestibility of gross energy was implemented in the following equation to estimate the *in vivo* AME (Apparent metabolizable energy) of test samples (Equation 6) (Yegani *et al.*, 2013):

$$\begin{aligned} &\textit{In vivo}\ \text{AME (MJ/kg of DM)} \\ &\quad = \textit{in vitro}\ \text{digestibility of GE of test sample} \\ &\qquad \times\ \text{GE of test sample} \end{aligned} \tag{6}$$

Statistical analyses

Data on larval growth (number of days, survival, larval yield), bioconversion efficiency, chemical composition of substrates and larvae were analyzed by SAS software ver. 9.4 (SAS Institute, 2013) by GLM procedure consider the fixed effect of pretreatment (n = 2), the fixed effect of season (n = 4), the fixed effect of production site (n = 2) and the interaction season*pretreatment (n = 16). Data are reported as LS-MEANS; significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

3 Results

Chemical analyses of the substrates

The chemical characteristics of the rearing substrates provided to BSF larvae are shown in Table 1. No significant interaction was observed between pretreatment and season; for this reason, the data are presented without interaction. The results were significantly influenced by the type of pretreatment (U vs P) and by the season. Regarding the pretreatment, NDF and NFC ($P = 0.007$, $P = 0.005$) were higher in untreated than pulped samples ($P = 0.007$ and $P = 0.005$, respectively, for NDF and NFC) whereas ash had the opposite trend (higher in P and lower in U; $P = 0.01$). Considering the season effect, a lower level of NDF was registered in spring although not different than autumn, while a higher content of ADL was determined in autumn; a lower NFC content was found in winter in comparison with the other seasons ($P = 0.002$). The ether extract was also significantly affected by the season ($P = 0.05$) with higher values in winter and spring (14.5% and 15.7%) than those registered in summer and autumn (11.3% and 11.8%).

In Figure 2, it is reported the average values (by season and pretreatment) of chemical composition of the substrates considering the effect of the time (before the larval rearing and after the larval rearing). NDF significantly decreased during larval growth ($P = 0.001$), while ADL remained unchanged. Moreover, there was a significantly reduction in NFC, EE and CP ($P < 0.001$), indicating a high palatability of these components. On the other hand, dry matter increased ($P < 0.001$) and this can be explained by natural drying of substrate, to the losses of some volatile compounds as consequence of the temperature used for the rearing of the larvae, and for the activity of microbial population usually present in the substrate.

Influence of seasonality and OFMSW pretreatment on larval growth

Figures 3 and 4 show the larval growth trend on the OFMSW collected in the four seasons and reared on the untreated and pulped waste. Larvae could grow on all the experimental substrates with few differences due to season or pretreatment. The larval growth curves shared

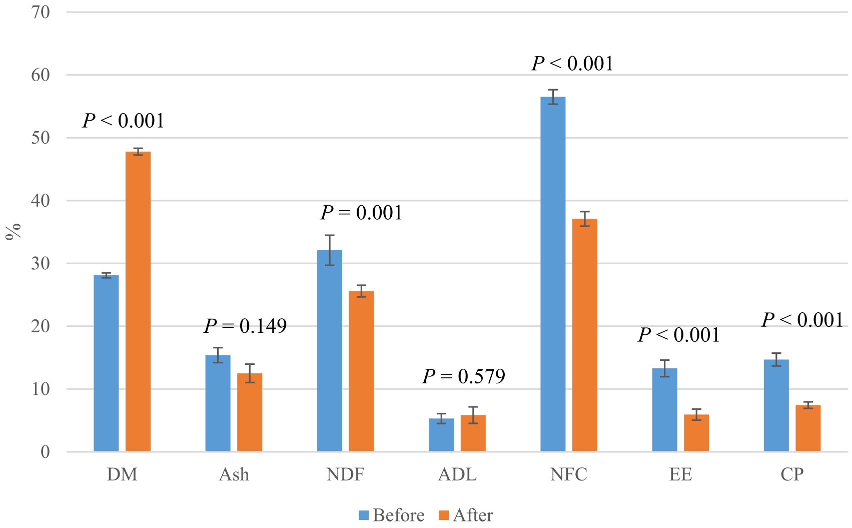

FIGURE 2 Chemical characteristics of OFMSW before and after BSF larval production.

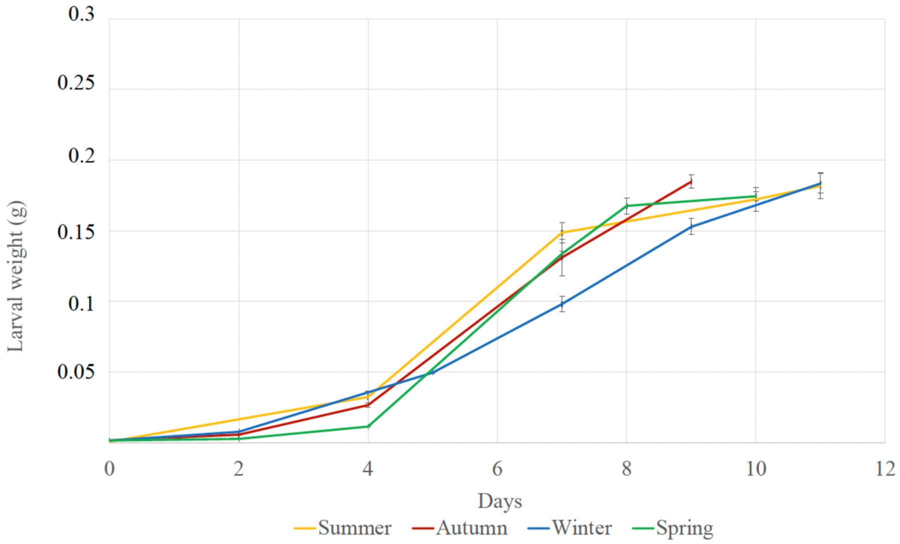

FIGURE 3 Larval growth in the untreated OFMSW during the four seasons.

a similar trend, with high growth rate mostly starting from the 4th day after the start of the trial (Figures 3 and 4). The highest number of days observed for reaching the end of the test, corresponding to the appearance of 5% of the prepupae, was 11, observed in summer in the untreated waste and in summer and winter in the pulped waste.

Table 2 shows the average values of the different parameters evaluated within the experimental trials, considering the influence of the pretreatment, the season and their interaction. The mean number of days necessary to get the end of the trial (5% of prepupae presence) varied between a mean minimum value of 8.50 ± 0.93 in spring and a maximum of 10.3 ± 0.10 in summer. This parameter was not influenced by pretreatment ($P = 0.175$), or the interaction between pretreatment and season ($P = 0.130$), while some differences due to the season ($P < 0.001$) were observed in summer for both untreated and pulped OFMSW, when larvae took more time to reach the end of the trial. Larval survival was always very high and comprises between 89.2 ± 3.56% in winter in the untreated and 98.8 ± 1.53% in spring in the pulped OFMSW. No differences on survival were observed between untreated and pulped waste in all the samples ($P = 0.648$), while the seasonality influenced the survival ($P < 0.001$). In fact, a lower survival was observed in winter on both untreated and pulped samples of OFMSW (89.2 ± 3.55 and 89.4 ± 10.2, respectively).

Concerning larval biomass yield, both the season and the pretreatment of the substrate influenced this parameter ($P < 0.001$ and $P = 0.035$, respectively), while their interaction did not have an impact ($P = 0.053$). The minimum (15.0 ± 3.70 g) and maximum

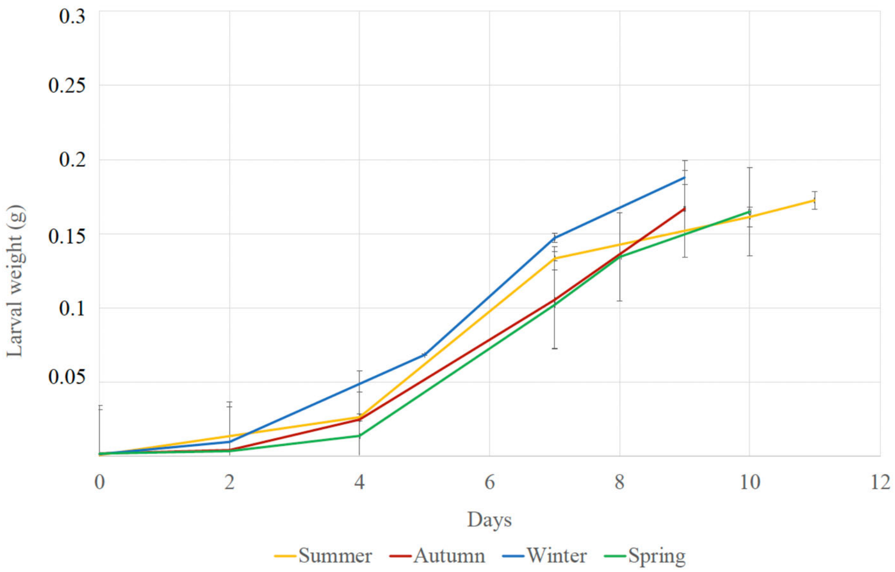

FIGURE 4 Larval growth in the pulped OFMSW during the four seasons.

TABLE 2 Mean values of the different growth and bioconversion parameters of BSF grown on Untreated (U) and Pulped (P) OFMSW in the four seasons

	Days	Larval survival (%)	Larval yield (g)	SR (%DW)	WRI (DM)	ECI (DM)	NCR
Summer							
U	10.3^{a}	96.6^{a}	30.2^{a}	69.1^{a}	6.02^{bc}	0.193^{c}	0.308
P	9.75^{a}	98.7^{a}	28.1^{abc}	51.2^{cd}	4.73^{d}	0.251^{bc}	0.327
Autumn							
U	8.63^{c}	96.4^{a}	15.0^{d}	44.2^{d}	5.12^{cd}	0.214^{bc}	0.261
P	9.00^{bc}	96.4^{a}	23.0^{bc}	51.1^{cd}	5.68^{bcd}	0.269^{b}	0.306
Winter							
U	9.00^{bc}	89.2^{b}	25.2^{abc}	57.2^{bc}	6.36^{ac}	0.256^{bc}	0.282
P	9.63^{ab}	89.4^{b}	25.7^{abc}	61.1^{b}	6.07^{bc}	0.191^{c}	0.284
Spring							
U	8.50^{c}	98.7^{a}	22.5^{c}	60.7^{b}	7.24^{a}	0.233^{bc}	0.402
P	9.00^{bc}	98.8^{a}	28.3^{ab}	48.8^{d}	5.43^{bcd}	0.360^{a}	0.328
SE	0.258	1.711	1.989	2.829	0.338	0.024	0.049
Season	<0.001	<0.001	<0.0001	<0.001	0.006	0.011	0.478
Pretreat.	0.175	0.648	0.035	0.021	0.004	0.015	0.961
Pretreat.*Season	0.130	0.920	0.053	<0.0001	0.005	0.003	0.753

SR = substrate reduction, WRI = waste reduction index, ECI = efficiency of conversion of ingested feed, DM = dry matter, U= larvae reared on untreated organic fraction of municipal solid waste, P = larvae reared on pulped organic fraction of municipal solid waste, NCR = Nitrogen conversion ratio.

[a,b,c] Means in the same column with different superscript letters differ ($P < 0.05$).

(28.3 ± 4.23 g) larval biomass was observed for the untreated OFMSW in autumn and in the pulped in spring, respectively. The larval yield was slightly higher on pulped than untreated waste (on average: 26.3 g and 23.2 g, respectively, for P and U; $P = 0.035$).

Considering the substrate reduction, the waste reduction index, and the efficiency of conversion of the ingested food, they were all influenced by the season ($P < 0.001$, $P = 0.006$ and $P = 0.011$, respectively), the pretreatment ($P = 0.021$, $P = 0.004$ and $P = 0.015$, respectively) and their interaction ($P < 0.001$, $P = 0.005$ and $P = 0.003$, respectively). Substrate reduction was between 44.2 ± 9.41% up to 69.1 ± 5.06% in the untreated substrate in autumn and summer, respectively, and between 48.9 ± 3.30% and 61.1 ± 11.2% in the pulped group in spring and winter. In two seasons (summer and spring) the pulping process of the OFMSW lead to a lower reduction of the substrate from the larvae, while the opposite was observed in autumn and winter, where the pretreatment favored the substrate reduction. In addition, the untreated OFMSW collected in summer allowed to obtain the higher value of substrate reduction (69.1 ± 5.06%) comparing to the other seasons. When the substrate was given untreated to the larvae, the lowest bioconversion rate was observed in autumn (44.2 ± 9.41%). Waste reduction index varied between 4.73 ± 0.661 and 6.07 ± 0.447 in the pulped substrate in summer and winter, respectively, and between 5.12 ± 1.03 (autumn) and 7.24 ± 1.17 (spring) in the untreated substrate; highest value were always observed in winter and spring. In all seasons except autumn, the homogenization of the OFMSW negatively influenced the index with lower values of reduction. The efficiency of conversion was comprised from a minimum of 0.191 ± 0.103 and a maximum of 0.360 ± 0.054. In general, the pretreatment of the OFMSW increased the ECI index; only in winter the index was lower in the pulped waste compared to the untreated. No statistical differences were observed for NCR index, that varied from a minimum of 0.261 in autumn in the untreated waste to a maximum of 0.420 in spring in the untreated.

***Larval chemical composition and digestibility* in vitro**

The chemical composition of BSF larvae grown on OFMSW subjected to two different pretreatments (Untreated and Pulped) over the four seasons is reported in Table 3. Both the pretreatment and the season affected the BSF larvae chemical composition.

Larvae grown on untreated samples had a higher fat and energy content compared to larvae on pulped samples (on average, for untreated and pulped groups, 34.4 ± 2.66 vs 28.5 ± 2.52% for EE and 26.8 ± 1.26 vs 24.7 ± 0.80 MJ kg^{-1} for gross energy); on the other hand the ash content was lower for BSF on untreated samples (9.26 ± 3.19) than for BSF on pulped samples (16.4 ± 2.58%).The ash content of BSF was the parameter with the highest variability among the chemical constituents as expressed by the coefficient of variation (overall coefficient of variation for ash content 36%).

The season affected the gross energy content (P = 0.049). In particular, in winter and autumn the energy level (MJ kg^{-1}) of BSF larvae was higher (on average 26.2 ± 1.70) whereas in spring and summer the larvae were characterized by a lower energy content (25.3 ± 1.06). The DM content (%) was significantly affected by the season showing a higher value in winter (34.7 ± 1.64) compared to the other season (overall mean 30.6 ± 3.67).

The protein content was not affected by pretreatment and season, and ranged between 34.4% and 39.6% DM (overall coefficient of varation for CP equal to 4.64%). The estimate chitin content (% DM) was not affected either by season or treatment (5.45 ± 1.38).

There was a significant interaction between pretreatment and season for DM, CP and GE ($P = 0.033$, $P = 0.021$ and $P = 0.016$, respectively). The CP (% DM) content was lower ($P < 0.05$) for BSF reared on pulped samples in spring (34.4 ± 0.35) compared to untreated samples (39.6 ±0.11); whereas for the other seasons no differences were observed depending on the pretreatment. On the other hand, GE was always higher for BSF on untreated samples except for spring season with similar values among untreatead and pulped samples.

The energy and protein digestibilities of BSF larvae, grown on two different substrates (untreated and pulped) in the four seasons, are reported in Table 3. The larvae grown on the untreated substrate, already found to be richer in lipids, had a higher *in vitro* energy digestibility (%) that was statistically different from the larvae grown on the pulped substrate (82.1 ± 6.29% vs 61.9 ± 6.93%; respectively, for untreated and pulped; $P = 0.001$). Consequently, the AME (MJ kg^{-1} DM) was affected by pretreatment ($P < 0.001$) with lower value for pulped (15.3) than untreated (22.0). However, there was a significant interaction ($P = 0.016$) treatment* season for *in vitro* energy digestibility with similar values for untreated and pulped samples in spring, although always numerically lower for pulped samples.

The *in vitro* protein digestibility was affected by pretreatment ($P = 0.027$) and season ($P = 0.038$). The CP digestibily of BSF reared on untreated samples was lower than that on pulped samples (68.8 ± 8.57 vs 73.3 ± 6.02%; respectively; $P < 0.05$). The BSF had the highest protein digestibility (%) in autumn (78.6 ± 3.6), intermediate in summer (72.2 ± 8.0) and spring (70.3 ± 2.4) and the lowest in winter (62.9 ± 5.5) ($P < 0.05$).

Fatty acid (FA) composition of larvae is resumed in Table 4. As mentioned before, lipid content was significantly affected by the pretreatment, while fatty acid composition varied according to the type of substrates, i.e. lauric, palmitic and stearic acids were significantly higher in the pulped samples. The untreated samples, instead, had the same values of saturated fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of pulped samples. In general, the FA profile of larvae was dominated by oleic

TABLE 3 Chemical composition and *in vitro* crude protein energy and protein digestibility of BSF larvae grown on untreated (U) and pulped (P) OFMSW in the four seasons

	DM (%)	Ash (% DM)	CP (% DM)	EE (% DM)	Chitin (% DM)	GE (MJ/kg)	DIG Energy (%)	AME (MJ/kg DM)	DIG CP (%)
Summer									
U	32.1^{ab}	13.4^{ab}	36.5^{bc}	35.0^{ab}	5.63	24.7^{b}	75.6^{bc}	19.8^{ab}	64.3^{bc}
P	30.8^{bc}	16.3^{a}	38.0^{ab}	31.3^{bcde}	4.20	23.5^{cd}	57.6^{e}	15.2^{bc}	75.1^{a}
Autumn									
U	29.0^{c}	6.93^{c}	37.3^{abc}	33.4^{abc}	5.35	26.1^{a}	86.4^{ab}	24.3^{a}	71.4^{ab}
P	28.0^{c}	16.5^{a}	35.7^{bc}	27.8^{de}	4.95	23.3^{cd}	59.4^{de}	14.7^{c}	75.9^{a}
Winter									
U	34.8^{a}	7.27^{c}	36.0^{bc}	37.6^{a}	5.16	25.8^{c}	88.5^{a}	24.2^{a}	59.4^{c}
P	34.6^{a}	17.9^{a}	37.6^{ab}	25.9^{e}	4.28	23.0^{d}	69.6^{cd}	14.1^{c}	66.4^{abc}
Spring									
U	29.8^{bc}	9.50^{bc}	39.6^{a}	31.7^{bcd}	7.52	24.2^{bc}	78.0^{abc}	19.8^{ab}	70.0^{ab}
P	34.1^{a}	14.7^{ab}	34.4^{c}	29.0^{cde}	6.54	23.4^{cd}	69.6^{cd}	17.1^{bc}	70.6^{ab}
SE	1.05	1.56	0.889	1.63	0.879	0.277	3.29	1.47	2.99
Season	<0.001	0.282	0.846	0.371	0.109	0.049	0.374	0.563	0.038
Pretreat.	0.54	<0.001	0.193	0.001	0.181	<0.001	<0.001	<0.001	0.027
Pretreat.*Season	0.033	0.130	0.021	0.104	0.947	0.016	0.016	0.090	0.431

CP = crude protein, EE = ether extract, GE = gross energy, AME = apparent metabolizable energy, U = larvae reared on untreated organic fraction of municipal solid waste, P = larvae reared on pulped organic fraction of municipal solid waste.
[a,b,c,d,e] Means in the same column with different superscript letters differ ($P < 0.05$).

acid (from 22.4 to 26.9 g 100 g^{-1} FAs) and by palmitic acid (from 21.6 to 26.1 g 100 g^{-1} FAs), followed by lauric acid (from 12.4 to 19.0 g 100 g^{-1} FAs). The FA profile of larvae was significantly affected by the season; in particular, the values of C12, C16, C18, and C18:2 varied with the season. The samples collected in summer presented higher values for C12 and C18:2, while higher values of palmitic and stearic acid were registered in spring.

Moreover, statistical differences were recorded between the season and the pretreatment for lauric, palmitic, stearic, linoleic acids, SFA, MUFA and PUFA. The interaction between pretreatment and season for SFA was significant in winter and spring; in particular, in winter, the content of SFA was significantly higher in the untreated samples in comparison to the pulped samples (57.0 vs 51.6%; respectively), while in spring the higher value of SFA was registered in the pulped samples (57.5%).

4 Discussion

Characteristics of the rearing substrate and larval growth performances

The aim of the study was to evaluate the growth performance of BSF larvae grown on municipal organic waste and their nutritive value. It is well known that the rearing substrate, along with other environmental factors, such as temperature and moisture, affects the larval growth, the larvae chemical composition and its chemical composition value (Harnden and Tomberlin, 2016; Meneguz *et al.*, 2018; Oonincx and Finke, 2021; Ribeiro *et al.*, 2022).

OFMSW typically consists of various organic materials of vegetal and animal origin, from kitchen and canteen waste, market waste, green waste (leaves, grass and pruning waste) and it contains various nutrients, in particular carbohydrates (i.e. starch, cellulose and hemicellulose) (30-69%), proteins (5-10%) and lipids (10-40%) (Paritosh *et al.*, 2018; Pleissner and Lin, 2013). Due to its heterogeneous origin, OFMSW exhibits a wide variability in chemical composition, as confirmed by this study, which compared samples collected during four seasons of the year. In particular, the ether extract, the NFC and the fiber fractions were significantly affected

TABLE 4 Fatty acid profile of BSF larvae grown on untreated (U) and pulped (P) OFMSW in the four seasons

Fatty acids	C12:0	C14:0	C16:0	C16:1 (cis-9)	C18:0	C18:1 (cis-9)	C18:2(*cis-9*, *cis-12*)	C18:2 (trans-9, trans-12)	C18:3 (cis-9, cis-12, cis-15)	SFA	MUFA	PUFA
	$g\ 100\ g^{-1}$ FAs											
Summer												
U	18.8^{a}	8.60^{a}	21.6^{d}	3.12	4.27^{cd}	25.4	13.6^{ab}	0.96^{a}	1.04	59.6^{a}	27.7^{b}	12.6^{cd}
P	18.5^{ab}	8.47^{a}	24.0^{bc}	3.35	4.26^{cd}	23.3	14.5^{a}	0.00^{c}	1.05	56.8^{a}	27.6^{b}	15.5^{ab}
Autumn												
U	16.6^{b}	8.79^{a}	22.4^{dc}	3.13	4.51^{bcd}	25.0	15.0^{a}	0.42^{bc}	1.18	56.5^{ab}	27.2^{b}	16.2^{ab}
P	19.0^{a}	9.23^{a}	24.4^{ab}	3.67	4.28^{bcd}	22.4	12.3^{bc}	0.21^{bc}	1.13	60.0^{a}	27.8^{b}	12.2^{d}
Winter												
U	16.9^{ab}	9.09^{a}	23.9^{bc}	3.45	5.07^{bc}	26.9	10.4^{c}	0.57^{ab}	0.95	57.0^{a}	25.7^{b}	17.3^{a}
P	17.9^{ab}	8.80^{a}	26.1^{a}	2.86	3.68^{d}	23.7	13.0^{ab}	0.00^{c}	0.89	51.6^{c}	33.3^{a}	15.0^{abc}
Spring												
U	12.4^{c}	7.17^{b}	24.6^{ab}	2.92	8.34^{a}	25.0	14.6^{a}	0.65^{ab}	1.05	52.8^{bc}	33.0^{a}	14.2^{bcd}
P	16.8^{ab}	9.22^{a}	25.4^{ab}	2.94	5.38^{b}	23.1	12.2^{bc}	1.06^{a}	1.17	57.5^{a}	28.3^{b}	14.0^{bcd}
SE	0.84	0.47	0.68	0.36	0.43	2.12	0.80	0.20	0.12	1.33	1.53	0.95
Season	<0.0001	0.214	0.001	0.573	<0.0001	0.857	0.014	0.013	0.149	0.006	0.136	0.097
Pretreat.	0.002	0.099	0.0002	0.834	0.0002	0.083	0.455	0.016	0.947	0.991	0.436	0.179
Pretreat.*Season	0.027	0.037	0.561	0.401	0.002	0.982	0.002	0.005	0.825	0.001	0.003	0.006

MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid.
U = larvae reared on untreated organic fraction of municipal solid waste, P = larvae reared on pulped organic fraction of municipal solid waste.
[a,b,c,d,e] Means in the same column with different superscript letters differ ($P < 0.05$).

by the season. Pecorini *et al.* (2020) highlighted a correlation between the seasonality and the composition of the waste; in particular, the higher variations were observed in the percentages of lignin and carbohydrates. Moreover, other authors found some seasonal significant variations in the organic municipal waste with particular reference to the percentage of ash, even if differences in the collection system could explain most of the effects (Hansen *et al.*, 2007). The results of the present study showed that the pulp pretreatment significantly reduced the hemicellulose and NDF contents and increased the ash percentage; consequently, the nutritive value of pulped OFMSW was lower. The increase of ash content of pulped can be explained by pretreatment process provided by the bio-pulper, which determines a separation of OFMSW according to density and solubility.

Overall, the chemical analyses of the substrates are comparable to those found by Pecorini *et al.* (2020), where the carbohydrates resulted to be the predominant component in all season. Moreover, in accordance with these authors, the higher content of lipids of the rearing substrates in winter and spring could be due to the different composition of human diet that is richer in lipids during the colder months.

Considering larval growth performances, only few differences due to the seasonality of the organic waste or its pretreatment with a biopulper were observed. The development time needed to reach the prepupal stage (corresponding to the harvesting stage) greatly depends on environmental factors and in particular temperature and humidity, but it is also influenced by the chemical composition of the rearing substrates, resulting in a delay or acceleration of the development period. In particular, protein content of the substrate greatly influenced the development time by accelerating it when the content is high (Oonincx *et al.*, 2015). In fact, on vegetable substrates, where the protein content is low, the development of BSF larvae is longer, resulting between 19 and 28 days (Jucker *et al.*, 2017; Lalander *et al.*, 2019; Nguyen *et al.*, 2013). In these trials, the time for larval developmental assuming 5% of prepupal appearance was shorter than what observed by other authors on other organic matters (Lalander *et al.*, 2019; Liu *et al.*, 2019; Sphrangers *et al.*, 2017). For example, Spranghers *et al.* (2017) observed the capability of BSF larvae to grow on digestate, vegetable waste and restaurant waste, and larvae took from 15 to 19 days for the appearance of the first prepupa, while in Lalander *et al.* (2019), 12 days have been observed on abattoir waste, 14 in food waste and even 42 days on digested sludge (the latter characterized by low nutritional value).

During the experiment, a high survival of the larvae has always been observed, and it was comprised between 89.2% and 98.8%. Comparable results were observed feeding larvae with agro-food by-products by Liu *et al.* (2018) using brewer's waste (survival 98%), or by Galassi *et al.* (2021) on brewer's grains and okara (survival 95.9% and 98.5%, respectively). Again, Gold *et al.* (2020) experimented high survival rate (90-99%) by using a wide variety and mixture of biowaste. The humidity of the substrates is also an important factor influencing the larval survival, bioconversion efficiency and also the possibility to separate larvae from the residues by sieving. Fatchurochim *et al.* (1989) observed that the diet moisture content influenced the larval survival rates, and better results were obtained between 40% and 70% humidity, while in Cheng *et al.* (2017), the food waste moisture did not have an impact on the larval survival. The water content of the OFMSW used in this work varied between 71% and 73% FW, considered an *optimum* range for the larvae of BSF. Moreover, Cheng *et al.* (2017) demonstrated that residue separation is feasible when the food waste has a moisture content between 70% and 75% by using temperature-controlled rotary drum reactor.

Substrate reduction obtained in the present study ranged between 44% and 69% of the starting substrate, being higher than that observed for different organic wastes of vegetal and animal origin (Gold *et al.*, 2020), but in line with that reported by Lalander *et al.* (2019). Considering the ability of BSF larvae to bioconvert the waste, the highest conversion indexes were achieved in spring with the pulped waste; in general, ECI index was higher when the organic substrate was pretreated with the biopulp. This fact could be attributed to the lower content of hemicelluloses in the pulped OFMSW, that were converted during pulping treatment into more digestible simple sugars (Papa *et al.*, 2022). Moreover, the pulped treatment had a lower particle size than untretated samples and a lower particle size is expected to icrease the growth performance of larvae. Peguero *et al.* (2024) showed that, on spent grain and grass clipping, BSF larval weight and bioconversion rate increased with a lower particle size (from untreated substrate to 0.5 mm).

Considering the bioconversion efficiency, the carbon content was not determined in the present study; however, applying the Van Bemmelen factor of 1.724 to convert organic matter in carbon, an estimate value of C/N of the rearing substrate is 21:1 which is similar to that reported by Lu *et al.* (2021) for food waste; the same authors found that the C:N ratio significantly influenced larval development and bioconversion efficiency and range of 18:1-16:1 was identified as optimal for food waste treatment using BSF. Considering this, adding some nitrogen sources to the OFMSW could improve the growth performance and bioconversion efficiency of BSF. The same study (Lu *et al.*, 2021) indicated that the same C/N range (18:1 or 16:1) is optimal to enhance larval protein yield. Regarding the N bioconversion efficiency, the value was similar to that reported by Naser El Deen *et al.* (2023) for BSF reared on organic wet fraction of municipal household waste; some variability is reported for this parameter and the organic waste composition has a major effect on NCR; particulary, the C:N ratio, the moisture content, and the presence of inhibitory substances can significantly impact the efficiency. The results of the present study seem to indicate that also the NCR could be improved by the addition of other N sources to OFMSW.

The OFMSW was characterized by discrete amount of fiber (NDF) which could arise some concern in terms of bioconversion efficiency; however, as highlighted by Kim *et al.* (2014), the presence of intestinal bacteria capable of degrading cellulose in the gut of BSF larvae can enhance the bioconversion of substrates rich in fiber as observed in the present study where larvae could efficiently bio-convert OFMSW waste without compromise their growth performance.

Chemical composition and nutritional value of the larvae

A wide range of variation for chemical composition (ash and EE in particular) and in the nutritive value was observed on the collected larvae. The differences observed can be explained by the differences in chemical composition of the rearing substrate; therefore, the possibility of having a substrate that varies little in chemical composition allows greater standardization of larval production, such as also required by the feed industry. In this regard, the OFMSW did not allow to obtain larvae with a constant chemical composition and nutritive value, especially for what concerns lipid, ash, energy contents, energy digestibility and AME. Particularly, the energy digestibility was negatively correlated to ash content. The ash content, comprises between 6.93% and 17.9%, was higher in the larvae collected on the preatreated OFMSW probably due to their lower fiber content. Indeed, fibers seem to have negative impact on mineral accessibility as they bind minerals. Ash content appears an issue for the utilization of larvae as feed ingredient by the compound feed industry. The value is comparable to that obtained (19.7% on DM) by Spranghers *et al.* (2017) in prepupae grown on digestate. However, in this context, the use of chemical treatment to remove the exoskeleton (rich in mineral) can be a strategy to decrease the ash content of larvae and increase the nutritive value (Nafisah *et al.* 2019).

In accordance with data obtained by other authors (Caligiani *et al.*, 2018; Ewald *et al.*, 2020) the dry matter of larvae was found to be around 32% FW. The protein concentration was comparable with that found by Gold *et al.* (2020) in larvae reared on food waste, while the values of crude fat were in general lower. Although neither the pretreatment nor the season affected the larvae CP concentration, the larvae grown on pulped resulted in a lower CP than that grown on untreated treatment during spring. The larvae grown on pulped during the spring resulted in a higher ECI than untreated which means that the feed conversion was better for pulped substrate as shown also by higher larval biomass for pulped than untreated waste. Although the difference was not significant, the NDF content of the pulped rearing substrate in spring was lower than that of untreated (23.9% vs 32.7% DM, respectively, for P and U in spring) and the difference was higher than that observed in the other seasons (data not shown). The lower NDF content of pulped in spring diet may have favoured a higher growth in this period; hence it can be hypothesized that the larvae reared on pulped during spring proportionally accumulated more lipids (as confirmed by the lipid content of larvae not dissimilar between pulped and untreated in spring differently than other season) which "diluted" the CP.

In our trial, the fatty acids profiles of BSF larvae at the end of the larval development stage were characterized by a higher value of SFA and low amounts of MUFA and PUFA in agreement with the studies of Scieuzo *et al.* (2023) and Ramos-Bueno *et al.* (2016). In particular, the FA profile of larvae was dominated by oleic acid. Zheng *et al.* (2012) also found that the main fatty acid from larvae fed on residual fraction of solid restaurant waste is oleic acid, while other authors (Kaczor *et al.*, 2023; Spranghers *et al.*, 2017), reported that the FAME (fatty acid methyl esters) of BSF larvae changes depending on the substrate but the main component is lauric acid (C12:0) in all substrates. However, the difference of FA composition found in literature between and within species could be due to composition of the substrates, life stage of larvae, season, sex and environmental condition (Oonincx *et al.*, 2015; Suryati *et al.*, 2023).

The chitin content determined in this study was in line with that reported by other experiments (Finke *et al.*, 2007; Marono *et al.*, 2015; Schiavone *et al.*, 2017) and similar to that on larvae fed on vegetable mix diet (Cappellozza *et al.*, 2019) and on organic waste (Meneguz *et al.*, 2018). The amount of chitin is the factor affecting insect protein digestibility because the small intestine is not able to digest and abstorbe it (Vidanarachchi *et al.*, 2010). Despite this, the average value of *in vitro* crude protein digestibility was quite high (69.1%), although variable, and it was in line with those found in samples from three different producers of BSF meal (from 66% to 68.8%) (Marono *et al.*, 2015), but lower than the average value (81.6%) reported by Rodriguez *et al.* (2022). The same author (Rodriguez *et al.*, 2022) observed large differences for *in vitro* CP digestibility due to the species, the developmental stage and the digestion methods used.

In the trial performed in this work, *in vitro* protein digestibility values were slightly lower than those of important protein raw materials (soybean meal and fish meal) and those determined using other insects, such as termite (*Macrotermes subhylanus* Madzhulu) and grasshopper (*Ruspolia differens* Serville) (Kinyuru*et al.*, 2010). The *in vitro* crude protein digestibility was also affected by the pretreatment with higher value for pulped waste. Although this data was not significant, it has to be underlined that larvae grown on pulped had a lower chitin content (on average –15.7% as compared to U) which can partially explain the difference due to substrate pretreatment. Overall, despite the variation, the values of CP digestibility were noticeable for the lower particle size due to the milling process which increased the area available for the digestion (Slimen *et al.*, 2023).

Concerning the energy value, as reported by Mahomud *et al.* (2023), there is a rising interest on the use of BSF larvae but information on the AME for precise feed formulations is scarce. The results of the present study showed that there was a wide variability for the AME content, which was lower for the pulped OFMSW mainly due to the different ash and lipid contents. The average value (18.6 MJ kg^{-1} DM) was slightly higher than that (17.4 MJ kg^{-1} DM) reported by De Marco *et al.* (2015) in an *in vivo* study. Moreover, in this study the values of AME were higher or comparable than those of feeds used in poultry diets (although evaluated as AMEn) (https://www.feedtables.com/content/amen-broiler-mj).

5 Conclusion

OFMSW could be valorized through its bioconversion by BSF larvae into more valuable products, as it is still rich rich in carbohydrates, lipids and proteins. Seasonality and mechanical treatment influenced the chemical composition of the OFMSW thus affecting the growth performance and bioconversion of the BSF larvae as well as their chemical composition.

Considering the different seasons of waste sampling, a high variability was reported within the results, with some differences observed in particular on the ether extract content (higher values in winter and spring), the NDF content (lower in spring) and the ADL content (higher in autumn). Pretreatment, on the other hand, significantly reduced the hemicellulose and NDF contents and increased the ash percentage.

Seasonality of the waste also influenced the larval performance and the bioconversion ability of the larvae, with high variable results on the considered parameters. The mechanical treatment resulted in a better growth of the larvae (higher larval yield, higher ECI index), but negatively influenced their nutritional value due to the high final ash content, albeit a higher *in vitro* crude protein digestibility was registered compared to the untreated.

The overall variability in the composition of the OFMSW did not allow obtaining larvae characterised by a constant chemical composition, in particular with regard to the lipid, ash, energy, energy digestibility and AME content; only the protein content was not influenced by the variability and remained constant between 14.1% and 15.3%.

OFMSW has proven to be a suitable substrate for larvae growth, but the high variability observed underlines that it is necessary to standardize the production process to better valorize it, avoiding high variability of larval biomass that is not appreciated by the market.

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Author contributions

CJ, SS, SC, IT: conceptualization, formal analysis, writing – original draft, review and editing, data curation; SM, GP, GDI, BS: methodology, data collection, data curation; VC, MP: methodology, data collection, visualization, data curation, writing – original draft, formal analysis; FA review and editing, funding.

Conflicts of interest

The authors declare no conflict of interest.

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