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DESIGN AND ENVIRONMENTAL ASSESSMENT OF BIOPLASTICS FROM *Hermetia illucens prepupae* PROTEINS

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Abstract

Proteins from *Hermetia illucens* (or Black Soldier Fly, BSF) have been employed in this study as possible source for bioplastic formulation. This type of bioplastic can replace the actual materials employed in agriculture, avoiding the critical issues concerning the soil pollution due to conventional plastic end-life. Different plasticizing agents (glycerol and polyethylene glycol) have been tested and the ability to generate a homogenous film, through wet casting, has been evaluated. Characterizations on tensile properties and water absorbance have been performed to estimate the effect of different plasticizers employed. Bioplastic formed by proteins/glycerol ratio 50:50 has shown interesting properties, contributing to the formation of homogeneous and free-standing film with tensile stress at break near to 2.5MPa, almost constant during degradation profile test. At the same time the high degree of solubility in water has been verified for the same sample (~70%). The environmental impact of the laboratory scale production of bioplastics obtained from BSFs proteins has been evaluated through the Life Cycle Assessment (LCA) methodology. Inventory analysis has been conducted using primary data and Ecoinvent database. LCA analysis has been conducted using the SimaPro 8.3 software and the IMPACT 2002+ method of evaluation. The analysis show that the energy consumption is high (63%), but this can be mainly attributed to a laboratory-scale production process and related with the energy consumption of aspiration system (93%). Therefore, these results will help to the design of industrial production of innovative bioplastics in order to minimize these environmental issues.

Key words: bioplastics, glycerol, *Hermetia illucens*, LCA, waste bioconversion

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1. Introduction

The worldwide plastic production and consumption is continuously increasing, generating in Europe an economic return of trillions of dollars (PlasticEurope, 2018, Comăniţă et al. 2016). Plastic consumption for agricultural purpose is estimated as 2–3 million tons for year and at least half of these are employed for the cultivation's protection as soil mulching films, low tunnels, greenhouses and temporary coverings (Dilara and Briassoulis, 2000; Espi, 2006; Huang et al., 2017; IBAW, 2018). Low-density polyethylene (LDPE) is the most common materials employed for the protections of the cultivation, considering its low cost compared with good mechanical and optical properties (Briassoulis, 2005). One of the main drawbacks of LDPE employment is about lifetime that ranges from few months to 3–4 years depending on peculiar conditions near the soil and geometrical properties (e.g. thickness) (Desriac, 1991; Lemaire, 1993). In fact, LDPE degradation comes as a fragmentation of the materials in small pieces of plastic that are too small to be removed and which often can be founded as pollutant in the soil (Scott, 1999). To avoid this type of pollutant on soil and cultivation and to reduce the disposal costs, films based on biodegradable materials must be considered as favorable alternative, even if concerns arise from the vegetable exploitation for plastic production (Briassoulis, 2005; Jachowicz et al.,

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2017; Krzan et al., 2006; Malinconico et al., 2002; Wu and Wang, 2018). Moreover, biodegradable films, if properly studied, can act as fertilizers, releasing nutrients, such as nitrogen, during their decomposition, and therefore producing an innovative and totally green sustaining source for cultivation (Scott, 2000).

As innovative aspect, the present study investigates the exploitation of prepupae extracted from *Hermetia illucens (*Linnaeus 1758-Diptera: Stratiomydae), also known as black soldier fly (BSF), to generate a free-standing bioplastic film. BSF is a considerable source of nutrients, in fact its proteins are employed as base for animals feed, and fats in biodiesel production (Cummins et al., 2017; Newton et al., 1977). BSF is also know to act as safe bio-converter for the human being, acting as waste reducer without the transmission of pathogen agents, generating a strongly beneficial effect in the circular economy perspective (Caligiani et al., 2018; Diener et al., 2011; Sheppard et al., 2002; Spranghers et al., 2017). In the present study, the formulation of bioplastic for agricultural purpose has been studied taking in account proteins extracted from BSF prepupae, previously reared on poultry manure, by mixing with selected plasticizers. Therefore, insects are employed to reduce waste's volume, and theirs fractioned prepupae as source of proteins, useful as base for a new category of materials interesting for agricultural purpose following the scheme shown in Fig. 1.

In order to evaluate the environmental profile of insect-based products, the environmental impacts associated with the whole life cycle of these processes has been quantified through a Life cycle assessment (LCA) evaluation. LCA is essential to identify and assess entails identifying and assessing the potential environmental impact associated with a material, product, service or process throughout its entire life cycle, from the raw material extraction and processing, through manufacturing, transport, use and final disposal. However, the scientific literature on bioplastic obtained from insects is still limited. Using the LCA approach, Oonincx et al. (2012) published a detailed environmental impact assessment in terms of global warming, agricultural land use and energy consumption for the mass farming of two species of mealworms (*Tenebrio molitor* and *Zophobas morio*) in comparison with traditional protein sources for human consumption (milk, chicken, pork, beef). Their results highlighted greater GHG emissions and land use associated with milk, chicken, pork and beef systems, whereas similar amounts of energy are required in conventional and mealworm protein production. However, Oonincx et al. (2012) reported that the high energy consumption observed for rearing of insects is due in large part to the need to air-condition the breeding environment. Similar conclusions are reported by Van Zanten et al. (2015), that focused the attention on the use of housefly larvae grown on poultry manure and food waste as livestock feed. Although, Boer et al. (2014) employed LCA to found that mealworms seem to have little potential for inclusion in compounded feed without increasing the carbon footprint, they also concluded that the use of other insect species, with a low energy requirement during rearing and higher nutrition values, reared on waste products instead of feed ingredients, could increase the replacement potential of insects.

LCA methodology was employed by Salomone (2017) to evaluate possible environmental impacts of bioconversione from food-waste to *Hermetia illucens* dried larvae. Theirs results confirm that Energy Use category is the main burden, although significant benefits are related to Land Use category if data from food-waste conversion are compared with alternative sources for biodiesel or feed. Therefore, in the present study, the environmental impact evaluation has been carried out adopting the LCA methodology (ISO 14040, 2006; ISO14044, 2006) to evaluate the environmental performance of the production process of the best innovative bioplastic formulation obtained from the experimental work, in order to estimate if, as base material for plastic, the insect exploitation demonstrates a lower environmental impact, than as alternative for feeding. In this study the focus has been concentrated on the environmental impact associated with the laboratory-scale production. The aim has been to develop a detailed picture of the environmental profile of the bioconversion process, generating new data-set to be available for future similar studies.

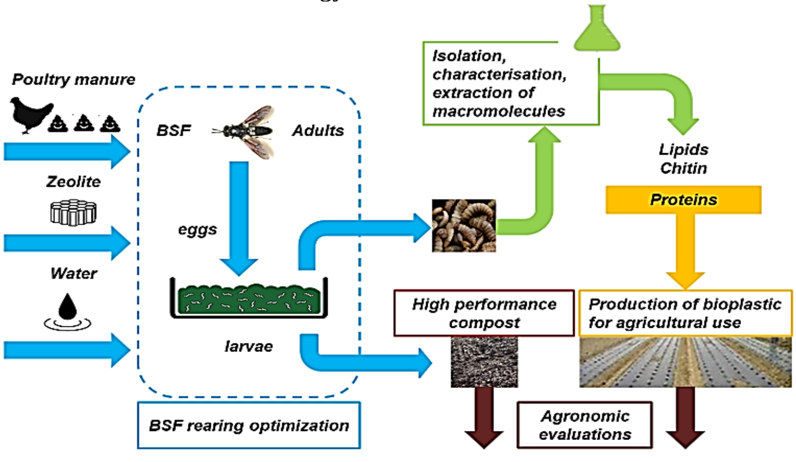

Fig. 1. BSF as renewable resource for bioplastic production

2. Material and methods

2.1. Films preparations

The BSF prepupae protein fraction was derived through a conventional chemical route, as reported in literature, obtaining a protein amount of 32wt% on the overall BSF protein content (Caligiani et al., 2018). The protein fraction was firstly ground with an analytical mill (IKA, A10 basic) and then sieved below 40 µm to obtain a powder with homogeneous grain size and able to react consistently with the selected plasticizers. Glycerol (GL, 99%, Sigma Aldrich), sodium hydroxide solution (NaOH, 1M, Sigma Aldrich) and polyethylene glycol (PEG400, 99%, Sigma Aldrich) were employed as additives for protein-based film formulation.

Films were obtained by mixing GL (or PEG 400) and protein, in distilled water (DI), adjusted to pH 10 with NaOH (1M) following the formulations expressed in Table 1. These suspensions were firstly heated for 30 min at 70°C and stirred at 200 rpm, then poured into aluminum dishes. The cooling was done under fume hood at room temperature for 24 h.

2.2. Films characterization

A dynamic mechanical analyzer (DMA, TA Q800) was employed in film tension set-up for tensile properties evaluation. Before measure each specimen was kept at standard conditions (50% Relative Humidity; 25°C) for 24 h. Rectangular specimen of 20×5 mm^2 were employed and run in duplicates for each mixture shown in Table 1. The effective sample length was measured in the film stage assembly under a pre-load force of 0.05 N at room temperature. During testing the load force was raised to 18 N at the rate of 0.05 N min^{-1} to measure the relative elongation.

Specimen's thickness and diameter were evaluated with a digital micrometer (Mitutoyo, YY-T1BD-2GYE) in fifteen different points, and the average value was taken as reference together with its calculated standard deviation. The sensibility of the instrument was 0.02 mm.

The moisture content (MC) was evaluated following Eq. 1 and measuring each sample's weigh before (w_0) and after (w_1) drying in oven at 105 °C for 24 h. (AOAC, 1995): (Eq. 1)

$$MC(\%) = \frac{(w_0 - w_1)}{w_0} * 100 \tag{1}$$

The water solubility (WS) was determined through Eq. (2), where w_2 is the weight of each sample after immersion in 200ml of distilled water for 24 h and drying in oven at 105 °C for 24 h (Gontard et al., 1994):

$$WS(\%) = \frac{(w_1 - w_2)}{w_1} * 100 \tag{2}$$

An analytical balance with sensibility of 0.00001g was employed to measure all the weights.

EN 17033:2018 and EN ISO 4892-2:2013 Method A cycle 1 were employed for the measurement of the degradation profile due to artificial weathering. According with these standards rectangular specimens (50x15mm) were employed and exposed in a closed chamber at irradiance of 0,51 W/(m^2 x nm), at 38°C with 65% of relative humidity 65% for 500 hours. At the same time deionized water was sprayed into the closed chamber for 18 minutes over 2 hours. After the exposure tensile mechanical properties were measured.

2.3. Life Cycle Assessment (LCA)

The functional unit was represented by the amount of bioplastic produced (0.403 gr). The system boundaries (Fig. 2) range from the obtained BSFs protein to the bioplastic production following the procedure mentioned in paragraph 2.1. Energies, materials, water, main equipment with their end of life, transport, waste and their treatment, emissions into the air at the continental level, the aspiration system and purification plant as well as the recovery and reuse of certain solvents were also considered.

Table 1. Material's formulations (wt%)

Experiment	***Protein***	***GL***	***PEG400***	***DI***	***Score***
GL1	12	12	/	76	5
GL2	14	10	/	76	3
GL3	16	8	/	76	3
PG1	12	/	12	76	3
PG2	14	/	10	76	2
PG3	16	/	8	76	1

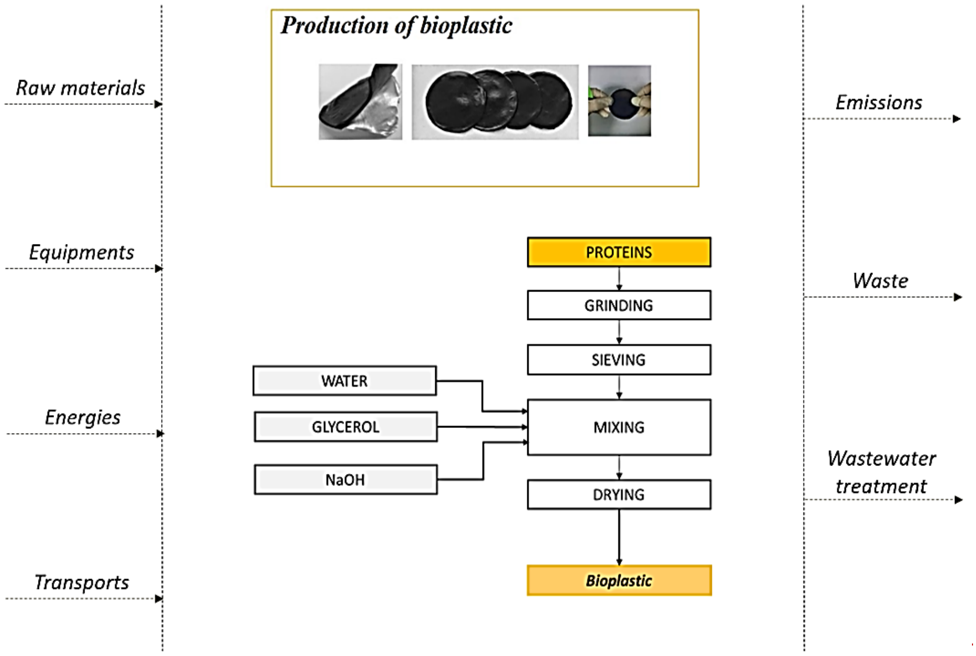

Fig. 2. LCA scheme for bioplastic production

2.3.1. Life Cycle Inventory

Regarding the quality of data used for the Life Cycle Inventory (LCI), lab-scale data were directly collected from the experimental procedure. Where the data were not available, the study was completed on the basis of secondary data obtained from the Ecoinvent databases v3 (Ecoinvent, 2013) which were used to model the background processes (land use, material production, fuel and electricity production, and transport). The emissions were calculated assuming that the laboratories were fully ventilated after the conclusion of each working day (8 hr). For these emissions, the indoor concentrations were calculated considering a total laboratory volume of 480 m^3 and assuming that 1% of the emissions come from the aspiration filters with 99% efficiency. The other extracted fraction, related with lipids and chitin content, were not used for the production of bioplastic, and thus were considered as co-products. A mass allocation was used. A composting process was hypothesized as bioplastic end of life.

2.3.2. Impact assessment methodology (LCIA)

The life cycle impact assessment (LCIA) results were modeled using the IMPACT 2002+ method (Jolliet et al., 2003) with Simapro 8.5 (PRè Consultants, 2013) to determine the environmental impact. This impact assessment method covers more impact categories than other methods and includes more substances. Since it is midpoint and endpoint oriented, it provides a complete overview of the environmental performance. However, the following additions and modifications were implemented to describe the system considered in a more representative manner i.e. modification to Land use (different types of land transformations were considered), Mineral extraction categories (additional resources were added) and Radioactive waste (radioactive waste and its occupied volume were evaluated) (Spinelli et al., 2014). The LCIA results were derived from both midpoint and endpoint levels. However, for the sake of brevity we report only the endpoint results. These are usually shown as the impact on human health, ecosystem quality, climate change and resource depletion. We decided to report only the endpoint results as the interpretation of these results does not require extensive knowledge of the environmental effects. Moreover, midpoint results can be more difficult to interpret because they consider many impacts which are often difficult to understand.

3. Results and discussion

3.1. Materials evaluation and characterization

The evaluation of the capability to constitute a free-standing plastic film has been performed firstly from a qualitative point of view through a consensual panel based on the judgment in blind of five people. The output of each experiment has been evaluated taking into account the homogeneity of the sample after drying and, therefore, its compactness and detachability from the aluminum support. Panel test grouped all experiments in 6 categories and a score from 1 to 6 has been attributed at each one category. In details, 1 is the value associated to the lowest quality (totally not homogeneous), whereas 6 is attributed to samples with the highest quality (completely compact, homogenous and with good detachability). From the collected scores, as expressed in Table 1, is clear that the decreasing of plasticizer content leads to the weakest quality in terms of homogeneity, compactness and detachability. Moreover, the type of plasticizer plays a key role, in fact, an improved compactness and detachability can be related to the employment of GL, whereas PEG400 leads to the worst overall result. Independently of plasticizer type and content, detachability has to be improved, in fact none of the samples studied in this work reached the highest score of 6.

Plastic integrity during processing, handling, usage and storage are governed by their mechanical properties. In the present work tensile behavior of the produced samples has been measured following the condition in section 2.2, confirming quantitatively the result from the panel test. In fact, as can be seen in Fig. 3 higher tensile stress at break (σ_b) corresponds to the employment of GL, reaching the highest value of 2.4 MPa for the sample GL1. A drop of σ_b value corresponds to a decreasing of the plasticizer content, for both GL and PEG400, whereas samples produced with plasticizer content <12wt%) show very similar values of σ_b (GL2 with GL3 and PG2 with PG3). This result confirms that at least 12 wt% of plasticizers (protein-based content) is necessary to achieve the highest results in term of tensile resistance at break, suggesting that, in other conditions, the microstructure configuration due to the additive content is not suitable to perform a strong binding between proteins due to poor chain linking density of the resulting polymer (Ganglani et al., 2002). Difference in GL and PEG400 behavior as plasticizer are consistent with their different functional groups that leads to a different protein-plasticizer interaction; more in details PEG 400 promotes the formation of more hydrophobic interactions that contributes to decrease the protein-plasticizer bonding (Knowles et al., 2015) whereas glycerol plays a chain linking action in the material's polymerization.

In fact, glycerol promotes its diffusion into proteins chain, because of its restrained molecular weight combined with high hydrophilic behavior, generating a restrained hydrogen-protein bonding (Awadhiya et al., 2016; Lunt and Shafer 2001; Martelli et al. 2006). Similar considerations can be done for the tensile stress at yield point (σ_y) that results lower than the tensile stress at break for all the investigated samples indicating that the elastic component of the strain is strongly higher than the plastic one, leading to poor ductile materials (even if the standard deviations suggests a partial overlap of the confidence ranges). The results from mechanical tests can be compared with data obtained in very similar conditions from bioplastic from others animal sources, such as crayfish, keratin and albumen. In particular, Ramakrishnan et al. (2018) considers the keratin employment together with glycerol to produce bioplastic through casting technique, obtaining lower tensile strength at break with respect to the results of the present study. Similar consideration can be drawn taking in account Felix et al. (2014) that consider crayfish as bases for bioplastic production, employing glycerol as plasticizer through injection molding.

Therefore, an overall increase of the mechanical tensile resistance at break can be obtained if protein from BSF are employed with respect to other protein from animal sources, although not reaching the values of materials already available on the market and deriving from vegetables, such as starch, that shown tensile stress at break around 20MPa and strain at break over 200% (Niaounakis, 2015).

The degradation profile against time of the investigated samples has been measured as shown in Fig. 4. For all the investigated samples, except GL1, an almost linear inverse proportion among tensile stress at break and exposure time has been measured with similar rate of degradation. In fact, GL1 not only shows the highest values of σ_b with respect to others but remains the sample with the highest tensile stress at break during and after weathering test. This confirms that the protein-additive combination of this sample is the most favorable to the obtainment of a polymeric material more stable during exposure to the weathering agent. This result is consistent with an only partial degradation, as described in Fig. 4, since weathering agents should be taken in consideration together with burial in soil test to achieve a complete result of degradation.

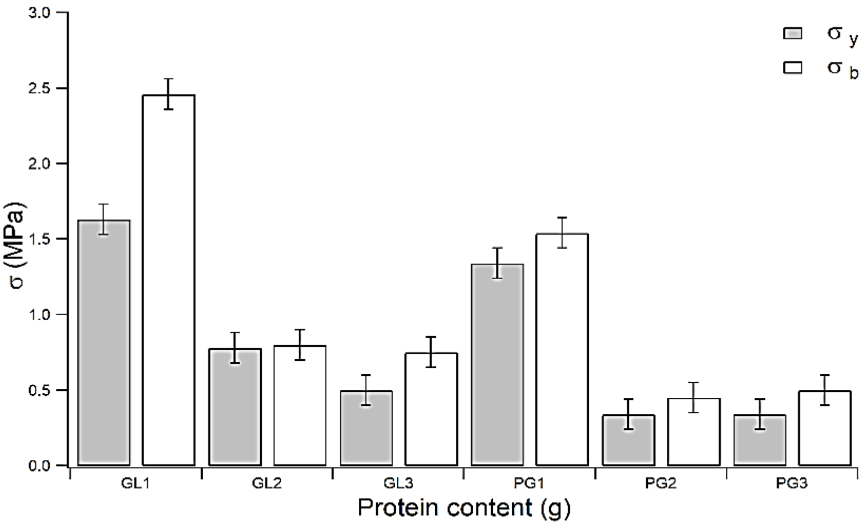

Fig. 3. Mechanical characterization

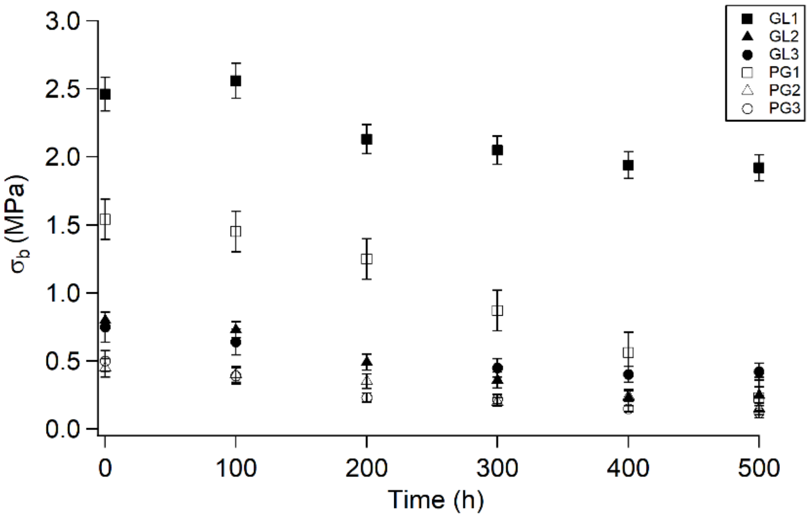

Fig. 4. Degradation profile

As shown in Fig. 5, the quantity of protein in plastic's formulation regulate both the moisture content and water solubility due to protein's high hydrophilic behavior.

Moisture content increases by decreasing the quantity of protein employed into material's formulation and this result is consistent with the strong hygroscopic behavior of the protein chains. This trend is true until 12 wt% of protein into the materials formulation, in fact after this value, considering the statistical error, samples have the same moisture content.

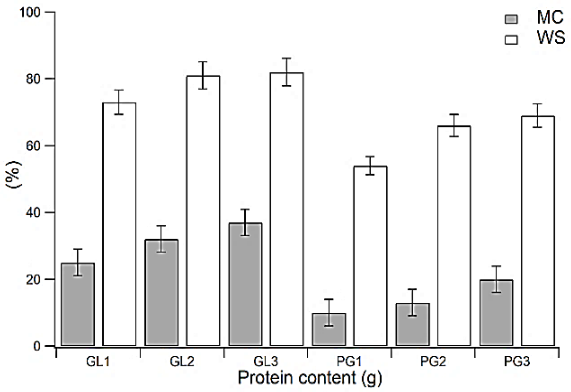

Fig. 5. Water absorbance and Moisture content comparison

Consequently, this protein quantity is a threshold for moisture content increment; In fact, over 12 wt% the packing of the protein chain becomes more open, due to restrained plasticizer's quantity, letting moisture be adsorbed more easily by the material.

The water solubility (WS), also known as total soluble matter (TSM), follows the same trend. As demonstrated in similar studies this parameter is strongly correlated with the amount of highly hydrophilic components into the materials formulation from which a high moisture content is derived.

High WS suggests also that proteins are not strongly bonded to the network structure (Felix at al., 2014). Similar trends can be associated with GL and PEG400 employment, even if lower values of WS and MC have been obtained with PEG400 due to the lower hygroscopic behavior if compared with GL. From these results the protein content > 12 wt% into material formulation promotes a higher content of nutrient not strongly bonded.

Therefore, in this condition higher is the protein content to be released in soil, acting as soil fertilizer during the plastic degradation (Chiellini et al. 2001; Gontard, 1994).

3.2. LCA

The analysis of results (Fig. 6) shows that the total damage associated to 1 gr bioplastic (following GL1 composition) production process is equal to 3.4296 mPt.

Furthermore, the main environmental impact is mainly due to energy consumption (63%), in particular related to the energy consumption of aspiration system (93%) needed for the drying of the specimens under aspiration hoods. In fact, the final part of the bioplastic production is related to the drying of the samples and it takes the greatest fraction of time of the overall process of production. Table 2 reports the environmental performance at end-point level (damage categories): Human health, Resources and Climate change categories afflict the total damage for 31.48%, 32.06% and 26.60% respectively.

In particular, in Human health category the major contribute to the total impact is due to particulates (<2.5 μm) emission in air; Resources category is mainly affected by natural gas and the emission that mainly contributes to the climate change is carbon dioxide. All these emissions are due to electric energy production employed by the aspiration system.

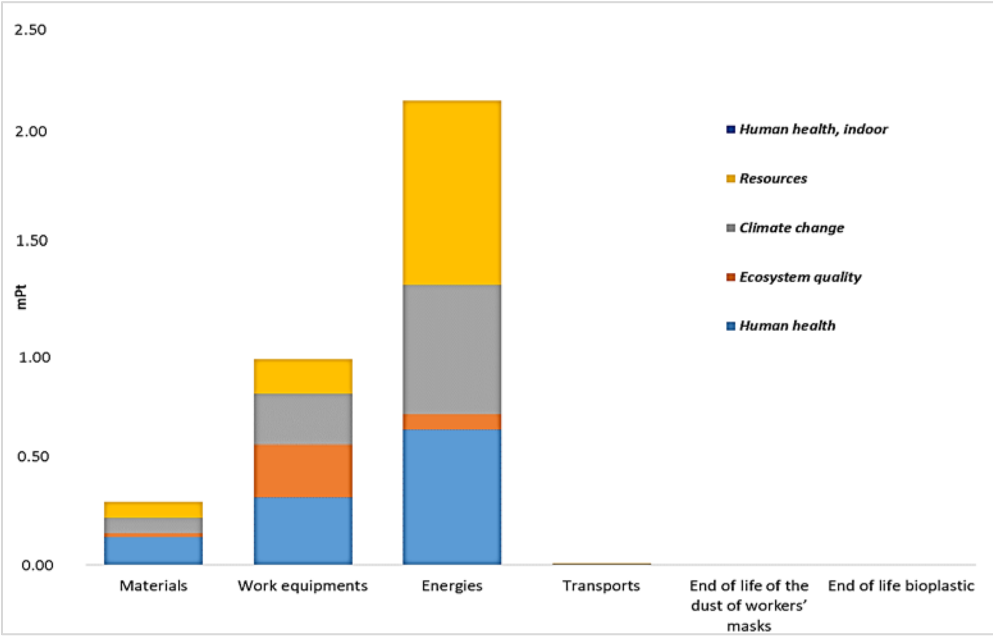

Fig. 6. Environmental damage of bioplastic production (1gr) by single score

Table 1. Environmental damage of bioplastic production process (1gr) by single score

Damage category	***Materials***	***Equipments***	***Energies***	***Transports***	***End of life of the dust of workers' masks***	***End of life of bioplastic***
Human Health (DALY)	1.29E-01	3.16E-01	6.32E-01	2.21E-03	1.60E-10	5.96E-06
Ecosystem quality (PDF*m2*yr)	1.93E-02	2.46E-01	7.16E-02	9.83E-04	2.05E-11	8.16E-07
Climate change (kg CO2 eq)	7.07E-02	2.37E-01	6.03E-01	1.89E-03	1.20E-10	3.61E-06
Resources MJ primary	7.70E-02	1.60E-01	8.61E-01	2.08E-03	5.54E-11	3.53E-06
Human Health Indoor (DALY)	3.32E-07	-	-	-	-	1.55E-07
Total [mPt]	2.96E-01	9.59E-01	2.167E+00	7.16E-03	3.57E-10	1.41E-05

"Occupation, forest, intensive" category affects Ecosystem quality damage (9.85% on the total damage) and it is generated by wood used for the activated carbon filter production (filter typology installed in the aspiration system).

These results are consistent with the available literature about life cycle assessment of possible new field of application for proteins that describe the energy consumption for air-condition/heating/drying as the category with the highest environmental impact. In particular, as already reported by Van Zaten (2015), the environmental impact is high nevertheless the rearing of the insect is made employing waste as livestock feed.

Moreover, the employment of Black Soldier Fly increases the energy consumption according to Salomone (2017) although insects are employed to produce plastic due to the lab scale condition of production, therefore in scale-up perspective the aspiration system employment should be strongly reduced or substituted.

4. Conclusions

This study confirms that proteins extracted from Black Soldier Flies prepupae can be employed to obtain bioplastic films, promising as bio-compostable plastics. The added value of these films is that they are generated by waste processing and reduction in volume by insects, increasing their positive effect in a circular economy perspective.

The addition of glycerol as plasticizer shows high potential due to favorable mechanical properties if the content of protein is near 12 wt% also after aging tests. Nevertheless, tensile stress at break must be increase in order to obtain values nearer to other bioplastic on the market, by employing other additives. PEG400 has shown very poor beneficial effect on film generation due to different functional group and bonding related to the interaction with protein with respect to glycerol.

LCA results show that the energy consumption of aspiration system is near to 63%, but this can be mainly attributed to a laboratory-scale production process and related with the energy consumption of aspiration system (93%). This consumption should be reduced in order to minimize the environmental impact, even if this process step it is necessary for the drying of the materials. This environmental impact is perhaps due to the production process is a laboratory-scale process and not yet an industrial one. Therefore, these results will help to the eco-design of industrial production of innovative bioplastics in order to minimize these environmental issues.

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