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Research Paper

Valorization of organic waste through black soldier fly: On the way of a real circular bioeconomy process

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ABSTRACT

The transition from a linear to a circular production system involves transforming waste into valuable resources. Insect-mediated bioconversion, particularly using black soldier fly (BSF) larvae, can offer a promising opportunity to convert the organic fraction of municipal solid waste (OFMSW) into protein-rich biomass. However, current regulatory restrictions do not allow the use of this substrate to obtain insect proteins for animal feed, prompting the exploration of other applications, such as the production of bioplastics. Here, we explored at laboratory scale an innovative and integrated circular supply chain which aims to valorize the OFMSW through BSF larvae for the production of biobased materials with high technological value. BSF larvae reared on this organic waste showed excellent growth performance and bioconversion rate of the substrate. The use of well-suited extraction methods allowed the isolation of high-purity lipids, proteins, and chitin fractions, which are building blocks to produce biobased materials. In particular, the protein fraction was used to develop biodegradable plastic films which showed potential for replacing traditional petroleum-based materials, with the possibility to be fully recycled back to amino acids. Socioeconomic analysis highlighted values generated along the entire supply chain, and life cycle assessment pointed out that lipid extraction was the most challenging step: implementation of more sustainable methods is thus needed to reduce the overall environmental impact of the proposed chain. In conclusion, this study represents a proof of concept gathering evidence to support the feasibility of an alternative supply chain that can promote circular economy while valorising organic waste.

1. Introduction

In recent years, various strategies are being considered worldwide to cope with environmental issues, including the implementation of a circular economy approach across sectors, aimed at halving carbon emissions within 2030 and achieving carbon neutrality by 2050 (Dantas

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et al., 2021). In this context, the disposal of the organic fraction of municipal solid waste (OFMSW) poses a significant challenge, reaching 900 million tonnes/year globally and expected to increase by 70 % within 2050 due to a rise in food waste (United Nations Environment Programme, 2021). The existing recovery systems of the OFMSW (i.e., anaerobic digestion and composting) are deemed unsatisfactory and somehow challenging as they depend on the chemical composition of the feedstock, offer minimal value, and raise environmental and public health issues (Paritosh et al., 2018), especially in developing countries (Adedara et al., 2023).

Among the recently proposed alternative strategies for recycling organic waste there is insect-mediated bioconversion. In particular, the black soldier fly (BSF), *Hermetia illucens*, is considered the most promising insect for valorising waste and by-products of the agri-food value chain (Athanassiou et al., 2024). In fact, thanks to their remarkable midgut plasticity (Bonelli et al., 2020; Bruno et al., 2024), BSF larvae can grow on a wide variety of organic leftovers and side streams, reducing the waste volume and transforming it into protein-rich biomass (Rehman et al., 2023). Numerous studies have been undertaken to characterize the growth performance of these larvae on different organic leftovers (Broeckx et al., 2021; Ceccotti et al., 2022), to define how the gut microbiota is shaped by the rearing substrate (Bruno et al, 2019a; Vandeweyer et al., 2023; De Filippis et al., 2024), and to evaluate the potential interference of contaminants in the substrate, such as heavy metals (Hu et al., 2023), pesticides (Meijer et al., 2021) and other chemicals (van der Fels-Klerx et al., 2020), demonstrating the safety and suitability of insect-mediated bioconversion for developing valuable circular supply chains. However, at least in Europe, the utilization of BSF meals in the feed sector is restricted by the current legislation which allows the use of insect proteins for animal feed production only if insects are reared on microbiologically and chemically safe substrates (European Commission, 2017). Therefore, while the use of other components derived from BSF larvae, such as lipids for the production of biodiesel and surfactants, does not pose particular limitations, alternative applications for proteins obtained from larvae grown on food waste must be explored.

A topic strictly related with waste management is plastic pollution. Indeed, the non-biodegradable nature of oil-based plastics has led to the catastrophic accumulation of a large amount of plastic waste and microplastics over recent decades, making plastic waste management one of today's most urgent environmental challenges (Shen et al., 2020; Ford et al., 2022). This issue is amplified by the widespread use of single-use plastics, especially in packaging, where products have a short life span between their production and disposal. In cases where plastic recycling is not feasible due to technical or economic constraints (Garcia and Robertson, 2017; Volk et al., 2021), governing institutions are thus increasingly promoting the use of renewable and biodegradable plastics. In this scenario, proteins are emerging as one of the most promising classes of biopolymers for the development of environmentally friendly bioplastics. The search for alternative sources of proteins (not in competition with the food and feed supply chains) and the generation of functional and biodegradable protein-based materials are thus two primary, leading-edge issues in research (Kamada et al., 2021; Shen et al., 2021; Peydayesh et al., 2021; Li et al., 2023; Peydayesh et al., 2023).

Few studies have pioneered the use of BSF proteins for producing bioplastics, but their performance was unsatisfactory, mainly due to their limited stability against chemical and physical agents, as well as the non-competitive costs of these derivatives, leaving large-scale application of such films substantially unexplored (Barbi et al., 2019; Barbi et al., 2021; Setti et al., 2020). Moreover, to the best of our knowledge, no studies have compared the production of bioplastics using proteins obtained from different developmental stages of BSF (i.e., larvae and pupae), which is one of the targets of the present work.

Here, we present an innovative and integrated circular supply chain that, starting from the biotransformation of the OFMSW through BSF larvae, leads to the targeted production of bioplastic films with high technological potential. In addition, a comprehensive assessment of the technological landscape, economic feasibility, and expected environmental impact of the whole production chain is presented.

2. Materials and methods

2.1. Insect-mediated bioconversion of the OFMSW

Insects were reared as reported in Pimentel et al. (2017). Batches of 300 six-day-old larvae were transferred to plastic containers (16 × 16 × 9 cm), fed with the experimental substrates, and kept in the dark at 27 ± 0.5 °C and 70 ± 5 % relative humidity (Bruno et al., 2019b). Two feeding substrates were used in this study: the OFMSW sampled from household waste (r-OFMSW, where "r" stands for "real") in five cities of Lombardy Region (Italy) and the surrogate-OFMSW (s-OFMSW), which was formulated to reproduce the OFMSW (see Bruno et al., 2024 for details) and selected to perform the experiments under reproducible and standardized conditions. Both substrates were minced before feeding the larvae. Insects were then collected at the appropriate developmental stage for the analyses (i.e., last instar larvae and 4-day-old pupae).

The growth performance of the larvae and the efficiency of the bioconversion of the r-OFMSW were evaluated through the following indexes: Relative Growth Rate (RGR), substrate reduction (D), Waste Reduction Index (WRI), Efficiency of Conversion of Ingested food (ECI), Nitrogen Conversion Efficiency (NCE), and Survival Rate (SR) (see Supplementary Table 1 for details). The rearing substrate, rearing residue (frass), and insects were frozen at −20 °C for 24 h and then dried at 60 °C overnight to determine the total content of dry matter for the calculation of the bioconversion indexes. All the experiments were conducted in triplicate.

The rearing substrate, larvae, and pupae were subjected to freezing and drying, as reported above, and finely minced. The samples were then analyzed by La-Chi laboratory (University of Padova, Italy) to determine their chemical composition. Crude protein, lipid and fibre, nitrogen-free extract, and ash content were determined according to AOAC International (Horwitz, 2000; Latimer, 2016). Crude protein content was determined considering nitrogen-to-protein conversion factor (Kp) of 6.25 for rearing substrate and 5.6 for insects (Janssen et al., 2017). Starch was determined by enzymatic digestion followed by glucose quantification by HPLC. Free glucose and fructose were quantified by HPLC, too. Chitin was calculated by using Van Soest acid detergent fibre method (Stelmock et al., 1985).

2.2. Extraction and quantification of components in protein and lipid fractions

2.2.1. Protein, lipid, and chitin extraction

After rearing on s-OFMSW and r-OFMSW, last instar larvae and 4-day-old pupae were sampled and used for lipids, proteins, and chitin extraction. The extraction procedure (Fig. 1) was carried out from five biological replicates for each condition and each analysis was repeated at least twice. For each replicate, the procedure used ≈20 g of dried biomass. Each sample was ground with an IKA A10 grinder for 2 min and immediately used for extraction.

Lipids were obtained by Soxhlet extraction (Caligiani et al., 2018) with petroleum ether (technical, boiling point 40–60 °C, Exacta + Optech Labcenter S.p.A., Modena, Italy) for 18 h. An alternative batch procedure for lipid extraction was also evaluated: briefly, grinded BSF biomass and petroleum ether were stirred at 1:2 w/v ratio for 1 h and then decanted for 5 min; the solvent containing fats was recovered by centrifugation at 2000 × g for 12 min (the procedure was repeated twice). Extracted lipids were stored at −20 °C under N_2 and unfrozen prior to the analysis.

Proteins were extracted from the lipid-free BSF-powder with a modified procedure from Smets et al. (2020). The defatted sample was dispersed in demineralized water (1:10 ratio w/v) under conditions of

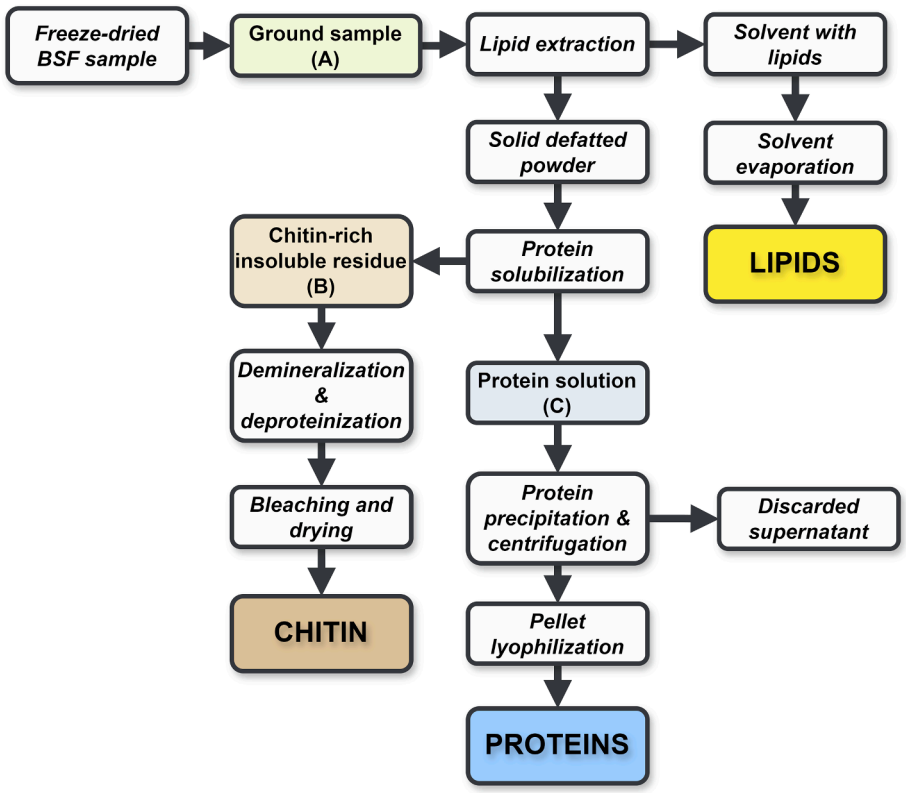


Fig. 1. Schematic representation of the biomolecule fractionation strategy set up in the present work. Intermediate compounds are indicated by A, B, and C. The final fractions extracted from BSF larvae and pupae are highlighted in brown, cyan, and yellow for chitin, proteins, and lipids, respectively. Processes are indicated in italics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

maximal protein solubility (pH 11.0, adjusted with 1.0 M NaOH) and kept at 45 °C for 2.5 h. The suspension was centrifuged at 1840 × g for 1 min and filtered; the solid fraction containing chitin was stored at −80 °C and then lyophilized. The protein-containing supernatant was adjusted with 1 M HCl solution to pH 4 to induce protein precipitation. The solution was centrifuged at 1840 × g for 45 min and the pellet was frozen at −80 °C and lyophilized.

2.2.2. Total carbohydrate content

The carbohydrate content of the ground sample (Fig. 1, sample "A") and of the final powder of protein extract (Fig. 1, sample "PROTEINS") was measured with the phenol–sulfuric acid method (Sadasivam and Manickam, 2005), using glucose as standard. Before the analysis, chitin was removed from ground sample by solubilization (100 mg) with 10 mL of 3 N HCl and boiling for 3 h; the solution was neutralized with Na_2CO_3 at room temperature and 1 mL spinned at 16060 × g for 15 min. The protein extracts were dispersed in 0.1 M phosphate buffer (pH 11).

2.2.3. Composition of lipid extracts

The lipid extraction yield was determined as the weight ratio (% w/w) to the initial dried insect sample, after petroleum ether evaporation at 50 °C using a Laborota 4000 rotary evaporator (Heidolph, Schwabach, Germany). All samples were analyzed at UNITECH OMICs (University of Milano, Italy) using ExionLC™ AD system (SCIEX) connected to TripleTOF™ 6600 System (SCIEX) equipped with Turbo V™ Ion Source with ESI Probe. Following extraction with 2-propanol:acetonitrile (90:10, v:v), 0.1 % formic acid, and 10 mM ammonium acetate, samples were added with 10 µL of Internal Standard (IS, from Splash Lipidomix 330707-1EA, Avanti Polar Lipids). Each sample was analyzed twice in ESI positive and ESI negative modes. Data were analyzed with MS-DIAL software (ver. 4.24) integrated by the LipidBlast database (version 68).

2.2.4. Composition of protein extracts

The protein extraction yield was calculated as the weight ratio (% w/w) of the extracted dried proteins to the initial dried insect sample weight. The total protein content was determined by acid hydrolysis followed by quantification of the amino acid content through the ninhydrin reagent (Starcher, 2001), measuring absorbance at 575 nm.

The residual carbohydrate and DNA contents (% w/w) in protein extracts was assessed on each sample (10 mg) dispersed in 1 mL of 0.1 M phosphate buffer (pH 11). The carbohydrate content was measured as reported above. DNA content was determined by the DNA Quantitation kit (Bisbenzimide fluorescent assay, Merck KGaA, Darmstadt, Germany). Soluble protein content at different pH values was analyzed by the Bradford method (see below).

2.2.5. Chitin and ash content

Chitin was extracted through a formic acid/sodium hydroxide method (Hahn et al., 2022). In detail, 3 g of the defatted, deproteinized samples (Fig. 1, sample "B") were added with 45 mL of 0.5 M formic acid (Merck, St. Louis, MO, USA) warmed at 40 °C and stirred at 200 rpm for 2 h for demineralization. Samples were centrifuged and the pellet was washed twice with Milli-Q water. After the addition of 30 mL of 1.25 M NaOH (for deproteinization), samples were heated up to 90 °C and stirred for 4 h, centrifuged, and washed again; then they were vacuum filtered and 150 mL boiling water were added to remove residual proteins and dyes. Samples were finally dispersed in 25 mL 4.5 % NaClO (v/v) and placed in a hybridization oven at 55 °C for 1 h. After the last spinning and washing step, the resulting white material was desiccated in the oven at 70 °C overnight. The extraction yield was calculated as the weight ratio (% w/w) of the dried and bleached chitin extract to the initial dried insect sample.

Ashes were determined according to UNI EN 14775. Ground samples (Fig. 1, sample "A") were transferred into small ceramic crucibles and

dried overnight at 70 °C, incinerated using a muffle furnace at 550 °C for 8 h, dried into a silica desiccator, and the weight was determined gravimetrically. Ashes value was calculated as the weight ratio (% w/w) of the ashes to the initial dried insect sample.

2.3. Proteomic analysis and physical/chemical characterization of protein extracts

2.3.1. Proteomic analysis

Proteomic analysis was performed on the protein fraction recovered from larvae and pupae grown on s-OFMSW, separated by SDS-PAGE, followed by trypsin digestion and nLC-MS/MS analysis. Briefly, dried protein extracts were suspended in Milli-Q water up to a final concentration of 5 mg/mL and 1 M NaOH was added dropwise to reach a pH of 12. Samples were heated at 80 °C for 20 min. A 10 µL aliquot of this solution was mixed with an equal volume of Laemmli buffer 2X and loaded onto the gel for one-dimensional electrophoresis (Boreggio et al., 2022).

For nLC-MS/MS analysis, thin gel slices (1 mm each) were cut from SDS-PAGE lanes: each slice was treated with 0.02 µg/µL trypsin (in 25 mM Ambic) at 37 °C overnight, as detailed in Boreggio et al. (2022). MS data were analyzed by the Mascot search engine (Version 2.3.01), using the Proteome Discoverer software (Version 1.2.0 Thermo), and UniProtKB/SwissProt as protein database (Uniprot_Insecta_Reviewed, total sequences 10974, total residues 5363805). MS analysis was performed in triplicate, both for larvae and pupae samples, and the final identifications were obtained after validation.

2.3.2. Protein extracts solubility and secondary structure content

The solubility of protein samples at different pH was assessed using a modified version of the method by Mshayisa et al. (2022). Briefly, each sample (10 mg) was dispersed in 1 mL phosphate buffer and adjusted at different pH values, then incubated on a rotary shaker at 9 rpm for 30 min, at room temperature. Samples were centrifuged at 16060 × g for 20 min at 4 °C. Protein content of the supernatant was determined by the Bradford assay (Sigma-Aldrich, Saint Louis, MO, USA). Protein solubility was calculated as percentage (% w/w) of the total protein content. For UV–visible spectroscopy measurements, extracts solubilized at 0.2 mg/mL at the pH of maximum solubility were analyzed in the 240–800 nm range using a HP 8452A Diode Array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Protein secondary structure in water was assessed through circular dichroism (CD), as reported in (Caldinelli et al., 2010), using a JASCO J-815 spectropolarimeter.

2.3.3. Protein charge (ζ-potential)

Protein ζ-potential was evaluated through electrophoretic light scattering (ELS) measurements. Protein extracts were dispersed at 1 mg/mL in Milli-Q water and brought to pH values ranging from 2 to 12 by adding small volumes of 1 N NaOH or HCl. Samples were then heated at 80 °C for 20 min. The analysis was performed in triplicate at room temperature (22 ± 2 °C) in DTS0012 type cuvettes by means of a Zetasizer Nano (Malvern Instruments, Malvern, UK). ζ-potential was automatically calculated from the electrophoretic mobility by means of the Hemholtz–Smoluchowski relation. 10 runs were performed for each measurement. All measurements were carried out in triplicate.

2.4. Production and characterization of bioplastics from insect proteins

2.4.1. Bioplastics production

Bioplastics production was carried out using proteins from larvae and pupae grown on either s-OFMSW or r-OFMSW (Fig. 1, sample "PROTEINS"), according to the protocol by Barbi et al. (2019) and Nuvoli et al. (2021) with slight modifications (Fig. 3A). Briefly, 250 mg of protein extracts were weighed and dispersed in 4.5 mL Milli-Q water. Extracts were solubilized by adding 0.5 mL of 1 N NaOH to a final pH of 11.5. The obtained protein suspensions (5 % w/v) were heated at 80 °C for 20 min and then brought to their initial volume by adding water; they were added with 125 mg of glycerol (50 % w/w on protein weight) and the resulting mixture mixed for 10 min under stirring at 400 rpm. The suspension was eventually cast onto silicone moulds: films were recovered after 24 h at 30 °C. Unless otherwise specified, films were placed in sealed plastic bags and stored in the dark at 20 °C, until further characterization.

2.4.2. Surface chemical groups of bioplastics

Surface chemical groups of bioplastics were investigated in ATR-FTIR mode using a Nicolet iS5 with KBr windows imaging system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Vee-MAX III ATR (Pyke technologies, Madison, WI, USA), with a germanium crystal. Background and sample spectra were collected after > 20 min of N_2 purge. The IR absorption spectra were recorded in the 4000–600 cm^{-1} region based on 64 scans and a resolution of 4 cm^{-1}, with a beam incident angle of 55°.

2.4.3. Mechanical properties of bioplastics

For mechanical characterization, rectangular specimens (l = 25 mm, w = 5.45 mm, n = 3 for each formulation) were punched out from cast bioplastic films, and conditioned at 30 °C and 40 % relative humidity for 24 h. Tensile stress/strain curves were obtained under quasi static conditions using a Dynamic Mechanical Analyzer (MCR702, Anton Paar) equipped with tension clamps. A preload of 0.1 N was applied, and elongation rate was set at a 2 mm min^{-1}.

2.5. Life cycle assessment

The LCA was carried out assessing bioplastics production from larvae and pupae with the goal to evaluate which source of protein was more effective in terms of (reduced) environmental impact of the system. Since the herein proposed value chain is not available on an industrial scale yet, primary data were collected for the analysis at laboratory scale, scaling up the electricity consumption. The functional unit (FU) of data collection referred to 1 kg of treated r-OFMSW, while results were presented per tonne of OFMSW potentially handled by a waste treatment facility. The analysis was carried out with SimaPro v.9.4 software and Ecoinvent 3.4 database. Impacts 2002+ was used as an impact assessment method. The system boundaries covered the processes from the OFMSW storage and its use for BSF larvae rearing to bioplastic production. The LCA did not consider the waste transport and the construction of treatment and valorization plants.

For the electricity mix the analysis referred to the Italian context while for the virgin materials to the European Union. The reference year was 2023, when the study was carried out. Details about the criteria and the complete life cycle inventory per FU are reported in Supplementary Text 1 and Supplementary Table 2, respectively.

2.6. Socioeconomic assessment

The socioeconomic analysis focused on three main frameworks: i) the innovation framework, with a patent landscape analysis (PLA); ii) the market opportunities framework, for the by-products and final products; and iii) the socioeconomic feasibility framework, aimed at identifying the elements of the Cost-Benefit Analysis (CBA).

PLA was conducted using Open data sources (Espacenet, Lens.org), in accordance with the WIPO guidelines (Trippe, 2015), to provide an overview of the main technological trends concerning the 3 main phases of the value chain: i) BSF-mediated bioconversion process of the OFMSW; ii) extraction of lipids, proteins, and chitin; and iii) preparation and characterization of protein-based bioplastics.

As to the two remaining frameworks, the analysis considered the process from the collection and management of the OFMSW (as the basis input for the larvae/pupae production line) to the choice of the main uses for insect-derived products and by-products, considering the

related economic sectors and final market opportunities. The CBA used these activities as the basis for the identification of the socioeconomic benefits that pile up along the production process, forming the so-called value chain.

If the process is evaluated in a circular bioeconomy framework, the final aim is the maximization of social welfare by weighting: i) externalities (i.e., positive or negative effects that the production has on the society) and ii) opportunity costs (i.e., benefits foregone by choosing a course of action different from what has been done before or from what might be done using the same materials, products or production processes) (OECD, 1993). Opportunity costs, that can be either positive or negative and are often related to externalities, are especially relevant from an environmental and social benefit perspective (Aleisa and Heijungs, 2022).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 7.00 (GraphPad software, La Jolla, SD, USA). Possible differences between bioconversion parameters, as well as between the solubility of protein derived from larvae and pupae, were checked by using the unpaired Student's *t*-test followed by Tukey's multiple-comparison *post hoc* test. Statistical differences between groups were considered significant at a p-value < 0.05.

3. Results and discussion

3.1. Bioconversion efficiency of the OFMSW

The s-OFMSW and r-OFMSW differed in terms of chemical composition (Table 1): the content of the main macronutrients (i.e., crude protein, lipid and fibre, and starch) was lower in r-OFMSW, while simple sugars, such as glucose and fructose, were more abundant.

The growth performance of the larvae and their efficiency in reducing and bioconverting the substrate are reported in Table 2. The maximum weight of the larvae on the two substrates was comparable, although three more days were necessary to insects reared on s-OFMSW to complete the larval period.

The difference in developmental time affected the RGR, which was higher in larvae reared on r-OFMSW. The comparable maximum weight reached by the larvae on substrates with differences in nutrient composition could be related to the different efficiency in the conversion of the ingested food. Actually, although larvae on r-OFMSW reduced the substrate to a lower extent (73.3 % compared to 83.5 % of s-OFMWS), their ECI was higher (24.1 % compared to 20.8 % of s-OFMSW). The higher conversion of r-OFMSW was likely due to an increase in the activity of digestive enzymes (i.e., proteases, amylases, and lipases), as already demonstrated for BSF larvae reared on the OFMSW (Bruno et al., 2024), although we cannot rule out a possible contribution of the microorganisms present in the substrate in its degradation (Bekker et al., 2021). Similarly, larvae grown on r-OFMSW converted nitrogen with a higher efficiency (50.4 %) compared to those on s-OFMSW (33.9 %), as shown by NCE. Finally, a considerable SR (higher than 90 %) was apparent for both diets (Table 2). However, despite variations in the bioconversion rate, all parameters were comparable to, or even better than, those reported in the literature for BSF-mediated bioconversion of this type of waste (Naser El Deen et al., 2023; Surendra et al., 2020), confirming the excellent growth and bioconversion performance of the larvae on both substrates. Moreover, despite the chemical composition of insects grown on the two rearing substrates showed slight differences in the protein, lipid, and chitin content (Supplementary Table 3), altogether these data demonstrate that the s-OFMSW well mimics the r-OFMSW.

Table 1
Chemical composition and moisture content of s-OFMSW and r-OFMSW. Values (g per 100 g of diet) are expressed on "as fed" and "dry matter" bases. The former is computed on the diet as fed to the larvae (taking into consideration the water content), the latter refers to a moisture-free basis. Values of s-OFMSW were obtained by Bruno et al. (2024).

	s-OFMSW		r-OFMSW	
Component	**As fed**	**Dry matter (%)**	**As fed**	**Dry matter (%)**
Crude proteins	7.9	22.1	3.0	14.6
Crude lipids	2.9	8.0	2.3	11.4
Crude fibre[a]	0.1	0.4	1.3	0.2
Nitrogen-free extract[b]	23.6	66.1	14.0	69.1
Ash	1.2	3.4	1.0	4.7
Starch	14.8	41.5	5.6	27.9
Glucose and fructose	2.7	7.4	2.9	14.5
Water content	64.3	–	78.4	–

[a] Includes most cellulose and insoluble lignin. [b]Includes sugars, organic acids, pectins, soluble lignin, hemicellulose, and a small percentage of cellulose.

Table 2
Developmental parameters and bioconversion efficiency of BSF larvae reared on s-OFMSW and r-OFMSW. The values are reported as mean ± SEM of at least 3 different experiments. Different letters indicate statistically significant differences between the two substrates (unpaired Student's t-test: $p < 0.05$). Values of s-OFMSW were obtained by Bruno et al. (2024). RGR: Relative Growth Rate; WRI: Waste Reduction Index; ECI: Efficiency of Conversion of Ingested Food; NCE: Nitrogen Conversion Efficiency; SR: Survival Rate.

Parameter	s-OFMSW	r-OFMSW
Larval period (days)	19.0 ± 0.6^{a}	16.3 ± 0.3^{b}
Maximum weight (mg)	260.4 ± 8.4^{a}	265.3 ± 5.8^{a}
RGR (mg mg^{-1} day^{-1})	0.07652 ± 0.00001^{a}	0.08927 ± 0.00002^{b}
Substrate reduction (%)	83.5 ± 1.6^{a}	73.3 ± 1.7^{b}
WRI (%)	6.4 ± 0.1^{a}	6.7 ± 0.2^{a}
ECI (%)	20.8 ± 0.5^{a}	24.1 ± 0.9^{b}
NCE (%)	33.9 ± 1.1^{a}	50.4 ± 1.3^{b}
SR (%)	91.1 ± 0.7^{a}	97.1 ± 1.7^{b}

3.2. Protein, lipid, and chitin extraction, and characterization of the different components

The extraction of the different biological components from larvae and pupae grown on s-OFMSW or r-OFMSW was carried out using methods aimed at maximising the purity of the protein fraction (Fig. 1, sample "PROTEINS").

For this reason, two alternative procedures for lipid extraction from samples at both developmental stages were compared: a two-step batch and a Soxhlet extraction procedure. Starting from 20 g of insect ground sample, the batch procedure required a total of 3 h and 80 mL of petroleum ether instead of 16 h ($\geq$ 16 cycles) and $\sim$ 300 mL necessary for the Soxhlet procedure; a $\sim$ 80 % purity degree was obtained with the batch procedure compared to Soxhlet. Notably, 70–80 % of petroleum ether was recovered. Since the obtainment of protein extracts with high purity required to minimize the amount of residual fats, the Soxhlet procedure was selected. It is worthy to note that for uses requiring protein samples at a lower degree of purity, the number of Soxhlet cycles can be decreased (e.g., after 6–8 cycles, $\geq$ 95 % of the total proteins are recovered with a 80 % purity) or the batch procedure can be taken into consideration.

Recovered lipids represented about $\sim$ 42 % of the dry starting material for insects reared on s-OFMSW and $\sim$ 30 % for those on r-OFMSW (Table 3), with no significant difference in lipid content between larvae and pupae reared on the same substrate. These values are higher than the average value (27 ± 11) reported from a meta-analysis of the current literature (Eriksen, 2022). Proteins were recovered by precipitation at pH of minimum solubility, as previously calculated (Smets et al., 2020): r-OFMSW larvae provided the highest amount of protein extract (16.4 %), which was 30 % more than all the other samples. A higher chitin content ($\sim$4.2 vs $\sim$ 2.9 %) was observed in pupae compared to larvae

Table 3
Extraction yields of the main BSF components. The results are expressed as the percentage of mass ratio on the starting dried sample (20 g). Values represent the mean ± SD of five replicates.

Component	s-OFMSW		r-OFMSW	
	Larvae (%)	Pupae (%)	Larvae (%)	Pupae (%)
Lipids	42.6 ± 2.9	41.9 ± 1.6	30.3 ± 3.2	30.7 ± 2.2
Protein	12.9 ± 1.0	12.9 ± 2.1	16.4 ± 3.5	12.6 ± 1.9
Defatted sample[a]	23.5 ± 2.7	36.1 ± 3.4	33.9 ± 1.0	38.0 ± 5.4
Chitin	2.9 ± 0.2	4.2 ± 0.2	5.1 ± 0.2	4.6 ± 0.1
Ash	3.4 ± 0.1	2.7 ± 0.1	5.6 ± 0.1	6.8 ± 0.3
Soluble carbohydrates[b]	1.9 ± 0.2	1.7 ± 0.8	1.3 ± 0.1	0.55 ± 0.06
Total carbohydrates[b]	2.9 ± 0.1	3.2 ± 0.1	1.8 ± 0.2	0.66 ± 0.02

[a] The dried solid pellet after removal of the fraction of soluble proteins, used for chitin extraction.
[b] Carbohydrates were determined as glucose, therefore chitin and N-acetyl-glucosamine were not included.

reared on s-OFMSW, while similar values were measured for pupae and larvae reared on r-OFMSW (4.6–5.1 %) (Table 3). The use of deep eutectic solvents, alone or coupled to physical pre-treatments, was also investigated and resulted in a ~ 3-fold lower protein recovery (data not shown).

In detail, from 1 kg of s-OFMSW a total of 73.4 g of larvae were produced, from which 9.5 g of proteins, 31.3 g of lipids, and 2.1 g of chitin were isolated; under the same conditions, 48.7 g of pupae were produced, from which 6.3 g of proteins, 20.4 g of lipids, and 2.0 g of chitin were isolated.

3.2.1. Analysis of the lipid fraction

The ten most abundant lipids in larvae and pupae reared on s-OFMSW (the condition yielding the highest lipid accumulation) detected through ESI-MS are reported in Table 4. No free C12-C18 fatty acids (FA) were present in larvae while they are present in pupae where vaccenic acid, an ω-7 FA, was the most abundant component. On the contrary, saturated triacylglycerols (TGs) were the most represented components in lipids from larvae and their amount was higher than in pupae. The same trend was recorded for mono- and poly-unsaturated lipids. These data are in agreement with the physiological processes occurring during metamorphosis in holometabolous insects. Indeed, larvae need to save lipids as energy reserve to sustain metabolic functions during pupal stage, in which the insect does not eat and organs and tissue are extensively remodelled or even completely rebuilt (Rolff et al., 2019). As a consequence, the insect developmental stage affects lipid composition: TGs, which are lipids related to storage, are the main components in larvae, while rapidly metabolically usable fatty acids are mainly present in pupae. Although we initially envisioned that BSF lipids could primarily be used for biodiesel production, the analysis of the main components of the lipid fractions prompts us to focus on alternative high-value uses, including applications for the pharmaceutical and cosmetics sectors (Tettamanti and Bruno, 2024).

Table 4
Lipidomic analysis of larvae and pupae reared on s-OFMSW. The list of the ten most represented molecules is reported. Values are reported as a percentage of the total lipid content. FA: fatty acid, TG: triacylglycerol.

	Larvae	Pupae
Lipid	**% on total lipids**	
FA 12:0 Lauric acid Dodecanoic acid		5.38
FA 16:0 Palmitic acid Hexadecanoic acid		5.00
FA 18:2 Linoleic acid (ω 6)		8.49
FA 18:1 Vaccenic acid (ω 7)		13.35
TG 34:0|TG 10:0_12:0_12:0	4.41	3.18
TG 36:0|TG 10:0_12:0_14:0	13.83	11.38
TG 38:0|TG 12:0_12:0_14:0	8.59	6.09
TG 42:2|TG 12:0_12:0_18:2	3.40	3.64
TG 42:1|TG 12:0_12:0_18:1	3.57	3.23
TG 42:0|TG 12:0_14:0_16:0	3.54	
TG 44:1|TG 12:0_14:0_18:1	3.42	
TG 46:2|TG 12:0_16:0_18:2	4.57	2.60
TG 48:2|TG 12:0_18:1_18:1	3.10	
TG 48:1|TG 14:0_16:0_18:1	3.28	

3.2.2. Analysis of the protein fraction

The analysis of the purified protein fraction (Fig. 1, sample "PROTEINS" dissolved at pH 11) for both larvae and pupae indicated a protein purity > 70 % (Fig. 2A), with extracts from insects reared on r-OFMSW possessing a lower degree of homogeneity, especially for pupae (Supplementary Table 4). The presence of a residual content of carbohydrates in the protein extracts, between 2–6 %, was apparent, with a higher amount in larval extracts. When the insoluble components were separated by centrifugation prior to the analysis, the carbohydrate content of the larvae decreased, reaching a pattern similar to that of pupae (~3%). The fraction of soluble proteins at pH 11 was > 80 %. The larvae protein suspension showed higher absorbance intensity in the UV–visible spectra (Fig. 2B), being also visibly more turbid (Fig. 2C, left). After centrifugation, a higher amount of precipitate was visible for larval protein suspensions (Fig. 2C, right), and absorbance spectra of the supernatant for larval protein suspensions became comparable to those from pupae. No nucleic acids were detected (Supplementary Table 4).

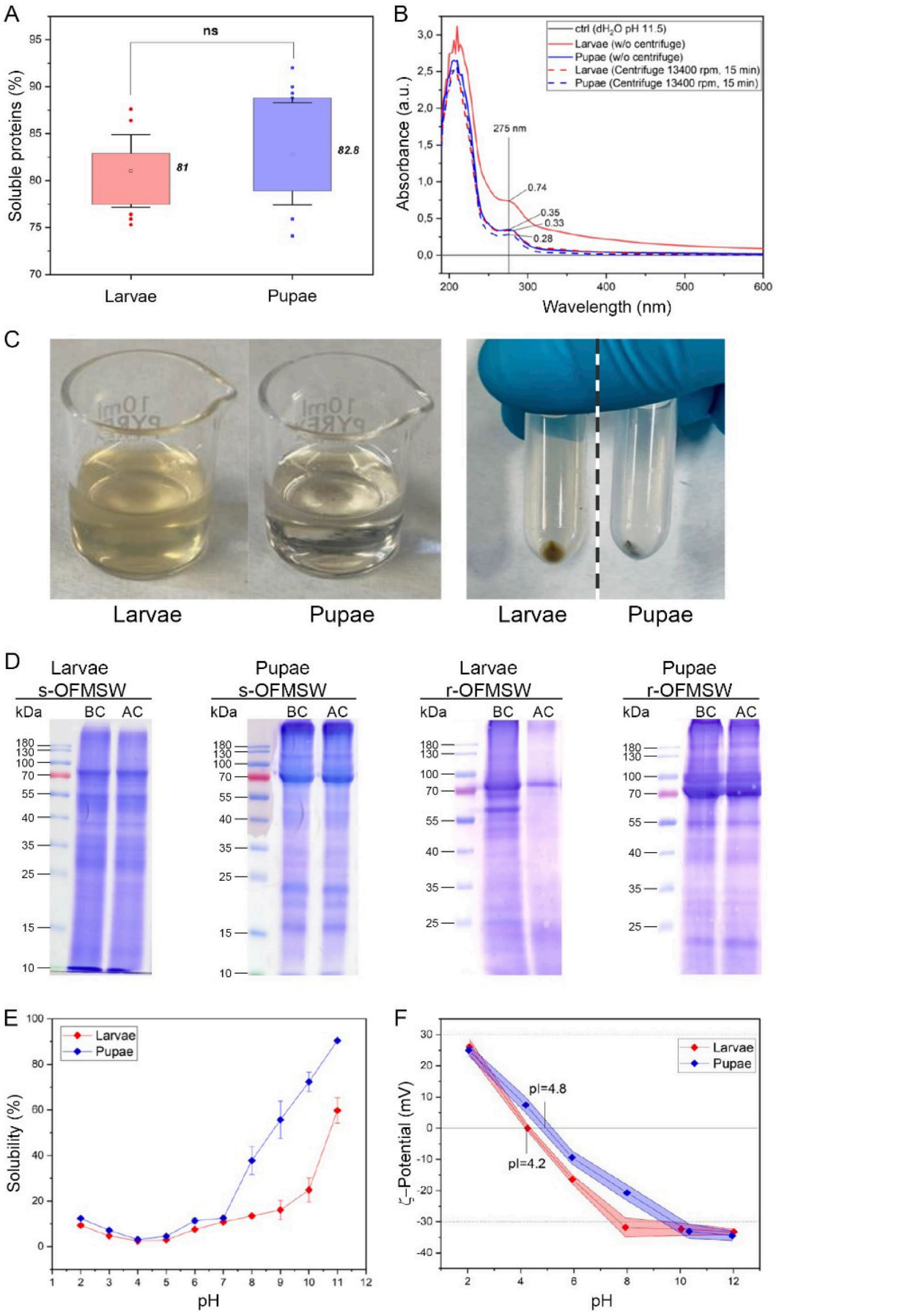
SDS-PAGE analysis showed marked differences in the band pattern between the two developmental stages, with proteins extracted from larvae more enriched in the 25 to 75 kDa region, and pupae extracts showing two main protein bands at ≈75 kDa and above 180 kDa, and three more bands in the 15–25 kDa region (Fig. 2D). Based on the quantification of NH_2 groups through TNBSA titration assay (Supplementary Table 5), longer polypeptides were present in pupal samples. CD spectra indicated that protein extracts from larvae and pupae were rich in proteins containing β-strands and turns as secondary structure elements (Supplementary Fig. 1), with differences between samples from insects reared on the two diets.

An insight on insect proteome by nLC-MS/MS analysis evidenced differences between protein extracts of larvae and pupae reared on s-OFMSW (Supplementary Tables 6 and 7). Among the most frequent validated proteins, a prevalence of structural and muscle proteins, belonging to actin, troponin, and tropomyosin families, was present in both samples, while a few enzymes (arginine kinase – for pupae only – and ATP synthase) were identified (Supplementary Table 8).

The solubility of proteins as a function of pH is reported in Fig. 2E: the solubility was lowest at pH 4 and was maximal at pH values ≥ 11 (in agreement with the fractionation method used, see Fig. 1). These values were supported by ζ-potential results (Fig. 2F). The strict correlation between particle precipitation stability and ζ-potential is indeed well described in the literature for a multitude of nanomaterials (Genovese and Lozano, 2001; Gharehbeglou et al., 2019; López-Zamora et al., 2018; Tabernero et al., 2017). In this case, an average isoelectric point of 4.2 and 4.8 was determined for larvae and pupae extracts, respectively, whilst the highest charge (absolute value) was found at alkaline pH. In particular, larvae extracts reached a ζ-potential plateau (−30 mV) at pH 8, whilst pupae showed a ζ-potential higher than 30 mV above pH 10.

3.3. Production and characterization of protein-based bioplastics

The bioplastics herein described were produced using a simple, scalable, water-based method, as outlined in Fig. 3A and detailed in Materials and methods. BSF larvae and pupae protein extracts were dispersed in alkaline water (0.1 M NaOH, pH 11.5) alongside a plasticizer (glycerol). Following the mixing process, yellowish to brownish, homogeneous, translucent and flexible free-standing films were generated by casting the solution onto polydimethylsiloxane substrates (Fig. 3B).



(caption on next page)

Fig. 2. Characterization of protein extracts. (A) Fraction of soluble proteins in larvae and pupae protein extracts, as determined by the Bradford assay. (B) Absorbance spectra of BSF larvae (red) and pupae (blue) protein extract suspensions before (continuous line) and after (dashed line) centrifugation at 13400 rpm for 15 min. (C) BSF larvae and pupae protein extracts water dispersed at pH 11.5 (left). Protein pellet after centrifugation at 13400 rpm for 15 min (right). (D) SDS-PAGE analysis of the resuspended protein extracts from larvae and pupae reared on r- and s-OFMSW. BC: before centrifugation; AC: after centrifugation. (E) Solubility of larvae (red) and pupae (blue) protein extract solutions at different pH values. (F) ζ-potential values for larvae (red) and pupae (blue) protein extract solutions at different pH. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

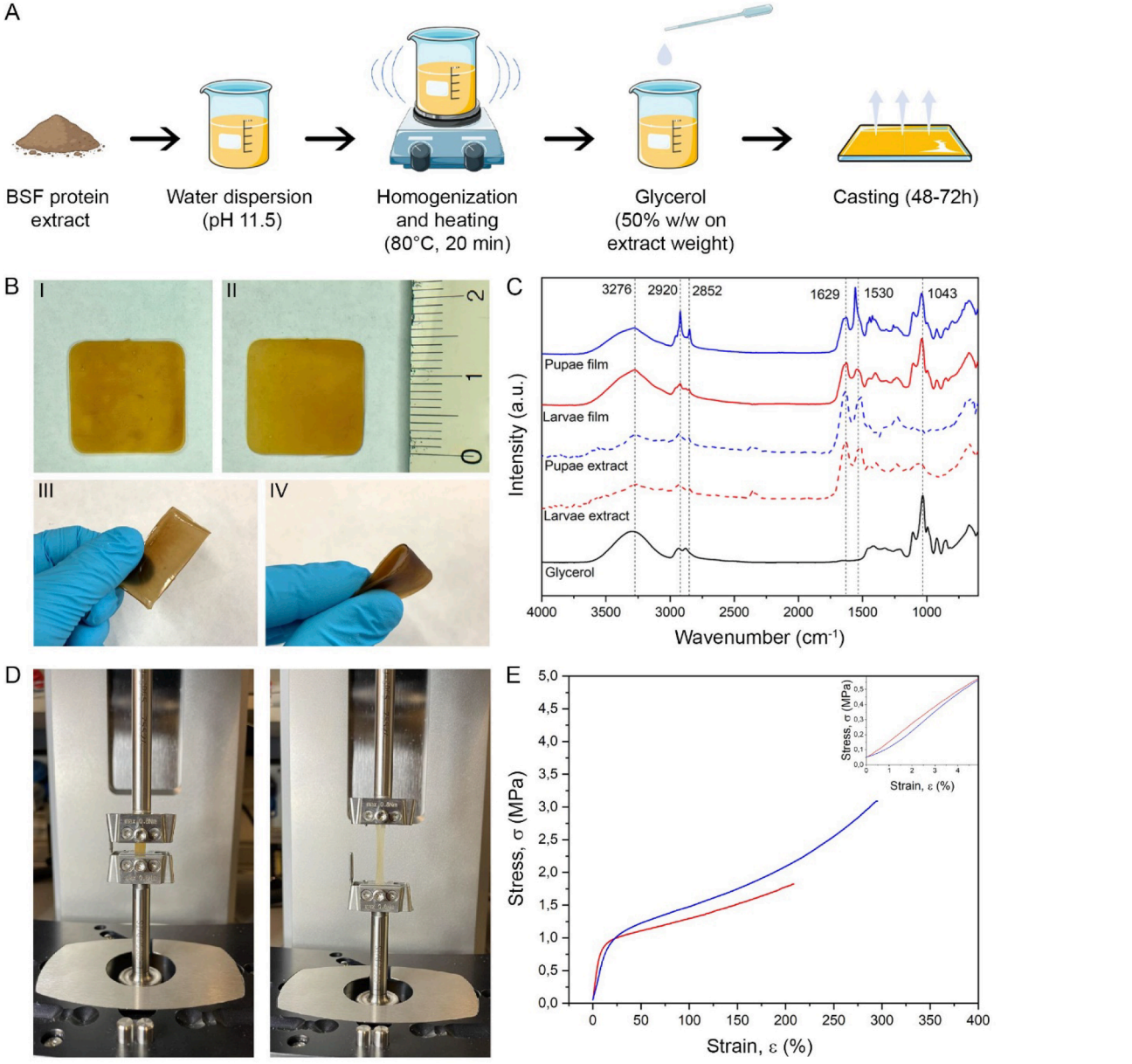


Fig. 3. Production and characterization of bioplastics from BSF larvae and pupae protein extracts. (A) Schematic process for the production of BSF protein-based bioplastics. (B) BSF protein-based bioplastics from larvae (I, III and IV) and pupae (II) extracts. (C) ATR-FTIR spectra of the protein extracts (larvae: red dashed line, pupae: blue dashed line), bioplastic films (larvae: red line, pupae: blue line) and glycerol (black line). (D) BSF pupae protein-derived film unloaded (left) and at maximum strain (right). (E) Stress–strain curves for bioplastics from BSF larvae (red line) and pupae (blue line) reared on the s-OFMSW. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

To investigate the presence of different chemical structures in films obtained from larvae and pupae, ATR-FTIR analysis was used (Fig. 3C). The analysis revealed, in both types of bioplastics, the protein characteristic Amide I (1700–1600 cm^{-1}) and Amide II (1600–1500 cm^{-1}) regions, while the strong signal at 1043 cm^{-1} was ascribed to –CO stretching of glycerol molecules (see Supplementary Text 2 for a broader outlook on the remaining modes and associated surface chemical groups). A focus on the Amide I region (Supplementary Fig. 2) revealed similar secondary structure profiles for the two groups of bioplastics, with a significant fraction of β-sheet (native (1690 and 1640 cm^{-1}) and aggregated (1624–1620 cm^{-1}) secondary structures (Supplementary Table 9), confirming the results gathered on the solubilized extracts (Supplementary Fig. 1).

Overall, these results suggest a similarity in the chemical profile of

proteins from extracts of larvae and pupae, as well as in the bioplastic films derived from them.

Mechanical tests showed that both materials had a rubber-like modulus and high deformation at break (Fig. 3D). However, films prepared from pupae protein extracts showed higher strain (ε_{break}) and stress at break (σ_{break}) compared to films from larvae (Fig. 3E), regardless of the rearing substrate (s-OFMSW or r-OFMSW) (Supplementary Fig. 3, Supplementary Table 10). We hypothesize that this different behaviour can be ascribed to the higher molecular mass of proteins from pupae extracts (Fig. 2D, Supplementary Table 5). Films' mechanical properties showed to be highly influenced by the conditioning method (Supplementary Fig. 4): for environments characterized by high relative humidity (e.g., 75 %), films displayed higher ε_{break} and lower σ_{break}. On the contrary, mimicking real conditions, after 8 days of exposure in open air (20 ± 2 °C, 50 ± 10 % R.H.) films displayed the highest values of σ_{break} and the lowest values of ε_{break}. Notably, when compared to BSF protein-based bioplastics reported in the literature (Supplementary Table 11), the pupae protein-based films herein produced displayed higher ε_{break} in each case (Supplementary Figure 5A), and comparable Young's modulus (E) and σ_{break} (Supplementary Figure 5B).

Regardless of the insect life stage, the obtained films possessed good chemical resistance and were insoluble in all tested organic solvents after 1 week (Supplementary Table 12). However, one of the major challenges associated with bioplastics (protein-based ones among them) is their inherently low water stability (Yashwant et al., 2023). In this regard, films dissolved in strong acidic (pH < 2) or alkaline (pH > 10) water solutions (Supplementary Text 2, Supplementary Figure 6, Supplementary Table 12). In neutral water, glycerol was rapidly released and high-water absorption was observed (Supplementary Figures 7 and 8), but films were macroscopically intact even after soaking for 6 days. Water uptake and volumetric variation after 24 h were higher for pupae bioplastic films, weight loss was higher for larvae films (44 % vs. 38 % in pupae) (Supplementary Table 13), and glycerol release was also faster in larvae-derived materials (25 % on dry film weight after 1 h, vs. 19 % for pupae) (Supplementary Table 14). For a detailed description of the methods behind swelling tests, quantification of water interaction parameters and glycerol release see Supplementary text 3, 4 and 5, respectively.

These results highlight the hydrophilic nature of BSF protein-based films. Notably, the macroscopic structure of each film was retained and none of them dissolved after prolonged immersion in water. This result suggests the formation of a robust protein network with water-stable interconnected structures. This network may derive from the formation of intermolecular β-sheets, as suggested by their high relative content (Supplementary Table 9). With these properties in mind, BSF protein-based bioplastics may be utilized in low-humidity contexts, as those found in printed and flexible electronics, where BSF proteins can be utilized as substrate materials or matrices for composites. Likewise, they can be used where long-term stability against environmental factors is not required, as in disposable packaging products, by exploiting the characteristic oxygen barrier properties of proteins (Purewal et al., 2024).

3.4. Life cycle assessment of the insect value chain

3.4.1. Normalization

Results of the normalization procedure are reported in Fig. 4A. The model suggested that non-renewable energy consumption, global warming potential (GWP), respiratory inorganics, and terrestrial ecotoxicity were mainly associated with the defatting procedure (Fig. 4B). This was due to energy consumption and depletion of fossil solvents like petroleum ether (modelled as Naphtha). Therefore, it seems that lipids chemical separation represents the most important process to be considered for mitigating environmental impacts.

For this reason, the search for alternative green solvents and/or solutions that allow the recovery of chemical reagents and, therefore, their

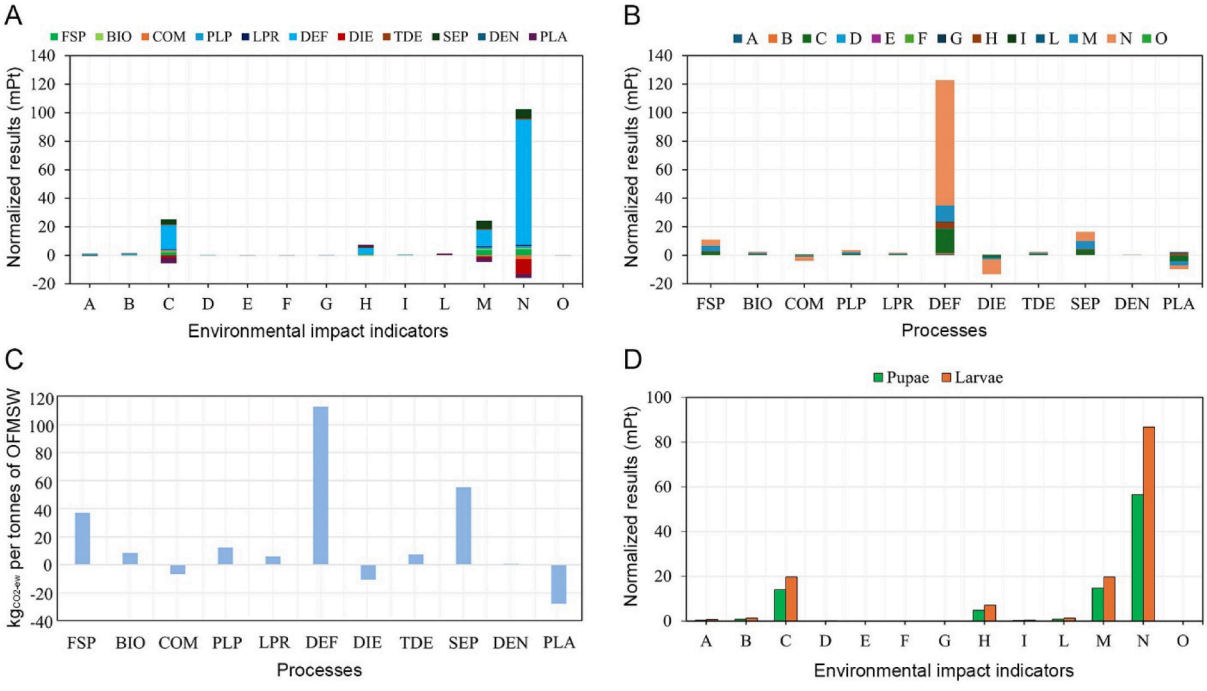


Fig. 4. Environmental profile of the OFMSW treatment by BSF larvae. Normalization of the results per (A) environmental impact indicator, and per (B) process. (C) GWP of the system and (D) comparison of the environmental impact indicators in scenarios with pupae or larvae. FSP: Feedstock pre-treatment; BIO: OFMSW bioconversion; COM: Composting; PLP: Insect growing and production; LPR: Larvae preparation for post treatment; DEF: Defatting; DIE: Biodiesel production; TDE: Treatment of defatted products; SEP: Protein separation and lyophilization; DEN: Protein denaturation; PLA: Bioplastics production. A: Carcinogens; B: Non-carcinogens; C: Respiratory inorganics; D: Ionizing radiation; E: Ozone layer depletion; F: Respiratory organics; G: Aquatic ecotoxicity; H: Terrestrial ecotoxicity; I: Terrestrial acid/nutri; L: Land occupation; M: Global warming; N: Non-renewable energy; O: Mineral extraction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

possible reuse are needed for scaling up the application. One of the main limitations of this analysis is related to the scale-up of the inventory, necessary because the industrial scale of the value chain is not yet available. Scale-up factors were added to the inventory, but this procedure can be error-prone and should be carefully assessed in future studies.

3.4.2. Contribution analysis: Focus on the global warming potential (GWP)

Quantitative results related to the GWP are reported in Fig. 4C. On balance, the system potentially contributed to generate a net GWP of almost 195 $kgCO_{2\text{-eq}}$ per ton of OFMSW, including avoided impacts. Only the defatting procedure contributed to about 57 % of the total GWP due to petroleum ether consumption. Electricity consumption was the second parameter to be considered, which contributed to the impacts during the feedstock pre-treatment and protein lyophilization. The OFMSW treatment and larvae rearing contributed with about 57.7 $kgCO_{2\text{-eq}}$ per ton of OFMSW, in line with the literature, although affected by uncertainty (Salomone et al., 2017). This represented about 30 % of the total GWP. Therefore, protein extraction for bioplastics production seems to increase the impacts of the OFMSW treatment process, while avoided impacts due to biodiesel and bioplastics production does not appear to compensate for the comprehensive impacts. Compared to conventional treatment systems, OFMSW treatment with BSF larvae seems to be an interesting option. However, the production of bioplastics from larvae does not allow to increase the environmental benefits compared to fishmeal production (Mondello et al., 2017).

3.4.3. Comparison between larvae and pupae

Different environmental profiles were obtained considering the larvae or the pupae as the input biomass of the processes (Fig. 4D). The analysis showed that pupae allowed decreasing the environmental impacts mainly due to the smaller amount of dried powder and therefore of chemical reagents needed for bioplastic and biodiesel production. On average, the environmental impacts for pupae were reduced by about 32.4 % (from 137.4 mPt with larvae to 92.8 mPt with pupae).

3.5. Socioeconomic assessment of the insect value chain

Despite the emergence of a quite recent and growing patenting trend concerning innovations in waste bioconversion processes using BSF larvae, PLA provided evidence of a substantial technological freedom to operate, finding no significant patent infringement risk, especially for what concerns the phases (ii) and (iii) of the value chain (see Section 2.6) (Supplementary Figure 9).

As for the analysis of costs, unitary costs must be considered as experimental, based on a huge desk-based research activity (Supplementary Tables 15 and 16). Only variable costs were considered on the basis of output quantities obtained on a very small and experimental scale. However, according to Pahmeyer et al. (2022), when scaled-up, the productivity of the inputs increases significantly, because of scale economies, and fixed costs can be amortized in a few years. Moreover, as the quantity of produced biodiesel does not seem suitable for sale because of the small amount, the possibility of using it as an energy source within the production process could be investigated in further research. Finally, value-added chitins from BSF were produced, as an alternative to other available chitin sources (Soetemans et al., 2020).

Concerning market opportunities, the bioplastic films produced from BSF proteins revealed a high quality and higher performance than other protein-based plastic films (Nuvoli et al., 2021). The production process of proteins was the outcome of a controlled mix of biobased elements which have been tested (s-OFMSW and r-OFMSW) and of a process guaranteeing constant performance of the biofilm. In a scale-up perspective, since the production of bioplastics directly starting from a variety of categories of organic wastes shows a high variability because of the different organic and chemical qualities of each material (Otoni et al., 2021), this process valorizes OFMSW in a circular bioeconomy and ecological transition framework and the final high and constant quality biofilm is expected to have a high market value. One of the uses, for instance, might be in the electronic device sector as a basis for biodegradable printed circuit boards, other components and, possibly, 3D printing (Luoma et al., 2022).

As to opportunity costs, a first element is related to the use of OFMSW for rearing BSF larvae instead of alternative uses like composting, and a second one to the production of bioplastics from BSF larvae/pupae instead of alternative types of biomasses (e.g., crops). Composting requires that OFMSW must be pre-processed to remove any impurity (according to the Italian law on biomasses) (Decreto Legislativo 75, 2010) and then delivered to large composting facilities. This involves non-negligible pre-processing and transport costs that the present process reduces (Montresori, 2022). The process allows the reduction of purification costs: as larvae spontaneously separate indigestible waste from digestible one, OFMSW pre-processing costs are lower than for composting. Health and environmental benefits can also rise from the reduction of pathogens in the insect-processed proteins (Chia et al., 2020) compared to those reported in OFMSW and, therefore, in compost. Furthermore, processing facilities can be smaller than composting ones and might be located near the OFMSW collection sites (Bruni et al., 2020), with a reduction of transport costs and pollution externalities. As to the second opportunity cost concerning bioplastic production, the reduced use of biomasses from the agri-food sector (that, differently from OFMSW, can also serve for human nutrition) might have an important benefit for food security policies (Food Systems Countdown Initiative, 2023).

4. Conclusions

The present study represents a proof of concept of a circular supply chain that prioritizes waste reduction and resource recovery, based on the valorization of the OFMSW through BSF larvae. This approach leverages the larvae feeding capabilities to manage waste in a sustainable manner and produce valuable insect-based bioproducts for various industrial uses. BSF larvae fed on OFMSW yielded high-purity biomolecules, which can be utilized in the frame of circular (bio)economy processes, such as the production of biodiesel from lipids, bioplastics from proteins, and food additives from chitin. LCA and socioeconomic analyses revealed that this application offers benefits beyond traditional waste uses like composting, including the production of high-quality and high-performance materials that can replace oil-based ones, such as bioplastic films with enhanced properties for flexible electronic devices, or high oxygen barrier packaging.

In this regard, bioplastics herein presented possess unprecedented stretchability when compared to current BSF protein-based bioplastics (Barbi et al, 2019; Barbi et al, 2021; Nuvoli et al, 2021). By adding active ingredients, blending with other polymers, or performing post-treatments, common issues related to protein-based materials (i.e., mechanical performance and low water stability) can be addressed, and their stability and properties further improved and expanded.

Scale-up opportunities for the herein reported process are feasible in a medium-term perspective, supported by sustainable finance and transition finance tools, highlighting the potential of these production methods in ecological transitions.

CRediT authorship contribution statement

Daniele Bruno: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marco Orlando:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Edoardo Testa:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marco Carnevale Miino:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Giulia Pesaro:** Writing – original draft,

Methodology, Data curation, Conceptualization. **Matteo Miceli:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Loredano Pollegioni:** Writing – review & editing, Writing – original draft, Conceptualization. **Vincenzina Barbera:** Investigation, Formal analysis. **Elisa Fasoli:** Investigation, Formal analysis. **Lorenza Draghi:** Investigation, Formal analysis. **Alberto Pietro Damiano Baltrocchi:** Writing – original draft, Software. **Navarro Ferronato:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raffaello Seri:** Writing – original draft, Conceptualization. **Elena Maggi:** Writing – original draft, Conceptualization. **Silvia Caccia:** Writing – original draft, Data curation. **Morena Casartelli:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Gianluca Molla:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Maurizio Stefano Galimberti:** Writing – original draft, Supervision, Conceptualization. **Vincenzo Torretta:** Writing – original draft, Supervision, Conceptualization. **Andrea Vezzulli:** Writing – original draft, Conceptualization. **Gianluca Tettamanti:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maurizio Stefano Galimberti, Vincenzina Barbera, Edoardo Testa, Elisa Fasoli, Gianluca Tettamanti, Daniele Bruno, Gianluca Molla, Marco Orlando, Loredano Pollegioni, and Morena Casartelli have patent BIO-NANOCOMPOSITE MATERIAL issued to 102022000019020. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2024.10.030.

Data availability

Data will be made available on request.

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Supplementary material – Supplementary texts

Supplementary Text 1. Criteria for the life cycle inventory.

Electricity consumption refers to the power of the machine multiplied by the hour or time of employment (primary data). This provided the first potential maximum value that could be obtained for quantifying the energy consumption as function of the time (no direct Watt measurements were conducted). For considering the real energy consumption for heating and cooling systems (e.g., air dryers and conditioners, refrigerators, heating systems), a "consumption rate" was calculated considering the maximum nominal annual energy spent and the declared annual energy consumed as provided by the suppliers. A factor equal to 5% was assumed. At the same time, a correction factor was used to scale up the laboratory devices to a potential industrial scale machine: a comparison with larger models of electric machinery (e.g., shredders, mixers, dryers…) was employed. A factor equal to 45% was used to allocate the energy consumption per process.

Operational materials and resources like water, chemicals, and solvents were collected with primary data obtained from the laboratory. For these, no conversion rates due to the scalability of the system were considered since it was assumed that chemical processes should be maintained equal also at industrial scale.

The potential avoided impacts were considered within the system. Compost production after treatment was considered to replace inorganic fertilisers with 15% phosphorus. A replacement rate equal to 0.2 was employed per kg of compost produced. At the same time, bioplastic production was considered to avoid bioplastics from polylactic acids (PLA): a replacement rate equal to 1 was used. Similarly, a replacement rate equal to 1 (optimistic scenario) was used for diesel substitution with biodiesel produced by the lipids extracted from the larvae or pupae. Secondary data were collected regarding lipids conversion from fatty acids into biodiesel (Dufour and Iribarren, 2012).

The results obtained in the LCA were presented and discussed in terms of normalisation (mPt), to identify the most impactful process and the most important environmental impact indicator. Then, the contribution analysis was carried out in relation to the global warming potential (GWP) (kgCO2-eq) which is considered the most important environmental impact indicator when assessing the BSF management system (Ferronato et al., 2023). Finally, the environmental impacts of using larvae or pupae were compared to understand the more sustainable source of proteins for the production of bioplastics.

Supplementary Text 2. ATR-FTIR analysis on bioplastic films.

Peaks maxima at 1629 cm^{-1} and 1530 cm^{-1} were assigned to C=O stretching and N-H bending vibrations, respectively (Haris and Chapman, 1995). Broad signals at 3276 cm^{-1} were associated with -OH stretching involved in H-bonds between glycerol, water, and polar groups of the polypeptide chains (Urakawa et al., 2017). Compared to films from larvae, those from pupae were characterised by more intense bands at 2840 and 2920 cm^{-1}, a region ascribed to C-H stretching patterns of methyl and methylene groups. Also, a narrow intense band occasionally occurred in the Amide II region with a maximum peak at 1560 cm^{-1}, usually associated with in-chain N-H bending vibration, COO- asymmetric stretching or single amino acids (Pereira et al., 2019).

Supplementary Text 3. Bioplastics resistance to organic solvents.

Bioplastic resistance to organic solvents was assessed by soaking films in selected common organic solvents with a wide range of Hildebrand solubility parameters. Circular specimens (f = 5 mm) were punched out of films and soaked for up to 1 week into the following solvents: hexane, toluene, isopropanol, methanol, ethanol, acetone, ethyl acetate, DCM, THF, DMSO, and DMF. Bioplastic resistance to each solvent was then assessed by visual observation of the sample integrity.

Supplementary Text 4. Description of swelling tests.

Water uptake tests were conducted in Milli-Q water solutions at room temperature. Films were weighed at time zero (W_0), then submitted to dehydration in a ventilated oven at 80 °C for 24 h and weighed again (W_D). Films were next immersed in Milli-Q water containing 100 µg/mL with benzalkonium chloride (BAC) to prevent the growth of microorganisms. Weights of swollen films (W_S) were recorded at 5 and 30 minutes, then after 1, 2, 4, 6 h and every 24 h for 6 days. Before weighing, samples were taken out the solution and gently dabbed with absorbing paper. At the end of the test (i.e., 6 days), films were dried in a ventilated oven at 80 °C for 24 h and weighed (W_F). At least six different specimens were tested for each sample.

Supplementary Text 5. **Quantification of water interaction parameters from gravimetric data of swelling tests.**

1. Moisture content (M.C.%), i.e., the amount of water naturally presents in films after the production process:

$$M.C.\% = \frac{W_0 - W_D}{W_0}$$

2. Water uptake (W.U.%), i.e., the amount of water absorbed by the film after specific times of immersion in water:

$$W.U.\% = \frac{W_S - W_G}{W_G}$$

3. Film solubility (F.S.%), i.e., the amount of material lost (i.e., solubilized or precipitated from the sample) after the swelling test:

$$F.S.\% = \frac{W_D - W_F}{W_D}$$

4. Volumetric variation (ΔV%), i.e., the variation of volume of the film after 4 h of immersion in water compared to the initial volume of the dry film:

$$\Delta V\% = \frac{V_S - V_D}{V_D}$$

Where V_S is the volume of the swollen sample and V_D is the volume of the dry sample.

Supplementary Text 6. Quantification of glycerol release from bioplastic films after water swelling.

The quantification of glycerol release from larvae and pupae-derived bioplastic films was carried out by a modified version of swelling tests. Briefly, films were cut into pieces of standardized size (5x10 mm) and conditioned for 24 h at 80 °C in a ventilated oven, to remove excess water. The film was then weighed (W_D) and immediately immersed in a known volume (2 mL) of deuterated water (D_2O) for 1 h. A known amount of benzamide (approximately 2 mg) was solubilized with 1 mL of eluates internal standard for NMR analyses. The amount of glycerol in the eluate (W_{gly}) was calculated according to Gaeta et al. (2012):

$$W_{gly} = W_{benz} \times \frac{I_{gly}}{I_{benz}} \times \frac{N_{benz}}{N_{gly}} \times \frac{M_{gly}}{M_{benz}}$$

where W_{benz} is the weighed mass of benzamide; I_{gly} and I_{benz} are the integrals of the signals of glycerol and benzamide, respectively; N_{gly} and N_{benz} are the numbers of nuclei responsible for the integrated signals (N_{benz} for benzamide = 1; N_{gly} for glycerol = 1); M_{gly} and M_{benz} are the molecular masses of glycerol and benzamide, respectively.

Glycerol loss relative to the total weight of the film (G.R. (%)) was calculated as follows:

$$G.R.(\%) = \frac{W_{gly} \times 2}{W_D} \times 100$$

Supplementary material – Supplementary tables

Supplementary Table 1. Description of the indexes used to evaluate the efficiency of the bioconversion process and growth performance.

Index Name	Equation	Explanation
Relative Growth Rate (RGR)	$(B_{fin} - B_{ini}) / (t \times B_{fin})$	B: total amount of the insect biomass at the end (B_{fin}) and the beginning (B_{ini}) of the bioconversion process; t: days spent by the larvae on the substrates
Substrate reduction (D)	$((W - R) / W) \times 100$	W: total amount of substrate provided to the larvae; R: rearing residue (frass) at the end of bioconversion process
Waste Reduction Index (WRI)	(D / t)	
Efficiency of Conversion of Ingested food (ECI)	$[(B_{fin} - B_{ini}) / (W - R)] \times 100$	
Nitrogen Conversion Efficiency (NCE)	$[(N_{ins} \times B_{fin}) / (N_w \times W)] \times 100$	N: nitrogen content of insects at the end of the bioconversion process (N_{ins}) and of substrate provided to the larvae (N_w)
Survival rate (SR)	$(I_{fin} / I_{ini}) \times 100$	I: number of insects at the end (I_{fin}) and at the beginning (I_{ini}) of the bioconversion process

103 **Supplementary Table 2. Inventory analysis (FU = 1 kg of OFMSW).**
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Process		Unitary process	Description	Energy	Materials	Source of data (Ecoinvent database)
Phase 1 – OFMSW treatment and larvae breeding						
1.	Feedstock pretreatment	Cooling system (Fridge)	Food waste conservation	0.08993 kWh	-	
		Shredding	Waste size reduction with knife rotors	0.001008 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S
2.	OFMSW bioconversion	Air conditioning	Eggs and neonate larvae incubator	0.01722 kWh	-	
		Moisturizing	Substrate preparation	-	Water: 623 g	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Neonate larvae breeding	Broiler to start growing the first stage larvae and to maintain the colony.	-	Chicken feed: 1.62 g	Broiler compound feed, at processing {FR} Economic, S
3.	Composting	Waste post treatment	Composting of remaining waste in centralized treatment plant	-	Organic Waste: 136 g	Biowaste {RoW}| treatment of biowaste, industrial composting | Cut-off, S
		Substitution of chemical fertilizer	50% of composting yield, 20% substitution of chemical fertilizers (NPK) based on P content.	-	Substitution of Fertilizer: 13.6 g	NPK compound (NPK 15-15-15), at plant {RER} Economic, S
4.	Pupae growing and larvae production	Washing	Pupae washing for colony maintenance.	-	Water: 5 g	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Air conditioning	Constant temperature and humidity for pupae incubator and growing	0.01066 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S
		Lighting	Room lights for BSF mating	0.01851 kWh	-	
		Air conditioning	Constant temperature for adults growing	0.00737 kWh	-	
		Waste removal	Leftovers from the love cage (dead flies and CF left) to be disposed of.	-	Organic waste: 0.4 g	Municipal solid waste {IT}| treatment, incineration | Cut-off, S
		Humid chamber	Humidity ensured within the growing area	-	Water: 12.7 g	
5.	Larvae preparation for post treatment	Washing	Larvae cleaning for post treatment (residuals removal).	-	Water: 333.33	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Freezing	Larvae storing before drying and for post treatment.	0.00007 kWh	-	
		Drying	Larvae drying for post treatment.	0.00803 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S
		Shredding Output – dried larvae powder: 73.38 g	Trituration of the larvae for powder production.	0.00822 kWh	-	
Phase 2 – Proteins extraction						
6.	Defatting	Mixing	Solvent and larvae powder mixing for lipids separation.	0.00991 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S

		Centrifugation	Batch system for lipids separation from the defatted.	0.02580 kWh	-	
		Rotavapor	Process needed to recover the solvent.	0.03797 kWh	-	
		Solvent for lipids extraction Output – lipids: 31.19 g Output – defatted (57.5%): 42.19 g	Petroleum ether employed as a solvent in batch systems.	-	Naphtha: 366.9 mL (density = 0.666 g ml^{-1})	Naphtha {RoW}| market for | Cut-off, S
7.	Biodiesel production*	Methanol	Chemical compound to obtain biodiesel from lipids.	-	Methanol: 0.000111 kg	Methanol, from biomass {RoW}| methanol production, from synthetic gas | Cut-off, S
		Solvent	Water used for making the Esterification reaction.	-	Water: 0.00007 kg	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Neutralization	NaOH used for increasing the pH of the solution	-	NaOH: 0.0001 kg	Neutralising agent, sodium hydroxide-equivalent {GLO}| market for | Cut-off, S
		pH reduction	Acid employed for esterification/ transesterification	-	HCl: 0.00018 kg	Hydrochloric acid, without water, in 30% solution state {RER}| market for | Cut-off, S
		Mixing and heating	Heating system to complete the esterification and transesterification	0.00059 kWh	-	
8.	Treatment of defatted products	heating and mixing	System to separate the supernatant from the defatted.	0.01234 kWh		Electricity, medium voltage {IT}| market for | Cut-off, S
		Water consumption	Water for chemical reaction (solvent)	-	Water: 2.64 g	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Neutralization	NaOH consumption for the chemical reaction to obtain the supernatant.	-	NaOH: 1898.71 mg	Neutralising agent, sodium hydroxide-equivalent {GLO}| market for | Cut-off, S
		Centrifugation Output: Supernatant: (60.9%): 25.7 g	Residual removal from the defatted to obtain the supernatant.	0.00226 kWh	-	
9.	Proteins separation and lyophilization	Centrifugation	Separation of proteins from the supernatant after chemical treatment.	0.09034 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S •
		Acid solution	Chemical reaction able to separate proteins from the supernatant.	-	HCl: 796.57 mg	Hydrochloric acid, without water, in 30% solution state {RER}| hydrochloric acid production, from the reaction of hydrogen with chlorine | Cut-off, S
		Wastewater treatment	Treatment of residual liquid waste to be treated in centralized treatment plants.	-	Wastewater: 16.19 g	Wastewater, average {RoW}| market for wastewater, average | Cut-off, S

		Freezing	Cooling system to lyophilize the proteins obtained	0.0003 kWh	-	
		Drying	System able to minimize the water content within the sample	0.05608 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S
Phase 3 – Bioplastics production						
10.	Proteins denaturation	Chemical reaction	Water employed as a solvent needed to proceed with proteins denaturation.	-	Water: 44.69 g	Tap water {Europe without Switzerland} | market for | Cut-off, S
		Neutralization	NaOH dissolved in the solvent to increase the pH of the solution.	-	NaOH: 456.36 mg	Neutralising agent, sodium hydroxide-equivalent {GLO}| market for | Cut-off, S
		Heating	Denaturation of the proteins at high temperature.	0.0004 kWh	-	
11.	Bioplastics production	Mixing	Mingling the plasticizer and denaturised proteins to produce bioplastic.	0.00015 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S
		Addition of plasticizers	Addition of glycerol to the solution to start the reaction.	-	Glycerol: 4.75 g	Glycerine {RER}| market for glycerine | Cut-off, S
		Drying	Desiccation of the film to obtain plastic.	0.00479 kWh	-	Electricity, medium voltage {IT}| market for | Cut-off, S

*Data collected from (Dufour and Iribarren, 2012).

Supplementary Table 3. Chemical composition of larvae and pupae grown on s-OFMSW or r-OFMSW. Values are expressed as percentage on dry matter content. Values of s-OFMSW were from Bruno et al. (2024).

	s-OFMSW		r-OFMSW	
Component	**Larvae (%)**	**Pupae (%)**	**Larvae (%)**	**Pupae (%)**
Crude proteins	36.2	42.3	33.4	35.1
Crude lipids	43.7	40.1	38.5	41.3
Nitrogen-free extract[a]	11.2	7.1	16.7	11.9
Ash	3.7	2.8	4.8	4.9
Chitin	5.2	7.7	6.6	6.8

[a]Includes sugars, organic acids, pectins, soluble lignin, hemicellulose, and a small percentage of cellulose.

Supplementary Table 4. Protein extract composition. The results are expressed as the mass ratio percentage with respect to the dried protein extract. Values represent the mean ± SD of two replicates.

	Larvae		Pupae	
	s-OFMSW (%)	**r-OFMSW (%)**	**s-OFMSW (%)**	**r-OFMSW (%)**
Protein[a]	93.6 ± 16.8	70.6 ± 19.8	87.8 ± 11.5	93.3 ± 33.9
Carbohydrates	5.7 ± 0.7[b]	3.7 ± 1.3	3.4 ± 0.2	1.9 ± 0.7
DNA	b.d.	b.d.	b.d.	b.d.

[a] Determined using the ninhydrin method
[b] When the resuspended protein extract from larvae reared on s-OFMSW was centrifuged prior to measuring the carbohydrates recovery, the value decreased to 3.8 ± 0.1%.
b.d., below detection (< 0.1 %)

Supplementary Table 5. NH_2 titration results from TNBSA assay (mmoles/g of extract) for larvae and pupae protein extracts (insects reared on s-OFMSW). The amount of -NH_2 functional groups was assessed by the TNBSA assay following a standardised protocol provided by Thermo Fisher Scientific Inc., USA. Briefly, protein extracts were solubilized at a concentration of 200 µg/mL in Milli-Q water adjusted with 1 M NaOH to a pH value of 11.5. Protein suspensions were next heated for 20 minutes at 80 °C. 150 µL of solubilized extracts were mixed with 75 µL of 0.01% TNBSA (500x Stock solution, Sigma Aldrich) in a transparent 96-well plate. After 2 hours of incubation at 37 °C and 90% relative humidity, the absorbance intensity at 348 nm was recorded using a Synergy H1 microplate reader (BioTek). Standard curves were generated with known samples of glycine. Analyses were made in triplicate for each tested replicate.

	NH_2 [mmol/g]				
	200 µg/mL	**100 µg/mL**	**50 µg/mL**	**Mean**	**SD**
Larvae extracts	0.45	0.47	0.48	0.47	± 0.02
Pupae extracts	0.35	0.34	0.30	0.33	± 0.01

Supplementary Table 6. Lists of complete identifications of proteins from larvae reared on s-OFMSW, obtained by three replicates. Validated proteins, commonly present in all three replicates, are highlighted in yellow.

Supplementary Table 7. Lists of complete identifications of proteins from pupae reared on s-OFMSW, obtained by three replicates. Validated proteins, commonly present in all three replicates, are highlighted in yellow.

Supplementary Table 8. Proteins identified in all 3 biological replicates of protein fractions from larvae and pupae reared on s-OFMSW by nLC-MS/MS analysis, mainly belonging to structural and muscular protein families.

Larvae (s-OFMSW)				
Protein description	**Accession number**	**MW (Da)**	**Mascot score**	**Number of peptides sequences**
ATP-Synthase	Q05825	54074	571	7
Actin-2, muscle-specific	P45885	42118	3157	5
Actin-87E	P10981	42174	3148	6
Tropomyosin-2	Q1HPQ0	32823	589	6
Actin, muscle-type A2	P07837	42232	634	4
Pupae (s-OFMSW)				
Protein description	**Accession number**	**MW (Da)**	**Mascot score**	**Number of peptides sequences**
ATP-Synthase	Q05825	54074	564	8
Actin-2, muscle-specific	P45885	42118	1186	6
Actin-87E	P10981	42174	1207	6
Arginine Kinase	P48610	40126	173	2
Tropomyosin-2	Q1HPQ0	32823	1050	11
Tropomyosin-2 (Isoform 3)	Q1HPU0-3	32558	1499	15
Tropomyosin-1, muscle-specific	Q1HPU0	32603	1834	14
Tropomyosin Lep s 1.0101	Q8T380	32508	94	6
Troponin 1	P36188	30234	190	5

Troponin C	P47949	17742	274	3

Supplementary Table 9. Quantification of relative secondary structure for larvae and pupae-derived bioplastics. Relative secondary structure quantification was performed through deconvolution of secondary derivative peaks from Supplementary Figure 2, as described in previous research (Barreto et al., 2020; Kamada et al., 2021; Shimanovich et al., 2015; Yang et al., 2015). Data processing was performed with the software Origin PRO, 2019.

Secondary structure	Larvae (%)	Pupae (%)
β-sheet	19.4	18.1
β-turn	10.7	11.4
α-helix	11.1	11.9
Random coil	14.8	15.9
Intermolecular β-sheet	44.0	42.7
Total	100.0	100.0

Supplementary Table 10. Mechanical parameters derived from quasi-static tensile tests on bioplastic films from larvae and pupae reared on s-OFMSW and r-OFMSW.

	E (MPa)	ε_{break} (%)	σ_{break} (MPa)
Larvae (s-OFMSW)	16.5 ± 3.5	169 ± 58	1.8 ± 0.2
Pupae (s-OFMSW)	11.1 ± 1.9	280 ± 25	2.8 ± 0.5
Larvae (r-OFMSW)	12.5 ± 2.5	226 ± 55	2.0 ± 0.2
Pupae (r-OFMSW)	12.9 ± 1.8	317 ± 55	2.0 ± 0.4

Supplementary Table 11. Table of BSF protein-based bioplastics from reference studies and their main mechanical properties.

Protein	Strategy	σ_break (MPa)	ε_break (%)	E (MPa)	Ref.
BSF protein (prepupae)	Proteins plasticized with glycerol	1-2.5	30-45	n.a.	Barbi et al., 2019
BSF protein (prepupae)	Compression molding of BSF proteins (10% w/w) blended with LDPE	6.17	20.9	119	Barbi et al., 2021
BSF protein (prepupae)	Proteins plasticized with glycerol, crosslinked with citric acid	6.45	67.2	113	Nuvoli et al., 2021

Supplementary Table 12. Solvent resistance of larvae and pupae protein-based bioplastics after one week of incubation at 20 °C.

Solvent	Larvae	Pupae
Hexane	v	v
Toluene	v	v
2-propanol	v	v
Methanol	v	v
Ethanol	v	v
Acetone	v	v
Ethyl actetate	v	v
Dichloromethane (DCM)	v	v
Tetrahydrofuran (THF)	v	v
Dimethylsulfoxide (DMSO)	v	v
Dimethylformamide (DMF)	v	v
Water (pH 2)	x	x
Water (pH 4)	v	v
Water (pH 6)	v	v
Water (pH 8)	v	v
Water (pH 10)	x	x
Water (pH 12)	x	x

V = resistance

X = dissolution

Supplementary Table 13. Water interaction parameters of bioplastic films from proteins fraction of larvae and pupae reared on s-OFMSW.

	Larvae	Pupae
Moisture content (M.C., %)	21 ± 1	21 ± 2
Film solubility (F.S., %)	44 ± 1	38 ± 2
Water uptake at 24h ($W.U._{24h}$, %)	260 ± 42	305 ± 76
Sol/Gel fraction	0.77 ± 0.03	0.62 ± 0.06
Volumetric variation (ΔV%)	292 ± 26	355 ± 44

Supplementary Table 14. Quantification of glycerol release from bioplastic films after water swelling.

	Glycerol release (% on dry film weight)
Larvae	25 ± 3
Pupae	19 ± 2

Supplementary Table 15. Costs and revenues along the value chain - Unitary production costs and revenue prices.

COSTS	Costs	Measure unit	Source	Notes
Energy (industrial uses)	0.088	€/kWh	Price cited in Centro Studi Confindustria, Interest Rates, NRR, Superbonus, Energy: what will happen to Italian growth? Italian Economic Outlook 2024-2025 Spring 2024	For Italy, the average price of electricity for industrial uses, the so-called PUN, was 88 €/MWh in March 2024
Water	0.0021	€/L=kg	Elaboration by The European House – Ambrosetti on data from Global Water Intelligence, ARERA and DANVA, 2024.	For Italy, the estimated average tariff for water for the year 2022 is 2.1 €/m^3
Chicken feed	6.2	€/kg	Use of an experimental reference price. Poultry blood flour. https://www.cbs-carpfishing-boiles-selfmade.it/prodotto/farina-di-pollo-novita/	Reference measure: €62 for 10 kg
Substitution of fertilizer	3.6	€/kg	Use of an experimental reference price. Multipurpose organic fertilizer. https://www.flortis.it/ita/cura-del-verde/bio-naturali/concimi-solidi/biologico-universale-concime-pellet-5-kg?eshop=true	Reference measure: €17.99 for 5 kg
Naphtha	1.8	€/L	Price registered in Switzerland February 2024. https://www.ticinonews.ch/ticino/nafta-scende-il-prezzo-ma-aumentano-le-persone-che-rimangono-al-freddo-389924	0.74 kg/L In Italy Naphtha is quite not used normally and it was necessary to refer to a different market. CHF=EUR in June 2024
Methanol	1.688	€/kg	Use of an experimental reference price. https://www.letslab.it/metanolo-25-l.lab	0,790 kg/L Reference price €53.42 for 25 L

NaOH (plastic)	2.0496	€/kg	Use of an experimental reference price. https://tecnolatte.it/ita/e-commerce/prodotto/706/soda-caustica-in-soluzione-idrossido-di-sodio-30-in-fusto-da-25-kg	Reference measure €51.24 for 25 kg
Glycerol	8.605	€/L	Use of an experimental reference price. https://www.laboratoriumdiscounter.nl/it/glicerolo-997-puro-commestibile.html	1.26 kg/L Price without Tax
HCl	0.148	€/kg	Use of a reference price for Europe June 2023. https://www.pricepedia.it/it/magazine/article/2023/07/03/il-mercato-europeo-dellacido-cloridrico/	Reference measure €148/T
Price of chitin	135	€/kg	Use of a reference price for US June 2024. https://chitolytic.com/chitosan-products/chitin-supply/	USD=EUR in June 2024 The price level depends on chitin purity, which seems superior if extracted from BSF larvae than from crustacean. See Triunfo et al. (2022)
Price of bioplastic	5	€/kg	Average obtained using the prices mentioned in Barletta M. et al. (2022)	
Price of biodiesel	1.3	€/kg	Use of a reference price for US June 2024. https://afdc.energy.gov/fuels/prices.html	USD=EUR in June 2024

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233 **Supplementary Table 16. Variable costs and outputs along the value chain.**

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	INPUT per 1 kg OFMSW	Measure unit	Costs €/kg	Prices €/kg
OFMSW treatment and larvae breeding				
INPUT				
Energy	0.16102	kWh/kg	0.01416976	
Water	974.03	g/kg	0.00204546	
Substitution of Fertilizer	13.6	g/kg	0.04896	
Chicken feed	1.62	g/kg	0.010044	
OUTPUT				
Organic waste	136.4	g/kg		
Dried larvae powder	73.38	g/kg		
Proteins extraction – defatting per kg OFMSW				
INPUT				
Energy	0.07368	kWh/kg	0.00648384	
Naphtha	366.9	ml/kg	0.66042	
OUTPUT				
Lipids	31.19	g/kg		
Defatted	42.19	g/kg		
Proteins extraction - biodiesel production per kg OFMSW				
INPUT				
Energy	0.00059	kWh/kg	0.00005192	
Methanol	0.000111	g/kg	1.8737E-07	

Water	0.00007	g/kg	1.47E-10	
NaOH	0.0001	g/kg	2.0496E-07	
HCl	0.00018	g/kg	2.664E-08	
OUTPUT				
Biodiesel	0.98487236	g/kg		0.001280334
Glycerol*	0.00011389	g/kg		
Salts to landfill*	0.00000886	g/kg		
Hazardous liquid waste*	0.00002364	g/kg		
Proteins extraction - treatment of defatted products per kg OFMSW				
INPUT				
Energy	0.0146	kWh/kg	0.0012848	
Water	2.64	g/kg	5.544E-06	
NaOH	.89871	g/kg	0.0038916	
OUTPUT				
Supernatant* (60.9%)	25.7	g/kg		
Proteins extraction - proteins separation and lyophilization per kg OFMSW				
INPUT				
Energy	0.14672	kWh/kg	0.01291136	
HCl	0.79657	g/kg	0.00011789	
Wastewater	16.19	g/kg		
OUTPUT				
Chitin	0.0021	g/kg		0.0002835
Bioplastics production per kg OFMSW				

INPUT				
Energy	0.00534	kWh/kg	0.00046992	
Water	44.69	g/kg	9.3849E-05	
NaOH	0.45636	g/kg	0.00093536	
Glycerol	4.75	g/kg	0.03243948	
OUTPUT				
Bioplastics				0.001916869

* *The quantities of these outputs are negligible and have not therefore been considered in the Unitary Revenues Prices in the table*

Technical notes for Supplementary Tables 15 and 16:

- If not differently cited, the time reference for the data found in the web is mid June 2024
- In June 2024 the exchange rates USD/EUR and CHF/EUR were quite equal
- The Unitary Costs and Revenues Prices have to be considered experimental, based on a huge desk-based research activity
- The tables refer to a Cost-Revenue Analysis, which therefore do not consider the Benefits, that is the positive externalities and other values produced along the value chains and as final outputs, which cannot be quantified using the same measure units. Table 17 summarizes the main Benefits.
- Only variable costs have been considered in the tables. According to Pahmeyer et al. 2022, we assume that fixed costs can be amortized in a few years.
- The output quantities have been obtained on a very small and experimental scale. According to Pahmeyer et al. 2022, when scaled-up, the productivity of the inputs increases in a significant way, like for energy, because of the reaching of scale economies. Moreover, as the production of biodiesel seems not suitable for selling, a hypothesis could be assumed about the possibility of using it as an energy source inside the production process.
- The input quantities for the costs assessment have been considered in accordance with the system flows of the LCA Inventory analysis indicated in the Supplementary Table 2.

254 **Supplementary Table 17. Benefits which cannot be directly monetized.**

	Values	Main Source
Food Security	The production costs of bioplastics are dependent on the development of feedstock prices, so far contributing in the increase of food prices	Döhler et al., 2022; European Bioplastics, 2021; Food Systems Countdown Initiative, 2023
Bioconversion of fuels	Contribution in the production of high-quality biofuels over time reducing the dependency from fossil fuels	Kee et al., 2023
Bioconversion of plastics	Contribution in the production of high-quality bioplastics, over time reducing the dependency from oil derived plastics and contributing in the ecological transition	Cámara et al., 2022; Döhler et al., 2022; Luoma et al., 2022; Nuvoli et al., 2021
Bioconversion of other non-renewable or non-biological materials	Contribution in the production of high-quality and high-performance bioplastics to be used in many different sectors, replacing other fossil or non-renewable materials	Cámara et al., 2022; Luoma et al., 2022
OFMSW management	Increase in the values obtained from the separated collection of waste, reduction in the need for OFMSW purification before treatments and reduction of fixed costs and transportation distances of waste compared to the production of Compost and Biomethane	Bruni et al., 2020
OFMSW valorization	Increase in the value added of the OFMSW value chain, resulting in a system of high-quality and high-value added output (bioplastics, biofuels and chitins)	Evidence from the research activities; Cámara et al., 2022; Otoni et al., 2021
Health and environment	Increase in the quality and safety of products used for human needs, like in medicine and pharmacology	Cámara et al., 2022; Chia et al., 2020; Döhler et al., 2022
Research, knowledge and Innovation driver	Contribution to the development of new and innovative bioconversion processes	Patent Landscape Analysis conducted in the research activities; evidence from the research activities
Transition to circular bioeconomy	Contribution in the enhancement of circular economy and bioeconomy and reduction in the use of non-	Cámara et al., 2022; Triunfo et al., 2022

	biodegradable and non-biological plastics in many markets	
Sustainable development goals	Contribution in reaching SDGs 2, 3, 9, 11, 12	Ravi et al., 2020

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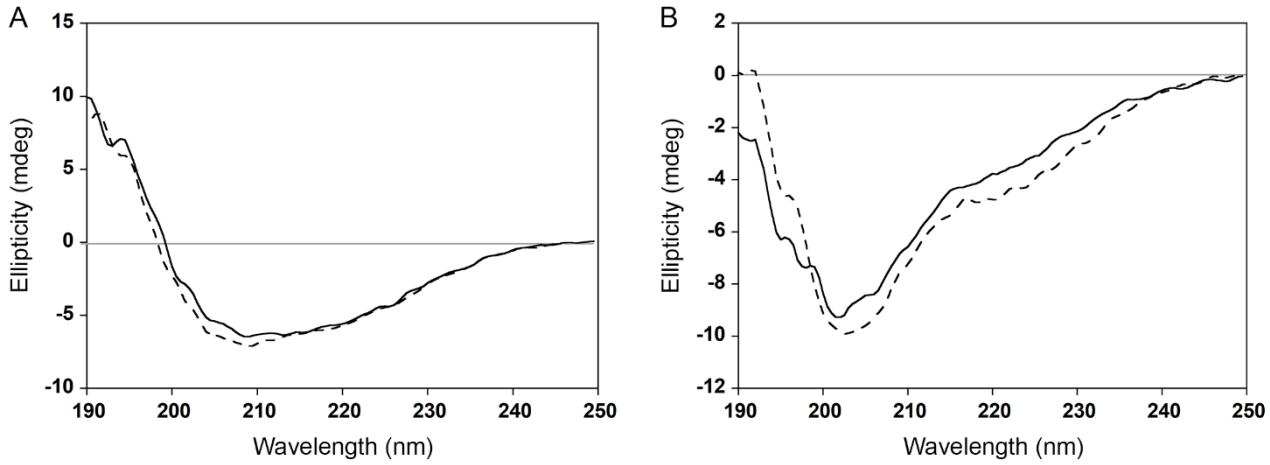
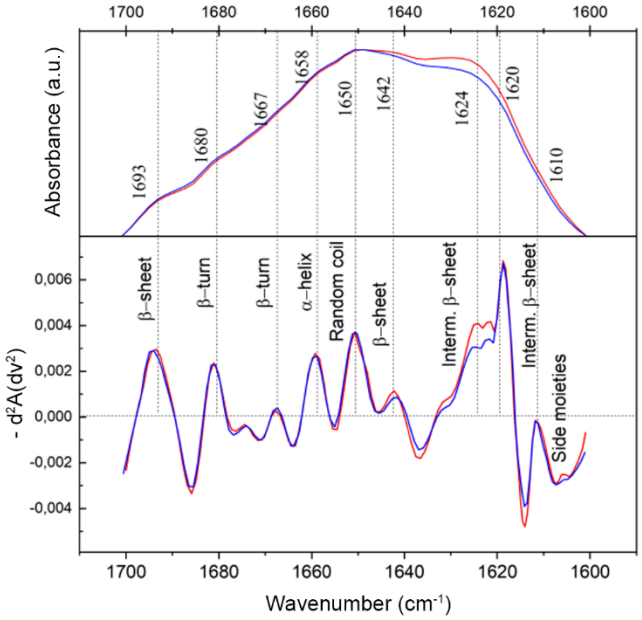
Supplementary material – Supplementary Figures

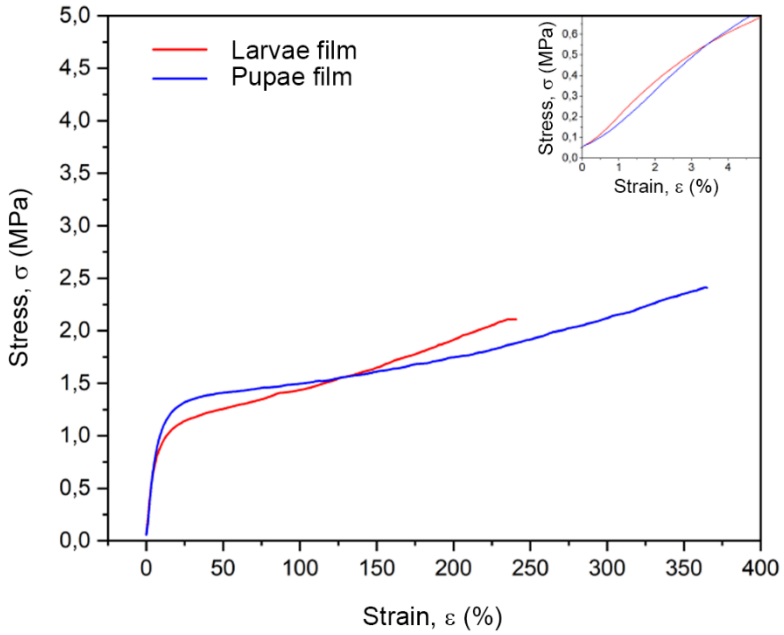
Supplementary Figure 1. Circular dichroism (CD) spectra of protein isolated from larvae (solid line) and pupae (dashed line) reared on s-OFMSW (A) and r-OFMSW (B). CD spectra were recorded at 20 °C in 10 mM potassium phosphate buffer, pH 8.5, and have been corrected for the buffer contribution. Samples at a final concentration of 0.2 mg protein/mL were incubated on a rotary shaker for 30 min, at room temperature. The samples were centrifuged at 16060 g for 20 min at 4 °C, and the supernatant used for the measurement.



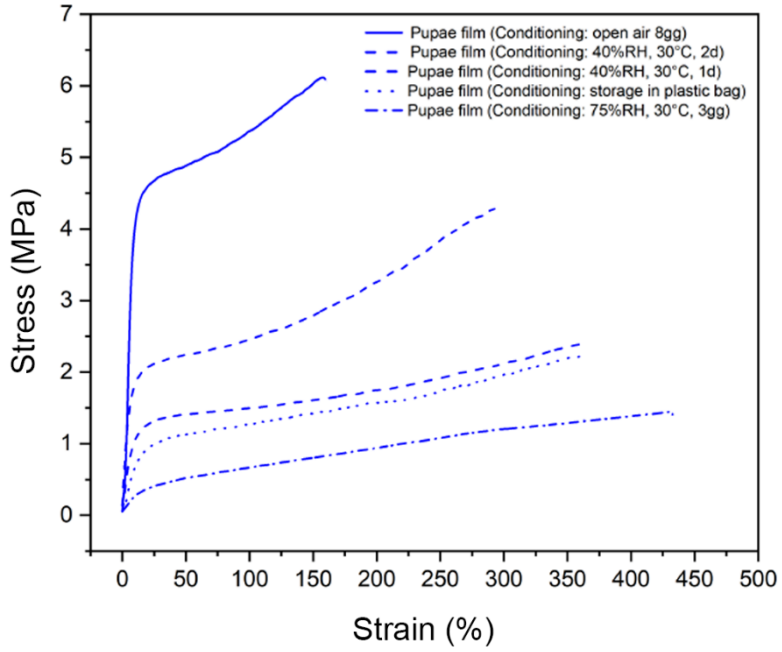
Supplementary Figure 2. FTIR-ATR spectra of the Amide I region (above) and its relative inverse secondary structure (below) of bioplastic films from larvae (red line) and pupae (blue line) (insects reared on s-OFMSW). Protein secondary structure analysis was performed using the second-derivative band narrowing method on original spectra in the 1700-1600 cm^{-1} region, as described in previous research (Barreto et al., 2020; Kamada et al., 2021; Shimanovich et al., 2015; Yang et al., 2015). Data processing was performed with the software Origin PRO, 2019.



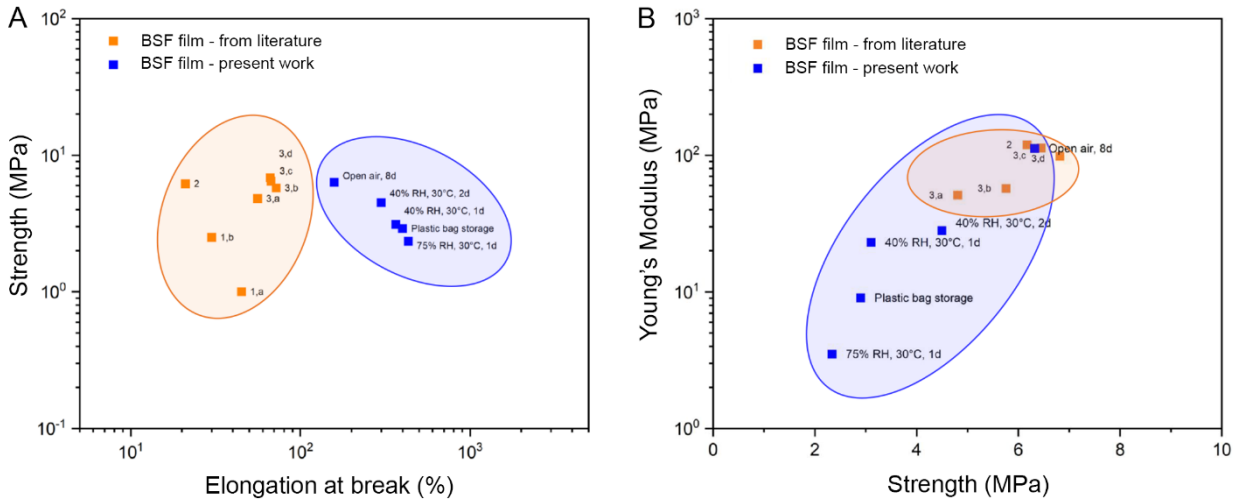
Supplementary Figure 3. Stress-strain curves for bioplastics from the protein fraction of BSF larvae (red line) and pupae (blue line) reared on r-OFMSW.



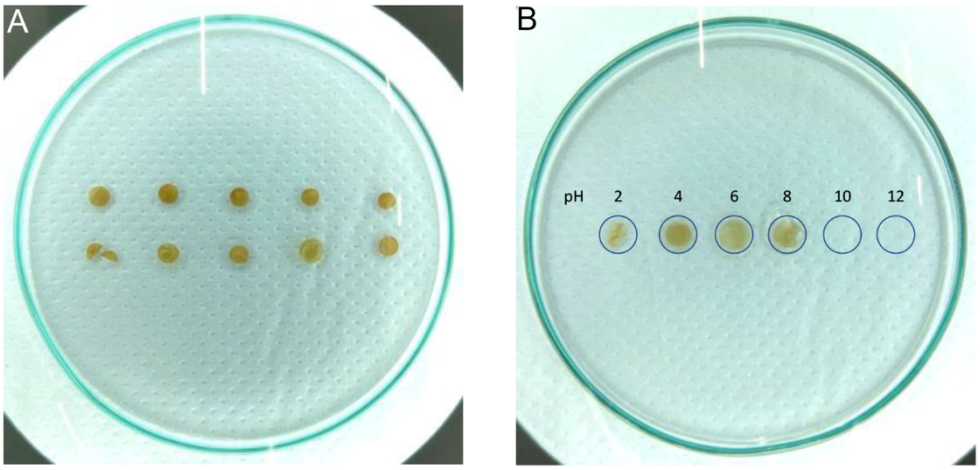
Supplementary Figure 4. Stress-strain curves for bioplastics from the protein fraction of BSF pupae after different conditioning methods.



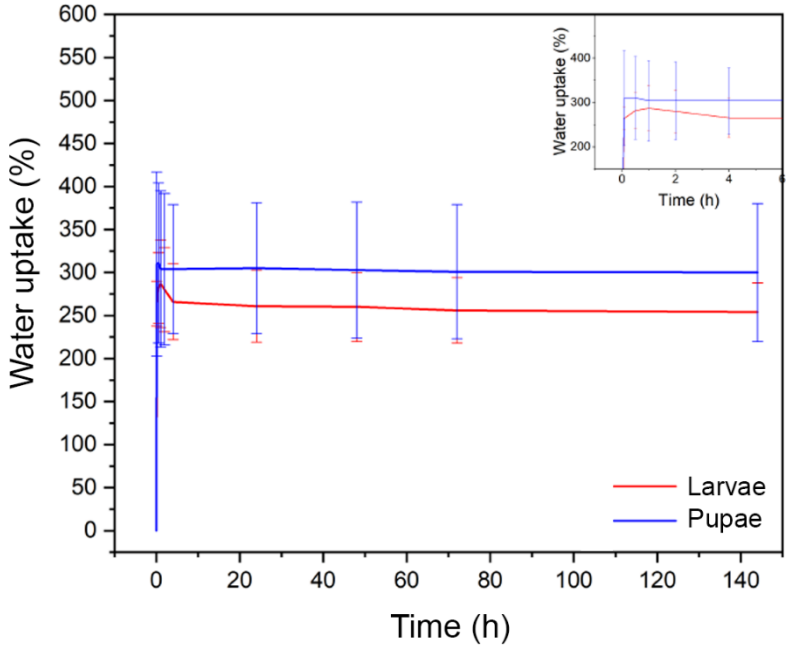
Supplementary Figure 5. Comparison of mechanical properties between BSF pupae protein-based films from current scientific literature (orange) and this study (blue). Mechanical properties of films from literature were taken from reference studies as reported in Supplementary Table 11. (A) Strength (MPa) versus elongation at break (%). (B) Young's Modulus (MPa) versus Strength (MPa).



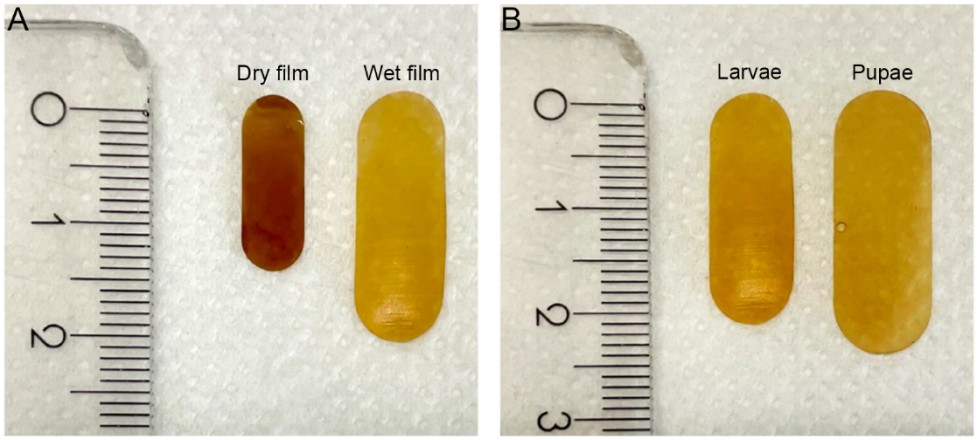
Supplementary Figure 6. Characterization of bioplastic films. (A) Pupae bioplastic film specimens after 1 week of immersion in organic solvents. Solvents from left to right, top to bottom: hexane, toluene, isopropanol, methanol, ethanol, acetone, ethyl acetate, DCM, THF, DMSO, and DMF. (B) Pupae bioplastic film specimens after 1 week of immersion in water at different pH values.



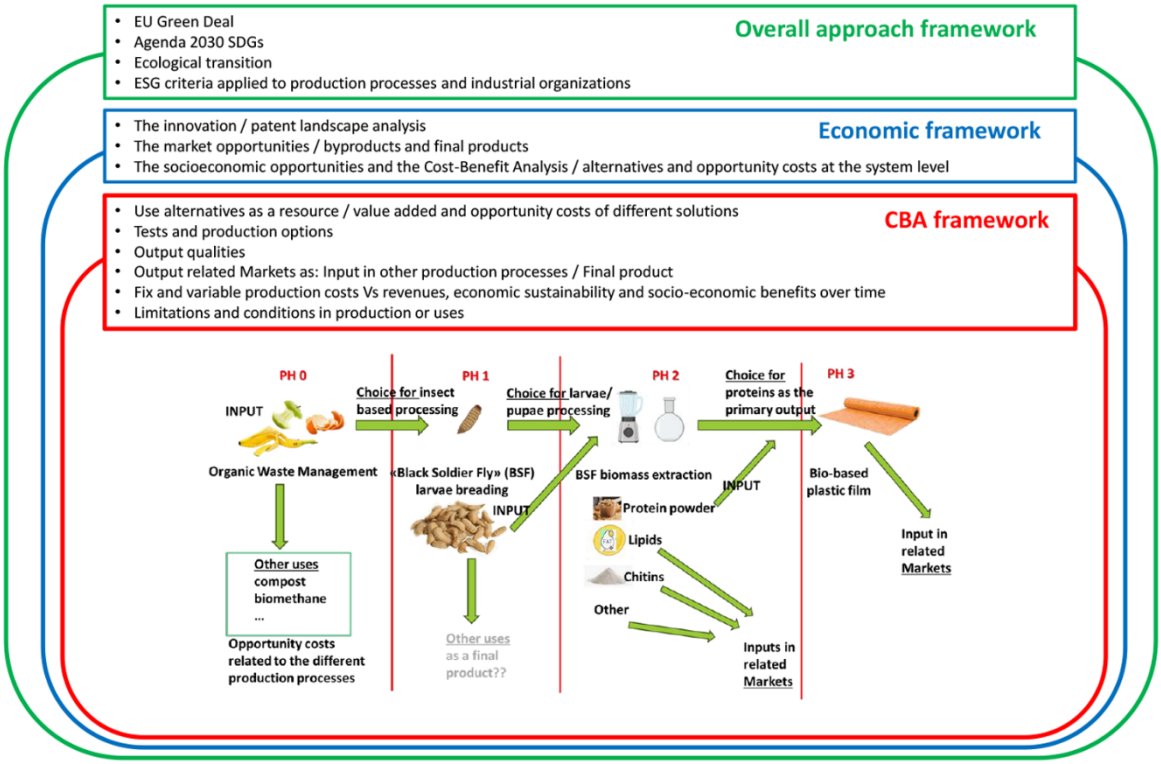
Supplementary Figure 7. Swelling profiles of bioplastic films from the protein fraction of larvae and pupae reared on s-OFMSW.



Supplementary Figure 8. Water-uptake from protein-based bioplastics. (A) Image of dry (as prepared) and swelled film (after 24 h in water). Samples are produced from the protein fraction of larvae reared s-OFMSW. (B) Image of larvae and pupae protein films after 24 h in water (samples from the protein fraction of larvae and pupae reared on s-OFMSW).



Supplementary Figure 9. Socioeconomic analysis frameworks.



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