The black soldier fly (*Hermetia illucens* L.) strategy within "SCALIBUR – Scalable technologies for bio-urban waste recovery" H2020 project

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The SCALIBUR project (Scalable Technologies for Bio-urban Waste Recovery – Horizon 2020 – CE-SFS-25-2018) aimed to investigate cutting-edge and sustainable approaches, including the use of black soldier fly larvae (BSFL, *Hermetia illucens*, Diptera, Stratiomyidae) for the bioconversion of leftovers deriving from the HO.RE.CA. sector to address the management of bio-urban waste. The technological objectives were focused on the achievement of a high TRL (7) and on the extraction, isolation, and assessment of bio-based products with a high value and commercial potential.

This report describes the main results of the research work carried out in recent years, in particular the design and optimization of the pilot plant for fractionation of the BSF larvae into their fundamental components, such as proteins, fats, and chitin. The proteins isolated from the larvae of BSF represent a product with a high nutritional value and are intended for animal and human use. Fats also have a considerable importance in the same sectors. Chitin and its derivatives (chitosan) represent biopolymers with many beneficial applications, such as development of nanofibers with potential interest in food applications.

An evaluation of the variability of water, proteins, fats, ashes, and carbohydrates present in HO.RE.CA. leftovers collected from a local canteen was carried out over a 12-month period (Montevecchi et al., 2023). A pilot plant based on horizontal centrifugation separation technology was designed and the critical parameters, temperature (T) and differential speed (Δn) were evaluated through a factorial design of the experiment (FDOE). A gross amount of 50 kg of BSF larvae was ground and thoroughly homogenized. For each of the ten total tests, 5 kg of ground larvae (standard lot) were used. Therefore, the ten batches tested can be considered perfectly homogeneous.

The protein fraction was analyzed to outline the nutritional (essential amino acids) and functional characteristics (Miron et al. a). Proteins were extracted under alkaline conditions using two different recovery methods, isoelectric precipitation and ultrafiltration. The effect of black soldier fly protein incorporation on the texture of meat analogs was determined and compared to meat analogs prepared using other alternative protein sources such as soy protein isolate and vital wheat gluten, whereas the beef round, chicken breast, and a commercial plant-based meat analogue were used as reference matrices (Miron et al. b). Finally, the chitin fraction was extracted fully characterized and then used to prepare nanofibers through mechanical treatment (Aragón-Gutiérrez et al., 2022).

Regarding the water content, it was completely random. However, a high amount of water (>80%) correlated with a higher larval mortality rate. Larval weight was significantly related to the amount of protein ($r = 0.80$; $p \leq 0.001$) present in the substrate and, to a lesser extent, the amount of fat ($r = 0.43$; $p \leq 0.05$). The feed conversion rate and bioconversion rate were both in agreement with literature data.

As for the optimization of the fractionation pilot plant, the results were subjected to statistical tests. A two-way ANOVA was performed using temperature and differential speed as statistical factors. ANOVA confirmed that both factors were statistically significant and had effects on extraction yields. Subsequently, a regression analysis was carried out to evaluate different models and choose the one with the greatest effectiveness, based on the coefficient of determination (R^2). Similar trends in the response surfaces were observed, the higher the temperature, the higher the yield, the lower the differential speed, the higher the yield. Once the parameters were optimized, tests were carried out to evaluate the system's separative effectiveness. These tests were also performed for the LCA requirements.

Comparison of the two protein isolation methods showed that ultrafiltration provided higher protein purity (96.4%) but lower extraction yield (24.3%), while isoelectric precipitation provided a lower protein content

(76.0%) but a higher extraction yield (37.2%). All essential amino acids were present in quantities high enough for human requirements (essential amino acid index = 1.72). The protein fraction obtained by ultrafiltration had significantly higher oil retention capacity and foaming capacity than the isoelectrically precipitated proteins. The proteins obtained by ultrafiltration and the precipitated proteins had an oil retention capacity of 125.8% and 81.6%, while the foaming capacity was 141.9% and 114.3%, respectively.

Texture parameters of meat analogues were used as response variables yielding robust regression models ($R^2 > 0.96$). The optimal incorporations of black soldier fly protein into meat analogues mimicking the texture characteristics of chicken breast and plant-based meat analogues were 6.7 g/100 g and 21.5 g/100 g, respectively.

The characterization of chitin gave the following results. FTIR spectra showed the characteristic vibration bands of chitin at 1650, 1620, and 1550 cm^{-1}, thus confirming the successful extraction of chitin from the fraction of insects. XRD patterns showed strong peaks at approximately 9.3° and 19.8° and small peaks at 12.0°, 23.0° and 26.0°, respectively. The TGA results revealed a highly stable behavior of the chitin fraction, with a maximum degradation temperature set at 396 °C. Finally, TEM analysis showed a very fine nanofiber network with a uniform width of 20-30 nm.

The main remarks can be summarized as follows: the protein content of the rearing substrate is an essential parameter for optimizing the weight gain of black soldier fly larvae. HO.RE.CA. leftovers are overall a suitable food substrate for larval rearing. The pilot plant with the horizontal centrifugation system proved to be suitable for carrying out the isolation of the larval fractions (fat, protein, and chitin).

Nutritional and functional properties of larval proteins can be used to improve the characteristics of human foods, resulting in improved consumer acceptance. In addition, larval protein can replace traditional proteins in meat analogues up to 21.5%. Finally, black soldier fly larvae represent a high potential to obtain chitin for the further development of nanofibers with potential interest for food applications.

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