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Assessing the environmental sustainability of insects as a source of functional proteins: A prospective LCA

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SUMMARY

Insect protein has properties that enable its use as a functional ingredient in food and feed formulations. This study provides a prospective life cycle assessment (LCA) of proteins of *Alphitobius diaperinus* from an upscaling of a lab-scale process. The functional unit is 1 kg protein, and the system boundaries are from the cradle to the processing gate. As the process yields fat as a coproduct, avoided loads and physicochemical allocation are applied. Different results are obtained regardless of how coproducts are dealt with; protein extraction is the unit process that contributes the most to the processing stage. Compared with whey protein isolate, the impacts of *A. diaperinus* are lower in five of six categories using allocation but only in three categories using avoided loads. This study highlights that the multifunctionality of food systems is crucial in determining the product's impacts, being also critical for comparison purposes.

1. Introduction

To meet the rising global demand for protein, the industry is actively searching for alternative sources that provide high-quality proteins, considering their availability and sustainability. Insects are a good source of lipids and proteins (Mishyna et al., 2020) with an efficient feed conversion ratio (van Broekhoven et al., 2015). In addition, they have shown lower environmental impacts than other proteins from animal sources (Oonincx and de Boer, 2012; Smetana et al., 2016; Ulmer et al., 2020), which can be even lower when by-products from other processes or waste are used as feed (Oonincx et al., 2015), like those from plants sources (Smetana et al., 2015). The macronutrient content of insects depends on factors such as the stage of development, diet, gender, and environmental factors, such as humidity, temperature, length of the day, and light intensity and spectral composition (Finke and Oonincx, 2013). Rumpold and Schlüter (2013) report the protein content of different insect orders with values ranging from 35.34% (dry basis) for Isoptera to 61.32% (d.b.) for Orthoptera. In addition to this high protein content, its digestibility ranges from 76% to 98%, depending on the species (Ramos-Elorduy et al., 1997). Fat is the second most significant portion of insect composition. Rumpold and Schlüter. (2013) reported fat content ranging from 13.41% (d.b.) for Orthoptera to 33.40% (d.b.) for Coleoptera. Fat from insects provides feed and food with essential fatty acids and supply energy (Rose et al., 2021). However, the composition of fatty acids in edible insects depends on their diet (Murugu et al., 2024).

Nowadays, the insect sector covers diverse activities, from producing insects for food purposes to processing (either the whole insect or extracting functional components) to implementing them into food products. Whole insects represent the highest market share (close to a 1/4th of the products on the market), followed by bars, snacks, speciality

Abbreviations: Ac, Acidification; As, The side surface area of the reactor; CCT, Climate change total; Cp_{mix}, Specific heat of the mixture; DLM, Dry lesser mealworm; DLMP, Defatted lesser mealworm powder; E_m, Energy to mill the dried insects; EtxF, Ecotoxicity freshwater total; EuF, Eutrophication freshwater; EuM, Eutrophication marine; EuT, Eutrophication terrestrial; FL, Fresh larvae; FU, Functional unit; HtcT, Human toxicity cancer total; HtncT, Human toxicity non-cancer total; Ir, Ionising radiation human health; Ka/S, Heat transfer coefficient of the insulation; LCA, Life cycle assessment; L_H, Latent heat of evaporation of hexane; LMP, Lesser mealworm powder; LU, Land use; M_{DLM}, Mass of dried lesser mealworms; M_H, Hexane mass; M_{mix}, Mass of the mixture; n, Energy efficiency; n_p, Efficiency of the heating element; OzD, Ozone depletion; PhoO, Photochemical ozone formation human health; pLCA, Prospective life cycle assessment; PM, Particulate matter; Q_d, Energy to dry fresh larvae; Q_f, Energy to separate the hexane from the fat; Q_p, The energy needed for protein extraction; Ruf, Resource use fossils; RuMM, Resource use mineral and metals; t, Time of the reaction; T_{0mix}, The initial temperature of the mixture; T_{Bmix}, The boiling point of the mixture; T_r, Reaction temperature; W, Amount of water evaporated; WPC, Whey protein concentrate; WPI, Whey protein isolates; WU, Water use; ΔH_v, Heat of vaporisation of water.

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food ingredients and pasta; these ingredients are expected to cover a 1/5th market by 2025 (IPIFF, 2020). In addition, the extraction of functional fractions from edible insects, such as fat and proteins, hides the shape of the entire insect, which can help gain consumers' acceptance (Kröger et al., 2022). In the last years, the techno-functional properties of insect proteins have been a topic of research, highlighting properties such as surface hydrophobicity, coagulation, gelling, solubility, foaming capacity, solubility, water- and oil-binding, and emulsifying ability (Bußler et al., 2016; Kim et al., 2021; Ranasinghe et al., 2023; Wang et al., 2021a). Techno-functional proteins play an important role in food processing and can be potentially used in feed and food formulations as gelling and emulsifying agents replacing conventional proteins such as those from whey (Wang et al., 2021b; Shokri et al., 2022) and also in pharmaceutical and cosmetic products (Wang et al., 2021a). Wang et al. (2021a) extracted a protein concentrate from *Alphitobius diaperinus*, which was used to stabilise water-in-oil-in-water emulsions, comparable to whey. Wang et al. (2021b) obtained a concentrate from *Hermetia illucens* with 62.44% protein and similar or even higher foamability and emulsification activity than whey protein isolates (WPI). Bußler et al. (2016) investigated proteins' extractability and techno functionality from *Tenebrio molitor and Hermetia illucens.* They concluded they could be added to high protein intermediates in food and feed production. Kim et al. (2021) studied the fat extraction of *Protaetia brevitarsis* by aqueous separation and using organic solvents. They found that n-hexane is the most efficient solvent, although ethanol also proved to be good, obtaining concentrates with similar emulsifying and foam capacity than those using hexane. Ranasinghe et al. (2023) used ultrafiltration as a physical alternative to get fractionated protein concentrates of *Tenebrio molitor* and *Hermetia illucens*. They observed that although the protein's extract foaming properties and emulsifying activity were not enhanced, it can still replace dairy protein for industrial applications. Other alternatives tested to extract fractions with enhanced techno-functional properties from insects include pH shifting, hydrolysis, and assisted extraction techniques (Ma et al., 2023).

Life cycle assessment (LCA) is a broadly used method to assess the environmental sustainability of products, which has been applied to determine the environmental impacts of insect rearing (Smetana et al., 2016; Suckling et al., 2020; Ulmer et al., 2020) and of new products using insects as raw material such as margarine, (Smetana et al., 2020) or milk (Tello et al., 2021). Nevertheless, to the best of the author's knowledge, no LCA studies have been conducted on the environmental impact of techno-functional proteins extracted from insects. It is worth noting that the application of LCA to assess new products or alternative processing technologies entails specific challenges, some of them related to the fact that available information corresponds to runs at the pilot or even laboratory scales (Silva and Sanjuán, 2019), which can differ from those at the industrial scale and make comparisons with already existing products not meaningful (Piccinno et al., 2016). The literature defines ex-ante or prospective LCA (pLCA) as assessing a product's or service's environmental impact at an early development stage, using likely scenarios of future performance at a full operational scale (Cucurachi et al., 2018). The development of a prospective LCA entails methodological issues such as temporal considerations (Langkau et al., 2023; Thonemann et al., 2020), as the assessed product or technology will need time until it is available for consumers, the availability of primary inventory data, which requires a process upscaling, or the lack of reliable background processes as they are expected to change with time (Steubing et al., 2023). An increasing body of research has been carried out to deal with these issues. The technology readiness level (TRL) has been proposed to determine the temporal issue of pLCA (Gavankar et al., 2015). The TRL assesses the technology maturity, from level 1, the observed basic principles, to level 9, for the fully devolved operational system (Mankins, 1995). Along these lines, Thonemann et al. (2020) highlight the need to use the same or consistent time frame and technological maturity levels to compare fairly with available products. Assessing the environmental impacts of a scale-up can help design and anticipate significant production problems (Thonemann et al., 2020; Tsoy et al., 2020). Up-scaling provides inventory data and has been tackled in the literature on pLCA (e.g., Caduff et al., 2014; Piccinno et al., 2016; Zhou et al., 2017). Tsoy et al. (2020) provide recommendations and a framework for up-scaling based on a literature review further adapted by Cucurachi et al. (2022). Multifunctionality can entail difficulties in pLCA. ISO 14044 prioritises system expansion when process subdivision is impossible. However, when the study aims to analyse a single product, this means performing a substitution, which raises some controversy as it implies deciding which is the displaced product with an equivalent function and collecting additional data (Heijungs et al., 2021). When allocation cannot be avoided, partitioning based on biophysical or economic criteria is recommended. Cucurachi et al. (2022) discuss the constraints of allocation methods when performing pLCA of bio-based systems. In particular, deciding on the representative biophysical relationship can be complex in these systems; conversely, in the context of pLCA, economic allocation can be challenging to implement when the output has no market value.

Knowing the environmental impacts of techno-functional proteins from insects can promote their use as sustainable ingredients in the food industry and other sectors in line with SDG 12, Sustainable Production and Consumption. Specifically, this assessment can contribute to target 12.6 since it can encourage food companies to adopt sustainable practices and integrate sustainability information into their reporting cycle. Having this information available is in line with the European Green Deal (European Commission, 2024a) and the Farm-to-Fork strategy (European Commission, 2024b), which is to reduce the environmental footprint of the EU and support the transition to sustainable food systems (IPIFF, 2022a).

Based on the study from Wang et al. (2021a), this paper aims to provide a prospective LCA of techno-functional proteins extracted from *A. diaperinus* (lesser mealworms or buffalo worms). As the process described in Wang et al. (2021a) was carried out at the lab scale, an upscaling will be implemented. Then, the environmental impacts of the insect proteins will be assessed and compared with those of conventional proteins with equivalent techno-functional properties.

2. Methodology

This study was carried out following the ISO 14040/44 standards (ISO 14040, 2009; ISO 14044, 2006) and literature on prospective LCA (Cucurachi et al., 2022; Piccinno et al., 2016; Sanjuán et al., 2014).

2.1. Goal and scope definition

This study aims to develop a prospective LCA to assess the potential environmental impacts of extracting protein from lesser mealworms and compare their environmental impacts with the ones of conventional proteins with similar techno-functional properties. Therefore, it is a comparative analysis oriented toward the product level, and according to Cucurachi et al. (2022), it requires using an output-based functional unit (FU). In particular, the FU of this study is 1 kg of protein extracted from *A. diaperinus*. Based also on the technical report of Cucurachi et al. (2022), the system boundaries are set from the "cradle to the processing gate" (*Fig. 1*). The foreground system is the protein extraction process, including the dehydration and milling of the fresh larvae and the fat extraction. The rearing process is included in the system boundaries for comparison purposes since conventional techno-functional proteins (e.g. whey proteins, legume proteins) are produced from different feedstocks. Subsequent life cycle stages after processing, such as the product's transport, distribution, and use or packaging waste management, have not been included.

In pLCA, the temporal boundaries of the study usually lie in the future, and therefore a time horizon for which the technology under investigation will reach maturity is defined (Langkau et al., 2023). However, in this case study, no emerging technologies are employed

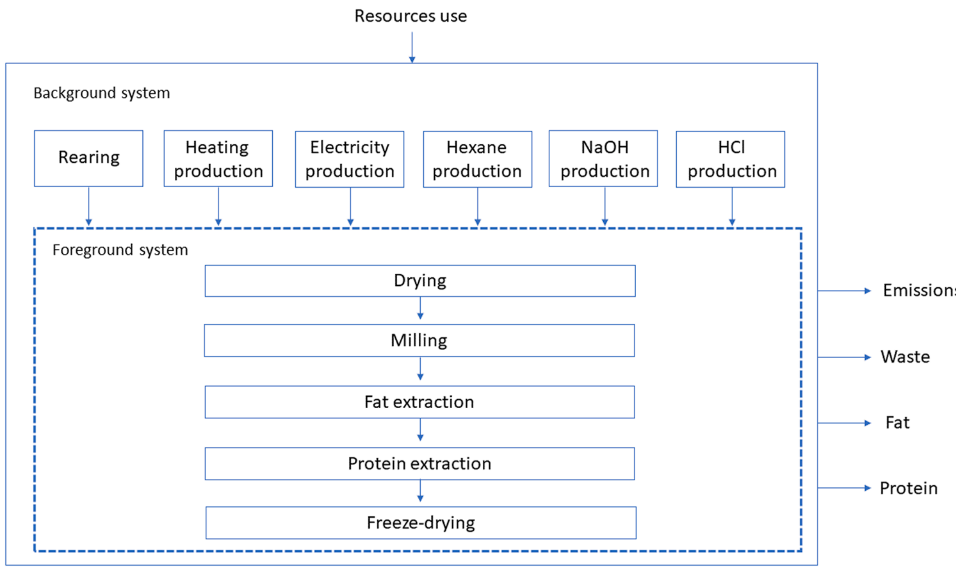

Fig. 1. System boundaries of the life cycle of protein extraction from *A. diaperinus*.

since the process is based on consolidated technologies, corresponding to TRL 8–9, and the main limitation to start the process is related to the need to develop more studies about the use of insect protein as a techno-functional ingredient; hence, the temporal-limit is the year 2029. Regarding the geographical boundaries, the production was assumed to occur in Spain.

It must be noted that the protein extraction process from *A. diaperinus* yields fat as a co-product that can be used in other applications. As ISO 14040/44 sets that system expansion should be done, it is considered that fat can potentially replace soybean oil, given that both have a high content of unsaturated acids (Taghi and Altintas, 2023; Alemida et al., 2015). Another alternative to isolating the impacts of protein production from those of fat is to carry out an allocation based on biophysical properties. In particular, the energy content provided by fat and protein mass is 9 kcal/g and 4 kcal/g, respectively (Tontisirin et al., 2003); as a result, 72.3% of the impacts are allocated to protein, considering the values in Table 1.

2.2. Description of the system under study and scaling-up procedure

To begin with the pLCA, the process of Wang et al. (2021a) was upscaled (Fig. 2). To this aim, the production scale was set at 5000 kg of fresh *A. diaperinus* larvae per batch, average weekly production of a medium-sized insect farm. Although the procedure described by Wang et al. (2021a) starts with commercial *A. diaperinus* flour, in the upscaling, the dehydration of the fresh larvae (FL) from 68% (OJEU, 2023) to 2.5% moisture content, the one of commercial flour (Kreca, 2023), has also been included. The dehydrated larvae (DLM) are then milled to obtain the lesser mealworm powder (LMP), followed by fat extraction. Fat can be extracted by mechanical separation, heat treatment, or organic solvents, according to the IPIFF Guide (IPIFF, 2022b). However, this guide recommends the extraction with organic solvents because the nutritional values and bioactivity of the dried protein are better preserved (IPIFF, 2022b). Wang et al. (2021a) set 2-MeTHF as the solvent to extract the fat. However, inventory data on the production of this solvent was not available in commercial databases. Thus, hexane was considered in this study since it is the common solvent used in the food industry. Organic solvents extract lipids (Rose et al., 2021), and hexane is a good hydrocarbon solvent for lipids of low polarity (Boulton and Baker, 1988). Next, a protein-rich solution is extracted from the defatted lesser mealworm powder (DLMP) using NaOH (0.25 M) at 40 °°C. NaOH is a common solvent used in the alkaline solubilisation of proteins (Hernández-Álvarez et al., 2023). Then, the proteins are separated using solid-liquid separation (Meng et al., 2018), and proteins are precipitated by lowering the pH to 4 with 37% HCl. The precipitated proteins are separated by centrifugation, and the extraction/precipitation process is repeated. The protein precipitate is then dehydrated in a freeze-dryer. The composition of FL, LMP, and DLMP is shown in Table 1.

Table 1
Percentage composition of the process flows: fresh larvae (FL), dehydrated lesser mealworm (DLM), lesser mealworm powder (LMP), and defatted lesser mealworm powder (DLMP).

	FL	DLM - LMP	DLMP
Protein (%)	18.64 [a]	56.8 [b]	77.40 [a]
Fat (%)	9.88 [a]	30.1 [b]	4.75 [a]
Moisture (%)	68 [c]	2.5 [b]	3.41 [a]
Carbohydrates (%)	1.48 [a]	4.5 [b]	6.13 [a]
Fibre (%)	2[a]	6.1 [b]	8.31 [a]

DLM and LMP share composition, as the difference between them is the milling.
[a] Calculated data.
[b] Kreca 2023.
[c] OJEU 2023.

Following Cucurachi et al. (2022), as operational parameters and governing equations of the extraction process are known and no simulation software is available, the inventory was built from an upscaling procedure based on detailed process calculations, as explained below.

The processing begins with the drying of the larvae. The energy required to dry the fresh larvae was estimated from the dryer efficiency, according to (Sanjuán et al. (2014):

$$Q_d = \frac{W^{*}\Delta H_v}{\left(\frac{n}{100}\right)} \tag{1}$$

Where Q_d is the energy to dry the fresh larvae (kJ); W is the amount of water evaporated from the FL (kg), calculated from a mass balance taking into account the initial and final moisture content of FL and LMP; ΔH_v is the heat of vaporisation of water (2260 kJ/kg); and n is the energy efficiency of the dryer (dimensionless), which is 85% for a drum dryer (Sanjuán et al., 2014), the equipment selected in this study.

The energy needed to mill the dry larvae was calculated according to Piccinno et al. (2016), who estimated that the average energy consumption of grinders ranges from 8 to 16 kWh/ton of milled material (Eq. (2)). Following the recommendation of Piccinno et al. (2016), the upper value was used as a conservative approximation because no information on the final particles' size was available.

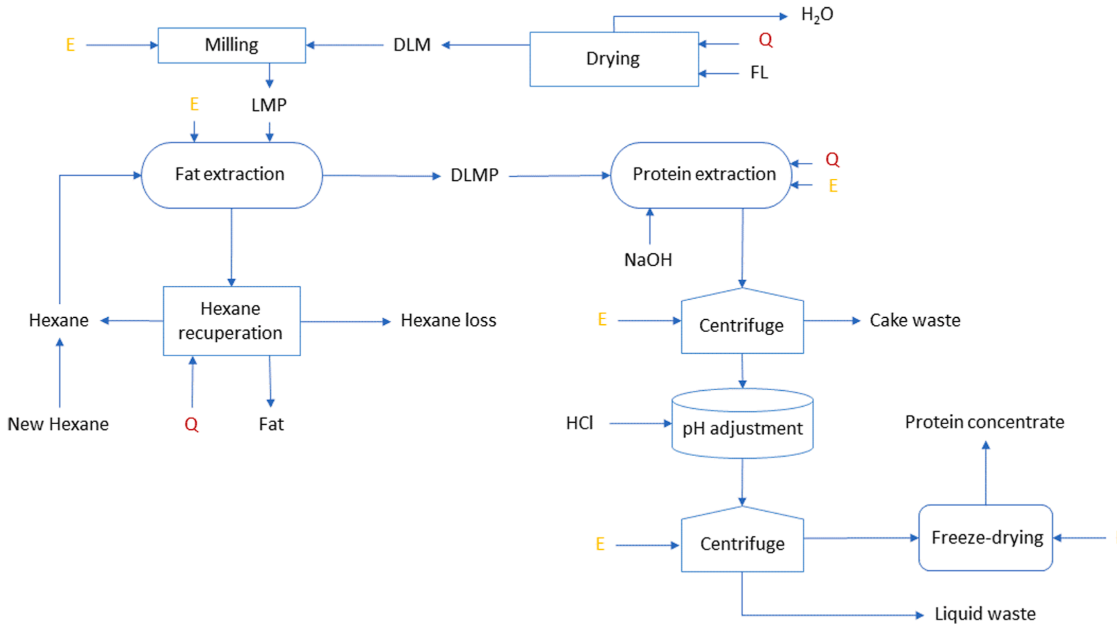

Fig. 2. Flow chart of the process for lesser mealworm fat and protein extraction at an industrial scale. E: electricity, Q: heat, FL: fresh larvae, DLM: dehydrated lesser mealworm, LMP: lesser mealworm powder, and DLMP: defatted lesser mealworm powder.

$$E_m = M_{DLM} {}^{*} 0.016 \tag{2}$$

Where E_m is the energy to mill the dried insects (kWh), and M_{DLM} is the mass of dried lesser mealworms (kg). It is assumed that no matter is lost between the drying and the milling steps.

Piccinno et al. (2016) recommend reducing 20% the amount of solvent for the scale-up. Therefore, instead of the 1:5 LMP-solvent ratio proposed by (Wang et al., 2021a), a 1:4 ratio was accounted for. The technology depends on the scale; thus, a rotary extractor was considered in which hexane is recirculated three times. According to Piccinno et al. (2016), the volume of the extraction tank must be 10% greater than that of the mixture, implying that the volume of the rotary extractor is 9 m^3. The energy needed to rotate the tank was calculated considering 6 kW (ABLE engineers, 2024) and an extraction time of 1 h (Wang et al., 2021a).

Hexane is separated from the extracted fat by evaporation and reused in subsequent extractions. Assuming a 95% recovery rate (KMEC, 2023), the energy to evaporate the hexane is calculated as:

$$Q_f = M_{mix} {}^{*} Cp_{mix} {}^{*} (T_{0mix} - T_{Bmix}) + M_H {}^{*} L_H \tag{3}$$

Where Q_f is the energy to separate the hexane from the fat (kJ); M_{mix} is the mass of the hexane-fat mixture (kg); Cp_{mix} is the specific heat of the mix (kJ/kg °C); T_{0mix} is the initial temperature of the mixture (°C); T_{Bmix} is the boiling point of the mix (°C); M_H is the hexane mass (kg); and L_H is the latent heat of evaporation of hexane (365 kJ/kg). The mixture's specific heat and boiling point were calculated as a weighted average of fat and hexane. For that purpose, data from soybean oil were considered for *A. diaperinus* fat Cruz-Forero et al. (2012) since, as explained above, both have a high content of unsaturated fatty acids.

Protein extraction is also carried out in a rotary extractor of 2 m height and 2 m diameter insulated with 0.08 m of thickness of glass fibre (Piccinno et al., 2016). Based on these premises, the energy needed for protein extraction was calculated from Eq. (4).

$$Q_p = \frac{M_{mix} {}^{*} Cp_{mix} {}^{*} (T_r - T_{0mix}) + As {}^{*} (Ka/S) {}^{*} (T_r - T_{0mix}) {}^{*} t}{n_p} \tag{4}$$

Where Q_p is the energy needed for protein extraction (J); M_{mix} is the mass of DLMP-NaOH mixture (kg); Cp_{mix} is the specific heat of the mix calculated as a weighted average, considering that Cp of DLMP is 1890.2 J/kg °C (Sanjuán et al., 2014); T_r is the reaction temperature, 40 °C following Wang et al. (2021a); T_{0mix} is the initial temperature of the mixture, established at 25 °C; *As* is the side surface area of the reactor (m^2); *Ka/S* is the heat transfer coefficient of the insulation, 0.56 W/m^2 °C (Piccinno et al., 2016); *t* is the time of the reaction, one h (Wang et al., 2021a); and n_p is the efficiency of the heating element, (dimensionless), 79% for a 10,000 L extractor (Piccinno et al., 2016).

The electricity consumed by the rotary extractor was estimated as that of fat extraction (ABLE engineers., 2024), considering 6 kW power and 1 h (Wang et al., 2021a). The mixture obtained is centrifugated for 15 min to separate the protein-rich solution from the solid cake waste. Then, HCl is added to the protein solution to adjust the pH between 4.0 and 4.3. and precipitate the protein. A second centrifugate separates the precipitated protein, obtaining a protein extract with a protein content of 61.08% (w/w), which is freeze-dried. The energy required in each centrifugation is calculated assuming an electricity consumption of 10 kWh per ton of centrifugated dry matter (Piccinno et al., 2016). An FD1800 Cuddon freeze dryer (Cuddon Freeze-dry, 2023) was assumed to estimate the energy needed for the subsequent freeze-drying, which consumes 1.6 kWh per kg of product to dehydrate. It must be noted that an additional 5% of electric consumption was considered globally, associated with ancillary equipment, such as pumps, valves, etc.

2.3. Life cycle inventory

From the process upscaling, inventory data of the foreground system was obtained as inputs and outputs per 1 kg of protein extracted from *A. diaperinus* (Table 2).

Background data (Table 3) was retrieved from ecoinvent 3.8 (Moreno Ruiz E, 2021) and LCA for experts (Sphera Solutions, Chicago, USA) databases. It is worth noting that the composition of the Spanish electricity grid mix will remain similar for 2029 (Navas-Anguita et al., 2020), and it is thus temporally and geographically coherent with the up-scale, as recommended by Cucurachi et al. (2022). As concerns the insect rearing stage, no specific LCA of *A. diaperinus* rearing was found in the literature; therefore, since *A. diaperinus* belongs to the Tenebrionidae family, rearing data of *Tenebrio molitor* from Dreyer et al. (2021) was used.

Table 2
The main inventory data for the upscaled protein extraction process is expressed per 1 kg of extracted protein.

	Input	Symbol	Amount	Unit
Drying	Heating energy	Q_d	11.13	MJ
	Fresh larvae	M_{FL}	6.39	kg
Milling	Electricity	E_m	0.12	MJ
	Dried larvae	M_{DLM}	2.10	kg
Fat extraction	Electricity	E_f	0.03	MJ
	Hexane	Hx	0.83	kg
	Lesser mealworms powder	M_{LMP}	2.10	kg
	Heating energy	Q_f	2.81	MJ
Protein extraction	Electricity	E_p	0.09	MJ
	Heating energy	Q_p	1.77	MJ
	NaOH	NaOH	23.10	L
	HCl	HCl	0.46	L
	Defatted lesser mealworm powder	M_{DLMP}	1.92	kg
Freeze drying	Electricity	E_{fd}	7.92	MJ
Ancillaries	Electricity		0.41	MJ

Table 3
Background processes for the protein extraction of *A. diaperinus* process.

Input	LCI Name	Type of process	Source
The heat from natural gas	Europe without Switzerland: heat production, natural gas, at industrial furnace >100 kW	agg LCI results, cut-off method	Ecoinvent 3.8
Electricity	ES: Electricity grid mix	agg LCI results, cut-off method	Sphera
Hexane	RoW: isohexane production	agg LCI results, cut-off method	Ecoinvent 3.8
Avoided Oil	RER: soybean meal and crude oil production	agg LCI results, cut-off method	Ecoinvent 3.8
Hydrochloric acid	DE: Hydrochloric acid (32%)	agg LCI results, cut-off method	Sphera
Sodium hydroxide	DE: Sodium hydroxide (caustic soda) mix (100%)	agg LCI results, cut-off method	Sphera
Fresh larvae	-	-	Dreyer et al. (2021)

2.4. Impact assessment

To assess the environmental impacts, the Environmental Footprint 3.1 method (OJEU, 2021) was used, covering the following impact categories (acronym and units in brackets): acidification (Ac, mole of *H+* eq.), climate change – total (CCT, kg CO_2 eq.), ecotoxicity freshwater total (EtxF, CTUe), eutrophication freshwater (EuF, kg P eq.), eutrophication marine (EuM, kg N eq.), eutrophication terrestrial (EuT, mole of N eq.), human toxicity cancer total (HtcT, CTUh), human toxicity non-cancer total (HtncT, CTUh), ionising radiation human health (Ir, kBq U235 eq.), land use (LU, Pt), ozone depletion (OzD, kg CFC-11 eq.), particulate matter (PM, Disease incidences), photochemical ozone formation human health (PhoO, kg NMVOC eq.), resource use fossils (Ruf, MJ), resource use mineral and metals (RuMM, kg Sb eq.), and water use (WU, m^3 world eq.).

3. Results

The impact scores show that rearing is the stage with the highest contribution in most impact categories, either applying avoided loads (Table 4) or allocation (Table 5). When using avoided loads, the rearing stage dominates the scores of 15 of the 16 impact categories, and only for Ruf does the processing stage have the highest share of the total impact score (56.46%). With allocation, the rearing stage has the highest contribution in 12 of 16 impact categories; in contrast, protein extraction is the main responsible for CCT, EtxF, Ir, and Ruf, namely contributing to 66.92%, 65.00%, 54.54%, and 72.16% of the total impact score, respectively.

Table 4
Environmental impacts of the rearing and processing of *A. diaperinus* per 1 kg of extracted protein by applying avoided loads. Rearing Impacts were calculated using inventory data for *Tenebrio molitor* from Dreyer et al. (2021).

Impact	Unit	Rearing	Processing	Total
Ac	Mole of *H+* eq.	$3.51{\cdot}10^{-1}$	$5.11{\cdot}10^{-2}$	$4.02{\cdot}10^{-1}$
CCT	kg CO_2 eq.	25.42	23.89	49.30
EtxF	CTUe	357.85	318.44	676.29
EuF	kg P eq.	$1.13{\cdot}10^{-2}$	$2.01{\cdot}10^{-4}$	$1.15{\cdot}10^{-2}$
EuM	kg N eq.	$1.97{\cdot}10^{-1}$	$1.32{\cdot}10^{-2}$	$2.10{\cdot}10^{-1}$
EuT	Mole of N eq.	1.44	$1.81{\cdot}10^{-1}$	1.62
HtcT	CTUh	$3.74{\cdot}10^{-8}$	$5.64{\cdot}10^{-9}$	$4.30{\cdot}10^{-8}$
HtncT	CTUh	$1.04{\cdot}10^{-6}$	$1.36{\cdot}10^{-7}$	$1.17{\cdot}10^{-6}$
Ir	kBq U235 eq.	4.57	2.68	7.25
LU	Pt	3179.78	109.35	3289.13
OzD	kg CFC-11 eq.	$1.46{\cdot}10^{-6}$	$7.05{\cdot}10^{-7}$	$2.16{\cdot}10^{-6}$
PM	Disease incidences	$2.47{\cdot}10^{-6}$	$5.17{\cdot}10^{-7}$	$2.99{\cdot}10^{-6}$
PhoO	kg NMVOC eq.	$8.94{\cdot}10^{-2}$	$4.91{\cdot}10^{-2}$	$1.38{\cdot}10^{-1}$
Ruf	MJ	306.30	397.18	703.48
RuMM	kg Sb eq.	$1.98{\cdot}10^{-4}$	$2.04{\cdot}10^{-5}$	$2.18{\cdot}10^{-4}$
WU	m^3 world equiv.	225.43	1.29	226.72

Table 5
Environmental impacts of the rearing and processing of *A. diaperinus* per 1 kg of extracted protein by applying allocation. Rearing Impacts were calculated using inventory data for *Tenebrio molitor* from Dreyer et al. (2021).

Impact	Unit	Rearing	Processing	Total
Ac	Mole of *H+* eq.	$1.68{\cdot}10^{-1}$	$5.01{\cdot}10^{-2}$	$2.18{\cdot}10^{-1}$
CCT	kg CO2 eq.	12.20	24.67	36.86
EtxF	CTUe	171.69	318.84	490.53
EuF	kg P eq.	$5.43{\cdot}10^{-3}$	$2.42{\cdot}10^{-4}$	$5.67{\cdot}10^{-3}$
EuM	kg N eq.	$9.44{\cdot}10^{-2}$	$1.58{\cdot}10^{-2}$	$1.10{\cdot}10^{-1}$
EuT	Mole of N eq.	$6.92{\cdot}10^{-1}$	$1.82{\cdot}10^{-1}$	$8.73{\cdot}10^{-1}$
HtcT	CTUh	$1.79{\cdot}10^{-8}$	$5.83{\cdot}10^{-9}$	$2.38{\cdot}10^{-8}$
HtncT	CTUh	$4.97{\cdot}10^{-7}$	$1.34{\cdot}10^{-7}$	$6.31{\cdot}10^{-7}$
Ir	kBq U235 eq.	2.19	2.63	4.82
LU	Pt	1525.58	153.10	1678.67
OzD	kg CFC-11 eq.	$6.99{\cdot}10^{-7}$	$4.92{\cdot}10^{-7}$	$1.19{\cdot}10^{-6}$
PM	Disease incidences	$1.19{\cdot}10^{-6}$	$5.13{\cdot}10^{-7}$	$1.70{\cdot}10^{-6}$
PhoO	kg NMVOC eq.	$4.29{\cdot}10^{-2}$	$4.56{\cdot}10^{-2}$	$8.85{\cdot}10^{-2}$
Ruf	MJ	146.95	380.84	527.79
RuMM	kg Sb eq.	$9.49{\cdot}10^{-5}$	$1.52{\cdot}10^{-5}$	$1.10{\cdot}10^{-4}$
WU	m^3 world equiv.	108.15	1.10	109.25

Concerning the processing stage, when the avoided loads are applied (Table 4), the impact scores are higher than those calculated using a biophysical allocation (Table 5). In particular, the impact scores of the processing with avoided loads mean between 24.97% of the total impact in Ruf and 51.81% of WU. The contribution analysis of insect processing without considering the rearing stage (Fig. 3), shows that protein extraction is the unit process with the highest contribution to the impacts in 14 of the 16 assessed categories, ranging from 70.10% in PhoO to 93.89% in EtxF. NaOH production explains these results, given that it is mixed with HCl and cannot be recovered. Fat extraction also has a relevant contribution in 4 of the 16 impact categories: 40.05% in EuF, 75.76% in OzD, 49.79% in WU and 81.70% in RuMM. The high contribution of the fat extraction phase is due to the production of hexane, even if it is recycled. The contribution of the remaining units' processes (drying, grinding, and freeze-drying) is low. Drying only has a remarkable contribution in OzD (15.8%) and freeze drying in Ir (11.47%) and WU (11%).

The results of the contribution analysis of insect processing when allocation is performed (Fig. 4) are similar to those obtained considering

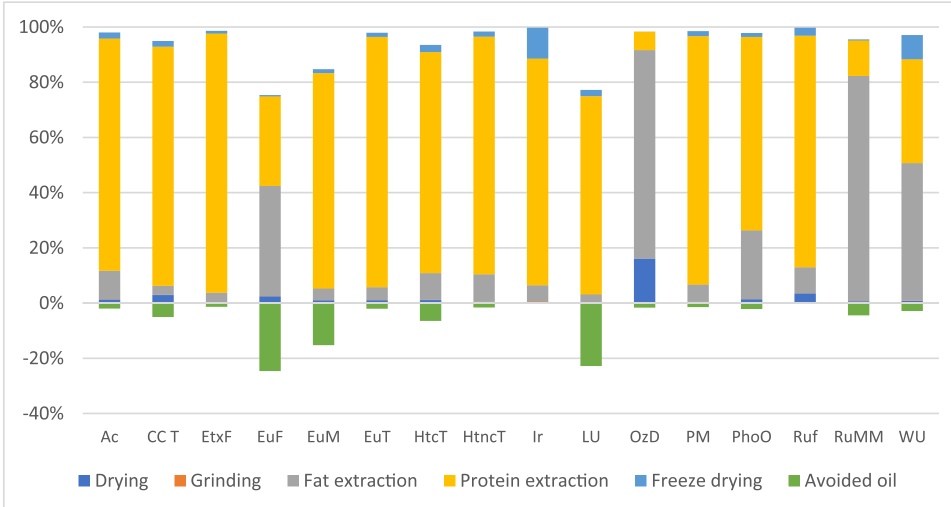

Fig. 3. Contribution analysis of insect processing considering avoided loads from fat extraction. FU: 1 kg of extracted protein.

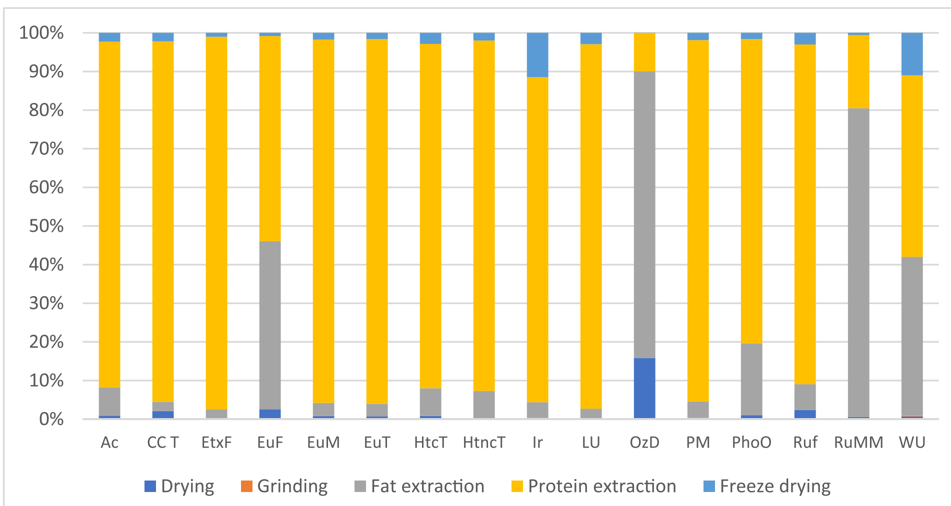

Fig. 4. Contribution analysis of insect processing performing allocation. FU: 1 kg of extracted protein.

avoided loads. Again, protein extraction is the unit process that contributes the most to the impact scores (in 14 of 16 impact categories), ranging from 46.99% in WU to 96.45% in EtxF. Fat extraction impacts the most in OzD and RuMM, with 74.27% and 79.91%, respectively. The remaining unit processes have a low contribution to all the impact categories.

4. Discussion

4.1. Comparison with conventional techno-functional proteins

Wang et al. (2021a) compared the protein concentrate from *A. diaperinus* with whey protein isolate (WPI) and pea protein. They concluded that the techno-functional properties of proteins extracted from *A. diaperinus* were similar to those from WPI, thus they can be a potential substitute. For this reason, in this study, the environmental impacts of the protein concentrate from *A. diaperinus* are compared with those of WPI using literature data for the latter. However, only the environmental impacts of whey protein concentrate (WPC) from Bacenetti et al. (2018) have been found in the literature. To obtain WPI, an additional concentration by ultrafiltration is required. For that reason, the impact results of Bacenetti et al. (2018) were recalculated by adding that additional filtration energy consumption to the baseline scenario WPC80. It must be noted that only the scores corresponding to the impact categories CCT, EuF, EuM, EuT, PhoO and RuMMa are compared since they are expressed using the same units as in Bacenetti et al. (2018). The results show that when applying avoided loads, the scores of WPI are higher than those of the proteins from *A. diaperinus* in EuM (11% higher), EuT (47%) and RuMM (41%). In contrast, the scores of *A. diaperinus* protein are higher for CCT (13%), EuF (67%) and PhoO (267%), which are categories in which the rearing stage has a higher contribution than the processing stage, as commented above. When comparing with the impacts of insect's protein applying allocation, the WPI shows higher impacts in 5 of 6 environmental scores, mainly in EuT and RuMM (ca. 70% higher scores), but also in EuM (53%), CCT (14%), PhoO (12.5%), and only the impact score of EuF is 34% lower. Under a life cycle approach, the focus to improve the environmental profile of the proteins from *A. diaperinus* concerning those of WPI should be on the rearing stage. Dreyer et al. (2021) found that feed supply is the stage with the highest contribution to most of the impact categories, and they recommend redesigning the diets using by-products from food processing and feed produced locally could decrease the impact scores of this stage. Therefore, it would be interesting to study the influence of the diet of the insects on their protein content and their environmental impacts per kg of obtained protein.

4.2. Improving the environmental profile of A. diaperinus protein concentrate

This section proposes recommendations to improve the environmental performance of *A. diaperinus* protein concentrates, mainly focusing on the processing stage. Given the high contribution of NaOH production to the impacts of the processing stage, it is essential to optimise its use or search for alternative solvents. The literature reviewed on protein extraction from insects shows that NaOH is the most widely used solvent (Thanakorn et al., 2023; Wang et al., 2021a, 2021b; Zhao et al., 2016). Gkinali et al. (2022) extracted the protein of *Tenebrio molitor* with three different methods: extraction of alkali-soluble proteins, isoelectric precipitation, and extraction of NaCl soluble proteins. In the two first methods, distilled water was used as the extraction solvent, and NaOH was used to adjust pH. In the third, a solution of NaCl was used for the extraction, and dialysis enabled the separation of the protein extract. The study found that the proteins' techno-functional properties differed depending on the extraction method. Thus, the environmental impact of these alternatives should be assessed, as well as their effect on functional properties.

Alternatives to reduce the impacts of the fat extraction unit process should also be tested to decrease processing impacts. It is worth noting that Wang et al. (2021a) used 2-MeTHF because it is a green, nontoxic, and biodegradable solvent (Jiménez-Gómez et al., 2020), but it was not considered in this study due to a lack of inventory data. However, it could positively affect the process's environmental impacts. Kim et al. (2021) used several defatting methods (aqueous, methanol, ethanol, and hexane) as a previous step in extracting protein from *Protaetia breviaries*. They determined their influence on the functional properties of the extracted proteins. They concluded that using ethanol allowed the extraction of proteins with properties similar to hexane. Although the environmental impact of using this alternative solvent should be assessed, a study in soybean oil extraction by Potrich et al. (2020) showed that replacing hexane with ethanol gave a potential 18.5% reduction in global warming. Enzyme-assisted aqueous extraction is another alternative to fat extraction. Cheng et al. (2018) assessed the environmental impacts of that alternative in soybean oil extraction, showing lower impact scores than the extraction with hexane. The IPIFF (2022a) also includes mechanical extraction by pressing among the alternatives for insect protein extraction; however, as commented in Section 2.2, this guide recommends organic solvents because the nutritional values and bioactivity of the dried protein are better preserved (IPIFF, 2022b). In addition, studies on the influence of this method on the quality of insect protein or its environmental impacts are required.

Despite the low contribution of larvae drying to the environmental impacts, applying pre-treatments can decrease the drying time with subsequent energy savings and better-preserving food quality (Llavata et al., 2020). Alternative drying technologies, such as atmospheric freeze-drying, are also recommended. For instance, Merone et al. (2020) observed a 70% reduction in total energy consumption in vegetable drying when using freeze-drying instead of conventional hot air drying, which entailed a 58 to 82% reduction in all impact categories. Freeze-drying experiments with insects are thus needed to study the effects of this technology on energy consumption and the characteristics of the dried product.

4.3. Methodological issues and limitations of the study

To evaluate the influence of the method used to isolate the two coproducts on the environmental results, allocation and avoided loads have been tested. The results show different impact scores depending on how the coproducts are dealt with. As commented in Section 1, system expansion requires choosing a product to replace the co-product and gathering the associated data, generating uncertainty (Heijungs et al., 2021). Conversely, applying biophysical allocation requires choosing a criterion, and economic allocation is not possible when assessing new products with no market price. An allocation based on biophysical parameters (in this case, the energy content of the coproducts) is in accordance with ISO:2006, given the physical relationship between products and co-products (Al-Zohairi et al., 2022).

The limitations of this study are mainly related to the upscaling of the process and the quality of the inventory data used in the LCA. It is widely recognised among pLCA practitioners that using lab or pilot data often overestimates the environmental burdens associated with the product (Shibasaki et al., 2006). The assumptions made in scale-up can greatly influence the environmental profile of a technology. Therefore, a comprehensive understanding of the technology and its materials and processes is decisive in increasing the reliability of the LCA results (Müller-Carneiro et al., 2024; Zhou et al., 2017). Different assumptions have been made in the process of upscaling that could influence the results, for instance, the dryer's efficiency, the energy of the mill, or the electricity consumption of the fat and protein extraction. A sensitivity analysis was made to check the influence of these assumptions on the impact scores. Nevertheless, as shown in Section 3, the impact score of the processing stage is dominated by the solvents used; therefore, the changes in the process parameters slightly influenced the impact scores of the process (for example, when changing the fat extractor, the total impact varies between 0.000003% in OzD and 0.02% in Ir when considering avoided loads). Previous studies have remarked on the effect of the process scale on the impact scores (Calero-Pastor et al., 2022); hence, providing results at another industrial scale could increase the reliability of the results.

As the upscaling is the source of the foreground inventory data, the Pedigree matrix adapted by Thonemann et al. (2020) for pLCA has been used to assess the data quality (Table 6). The matrix includes five semi-quantitative data quality indicators (reliability, completeness, temporal correlation, geographical correlation, and further technological correlation), where the lower, the better (Weidema and Wesnaes, 1996).

The data reliability is considered good, with a score of 2. That means that verified data partly based on assumptions have been considered. As for completeness, it has been set to 1 since data comes from specific equations. The temporal correlation is 3 since the data are less than 10 years different as the application is designed for 2029. The geographical correlation is set with a score of 1, given that the information was taken for Europe or specifically for Spain. In further technical correlation, most of the variables' scores are 3, as data from processes and materials under study, although from different technologies.

Another limitation of the study is related to the rearing stage. As commented in Section 2.2, no data on *A. diaperinus* were found in the literature, and a study on rearing an insect of the same family (*Tenebrio Molitor*) was used. Likewise, this study corresponds to an insect farm located in Austria, in which, after feed production, heating had a large contribution to the rearing impacts. Considering a different location, such as Spain, can influence the impact scores since the composition of the electricity mix is different, and heating requirements would decrease. The results have shown that this stage is crucial in the impact scores; hence, to increase the reliability of the results using actual data on the rearing process of *A. diaperinus,* taking into account the farm location would increase the result reliability.

4.4. Practical implications of the results and future prospects

The production of edible insects is expected to play a relevant role in the economy since demand will grow, the cost of insects will decrease, and the investment in the sector will triple (van Huis, 2022). Therefore, foreseeing additional applications and developing suitable processes can be decisive from a socio-economical point of view. As to the practical implications, the industry can benefit from this research since it aims to

Table 6
Pedigree matrix of the process variables.

Variable	Symbol	Reliability	Completeness	Temporal correlation	Geographic correlation	Further technical correlation
Fat extractor volume	Vreac	2	1	3	1	2
Electricity fat extractor	E_f	2	1	3	1	1
Mix boiling point with soybean oil	mixBp1	3	1	3	1	4
Protein extractor volume	Vreac	2	1	3	1	2
Specific heat DLMP – NaOH mixture	Cp	2	1	3	1	2
Thermal energy dryer	Q_d	2	1	3	1	3
Fresh larvae mass	M_{FL}	2	1	3	1	1
Electricity milling	E_m	2	1	3	1	3
Dried larvae mass	M_{DLM}	2	1	3	1	1
Hexane mass	Hx	2	1	3	1	4
Lesser mealworm powder mass	M_{LMP}	2	1	3	1	1
Thermal energy Hexane recuperation	Q_f	2	1	3	1	3
Electricity protein extractor	E_p	2	1	3	1	3
Electricity centrifuges	E_c	2	1	3	1	3
Thermal energy protein extractor	Q_p	2	1	3	1	3
NaOH volume	NaOH	2	1	3	1	1
HCl volume	HCl	2	1	3	1	1
Defatted lesser mealworm powder mass	M_{DLMP}	2	1	3	1	1
Electricity freeze-dryer	E_{fd}	2	1	3	1	3
Electricity ancillaries	E_a	2	1	3	1	3

guide the development of more environmentally sustainable products from insects, thus contributing to SDG 12. Specifically, the results show that the environmental impacts of *A. diaperinus* protein extraction are similar to those of WPI. Considering that lack of communication on the concerned products is one of the main limiting external factors for the development of the European insect sector (IPIFF, 2023), these results can be used for ecolabeling purposes such as Environmental Product Declarations (Schau et al., 2008). It is important to add that, to the authors' knowledge, no studies on the environmental impacts of fat and protein extraction from insects at any production scale are found. To improve the environmental profile of these proteins, future studies should implement the proposed environmental improvements and assess the techno-functional properties of the extracted proteins. Additional efforts are required to improve the sustainability of the rearing of insects, for example, through the design of insect diets. Also, testing other production capacities will be essential to choose the production scale with the greatest economic benefit and the lowest environmental impacts. Additionally, a life cycle costing (LCC) would allow the economic impacts of the product to be calculated. In addition, the eco-efficiency of this product can be estimated by integrating the environmental and economic results in an index using multicriteria decision-making methods. In addition, a comprehensive sustainability assessment should include specific studies that address the social impacts of developing functional proteins from insects at the industrial scale. This implies taking into account the specific community where the project would be deployed since the local context affects the social impacts (e.g. Zamagni et al. 2013). Along these lines, social life cycle assessment is an agreed method to assesses how humans are affected by products or systems throughout their life cycle (Tokede and Traverso, 2020).

5. Conclusions

In the last decade, the interest in insects to be consumed as food or food ingredients has grown worldwide, being the focus of multiple research studies and gaining entrepreneurs' interest. Previous studies have shown that edible insects can be considered an alternative source of techno-functional proteins. However, there are still some issues that remain to be explored, and this research gives a step forward in assessing the environmental profile of protein concentrate from *A. diaperinus*. The results of this pLCA show that regardless of how fat coproduct is dealt with, protein extraction is the unit process that contributes the most to the processing of *A. diaperinus*, mainly due to the use of NaOH for protein precipitation, followed by that of hexane for the insect defatting. Therefore, alternative methods for decreasing or avoiding these solvents should be studied. In addition, the effect of these alternatives on the techno-functional properties of proteins should be considered, being a key point for their use as an ingredient in the food industry. For comparison purposes, insect rearing has been included using literature data, and it proved to be the most impactful stage, implying that measures to decrease its impact should be implemented.

The environmental impacts of protein extracted from insects are of the same order of magnitude as those of WPI. It has to be remarked that the method used to deal with the coproducts, insect fat, influenced the comparison. From a methodological point of view, this study highlights that the multifunctionality of food systems is crucial when analysing the environmental impacts. It is critical to improve the product system's environmental performance in pLCAs and perform comparisons with similar products. Major barriers related to optimising the production scale and the market price still have to be overcome. Consequently, future studies should address these products' social and financial impacts to reach sustainable food systems aligned with environmental EU policies and SDG12.

Ethical Statement- Studies in humans and animals

For this research, no expirements were carried out in any way with humans or non-human animals.

CRediT authorship contribution statement

Alejandro Corona-Mariscal: Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Neus Sanjuan:** Writing – review & editing, Validation, Methodology, Conceptualization. **Carme Güell:** Methodology. **Gabriela Clemente:** Writing – review & editing, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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