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Prospective life cycle assessment for the full valorization of anchovy fillet leftovers: The LimoFish process

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ABSTRACT

Prospective life cycle assessment models were developed and applied at the laboratory and industrial scale with the aim to evaluate the environmental burdens associated with the LimoFish process used to produce the fish oil "AnchoiOil", the new organic fertilizer "AnchoisFert" or biogas (by means of anaerobic digestion) after treatment of anchovy fillet leftovers (AnLeft) with agro-solvent d-limonene. Potential impacts for climate change and freshwater eutrophication were estimated at 29.1 kg CO_2 eq/kg AnLeft and 1.7E−07 kg PO_4 eq/kg AnLeft at laboratory scale, and at 1.5 kg CO_2 eq/kg AnLeft and 2.2E−07 kg PO_4 eq/kg AnLeft at industrial scale. Electricity consumption is the main contributor to the environmental impact of the process and plays a significant role in the production of *d*-limonene, for which cold pressing extraction would reduce the related impacts by ~ 70 %. The use of the solid by-product as organic fertilizer or input to anaerobic digestion would provide additional environmental benefits to the process. The LimoFish process is a successful example of a low impacting strategy to reduce the demand for natural resources and maximize the application of the circular economy principles in the fishing industry.

1. Introduction

Fish and seafood processing leftovers are widely recognized as a potential source of value-added biomolecules and biomaterials, such as bio-lubricants (Angulo et al., 2018), biofuels (Eiroa et al., 2012; Karkal and Kudre, 2020), organic fertilizers (Brod and Øgaard, 2021), and bio-nano materials (Maschmeyer et al., 2020). Fish processing increases the stability and quality of products, removing parts such as the viscera containing bacteria and enzymes that would otherwise quickly deteriorate proteins or lead to oxidation of the omega-3 polyunsaturated fatty acids (PUFAs) abundant in sea life lipids. In 2011, more than 70 % of the total fish caught was subjected to processing before reaching the marketplace, with fish industry leftovers accounting up to 75 % (w/w) of the catch (depending on post-fishing and industrial processing

Abbreviations: AD, Anaerobic Digestion; AnFert, Anchois Fert; AnLeft, Anchois Leftovers; AnOil, Anchois Oil; CChl, Climate Change long term; CChs, Climate Change short term; CHP, Combined Heat & Power (Cogeneration unit); FAc, Freshwater Acidification; FEc, Freshwater Ecotoxicity; FEu, Freshwater Eutrophication; FNeu, Fossil and Nuclear energy use; FU, Functional Unit; HCT, Human Carcinogenic Toxicity; HnCT, Human non-Carcinogenic Toxicity; Ira, Ionizing Radiation; LCA, Life Cycle Assessment; LCI, Life Cycle Inventory; LCIA, Life Cycle Impact Assessment; LabS, Laboratory Scale; Meu, Marine Eutrophication; Lob, Land Occupation biodiversity; LTb, Land Transformation biodiversity; MRu, Mineral Resource use; Old, Ozone Layer depletion; PMF, fine Particulate Matter Formation; POf, Photochemical Oxidant formation; PUFAs, PolyUnsaturated Fatty Acids; SUp, Scale Up; SUp_{AD+CHP}, Scale Up w AD+CHP units; SUp_{CP}, Scale Up w cold pressing technology; TAc, Terrestrial Acidification; WSc, Water Scarcity.

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processes) and on average 50 % in weight (Rustad et al., 2011). Since then, the share of processed fish has further increased due to economic and demographic development of numerous countries. In 2020, global fish production was 179 million tonnes (FAO, 2020), with half of it intended for human consumption, but the fish processing industry generates a significant amount of waste, producing 300–500 kg of waste per tonne of fish processed. After a slight reduction in 2020 likely due to the COVID-19 pandemic (Bai et al., 2020; FAO, 2022), fish catches increased again in subsequent years.

New sustainable management of seafood processing biowaste is a truly global problem (Kannan et al., 2017). Plentiful research efforts have been devoted to the valorization of this biowaste including for fish heads, tails, fins, skin and bones (Ideia et al., 2020; Maschmeyer et al., 2020; Rakotoarisoa et al., 2021; Vázquez et al., 2022, Wang et al., 2021). A few bioeconomy companies started the production of valued bio-products from fishery leftovers, especially fish oil and fish proteins for the pet-food industry (FAO, 2022), increasing the recovery of waste material and promoting circular economy strategies (Bontempi, 2022). Rich in omega-3 lipids, indeed, the extraction of fish oil for the production of omega-3 food supplements significantly contributes to overfishing across the world (Rehan et al., 2018).

Amid the new processes to recover valued bioproducts, the extraction of a whole fish oil named “AnchoisOil” (AnOil in the following) from Mediterranean anchovy (*Engraulis encrasicolus*) fillet leftovers using the bio-based solvent *d*-limonene was first reported in 2019 (Ciriminna et al., 2019). AnOil can replace fish oil (a cheap and convenient a convenient way to incorporate omega-3 s into the diet) produced with conventional extraction and/or refining methods starting from the fatty tissues of oily fish such as salmon, mackerel, anchovies, and sardines. D-limonene, a valued bio-based terpene, is commercially derived from orange and lemon peels prior to fruit squeezing for the production of citrus juice (Calabrò et al., 2018; Lotito et al., 2018; Santiago et al., 2020; Teigiserova et al., 2021). In contrast to petroleum-derived solvents, d-limonene is an edible substance with exquisite smell with multiple applications in the food and cosmetic industries. Furthermore, the terpene has distinct antibacterial, antifungal, antioxidant and anti-carcinogenic properties for which it is increasingly used in the nutraceutical and biopesticide industries (Ciriminna et al., 2022; Rehan et al., 2018). Unlike low boiling solvents easily lost in the atmosphere such as widely employed *n*-hexane, d-limonene has a very high boiling point (176 °C). The only, current limit of this extracting agent is its high upfront cost, mitigated by a unique stability that allows it to be reused several times with minimal quality losses (Scurria et al., 2020).

The LimoFish process (Ciriminna et al., 2022) is waste-free thanks to nearly complete recovery of the extraction solvent and to the use of the milled anchovy leftovers residues of the extraction as an exceptional organic fertilizer named “AnchoisFert” (AnFert in the following) (Muscolo et al., 2022). Carried out under ultra-mild conditions (*i.e.*, room temperature and atmospheric pressure) the process exemplifies a circular economy strategy applied to reduce the demand for natural resources (*i.e.*, anchovies) and using anchovy fishery biowaste (Wang et al., 2012; Wu et al., 2013). The process cuts significantly the cost of conventional energy-intensive fish oil extraction and refinement multistep processes by shifting the production of fish oil rich in omega-3 lipids from blue fish or dedicated cultivations (Rubio-Rodríguez et al., 2010) to blue fish leftovers.

A quantitative analysis of environmental impacts associated with the LimoFish has not yet been performed. Life cycle assessment (LCA) is a widely applied methodology to evaluate the environmental performance of a product, process, or service processes based on the assessment of direct and indirect impacts, and possible consequences (ISO, 2006a). Prospective (or looking-forward) LCA integrates forecasting methods in its approach to assess technology at an early-stage (*i.e.*, lab scale, small-scale production) to full scale-up implementation (Arvidsson and Molander, 2017; Cespi et al., 2016; Elginoz et al., 2022; Patel et al., 2012; Pereira da Silva et al., 2021; Soratana, 2014; Thonemann and Schulte, 2019). Pereira da Silva et al. (2021), for instance, integrated LCA evaluation in the development stage of a process aimed at extracting starch from mango kernel. On a laboratory scale, the LCA methodology allows to identify the potential environmental hotspots and provide suggestions useful for to supporting future design. On the other hand, its adoption on industrial scale can be used to assess impacts under operating conditions close to the real process (e.g., energy use, emissions, and waste disposal). The application of the LCA at both scales can identify potential trade-offs and foster collaboration among stakeholders. In this study, we apply LCA to evaluate the potential environmental burdens associated to the AnFert and AnOil production via the LimoFish process in order to estimate the environmental impacts of the secondary production (*i.e.*, not from dedicated systems). LCA was first applied to laboratory data. Then a scale-up of the novel process modelled according to well-established methods (Maranghi et al., 2020; Piccinno et al., 2016) was evaluated using the LCA approach for modelling the production of chemicals for the overall evaluation of the process at industrial level.

2. Materials and methods

2.1. The life cycle assessment

According to the international standards ISO 14040-14044 (ISO, 2006b, 2006a) LCA is a strategic technique to identify and quantify the potential environmental impacts associated with a product, process or system throughout its life cycle. The common LCA framework (goal and scope definition, life cycle inventory - LCI, and life cycle impact assessment - LCIA), applies environmental mechanisms and characterization models to relate the LCI results to selected category indicators for a quantitative evaluation of environmental impacts. A fourth phase, *i.e.*, interpretation, transversal to the previous three, ensures consistency between the aims of the study and its execution to finally draw recommendations. In the following paragraphs, the four phases are described with reference to the LimoFish process.

2.1.1. LCA definitions and system boundaries

Fig. 1 depicts the main stages of the LimoFish at the laboratory scale (LabS) and after scale-up (SUp). Both operating scales are modelled by LCA in this study.

The functional unit (FU) of the study was set at 1 kg of anchovy leftovers inflow subjected to the LimoFish. The choice of using 1 kg of inflow material instead of 1 kg of product is made in order to focus the problem on the waste valorization, according to the main literature on waste management (Das et al., 2022; Mayanti et al., 2021; Provost-Savard et al., 2023). The system boundaries (Fig. 1) were defined following a “cradle-to-gate” approach, including therefore production and supply of raw materials and chemicals, energy requirements, and waste management occurring along the process. The infrastructure was excluded from the consideration, since its contribution is negligible for the case study. According to ecoinvent data (Wernet et al., 2016a), a reasonable estimation of the a chemical working plant, assuming an average life time of 30 years (Wernet et al., 2016a) and an annual production of 810kt (Wernet et al., 2016a) of a generic chemical, is 1.35E-03 kg CO_2 eq / kg of produced material. This value is accordingly considered not significant. The total impacts associated to the FU were calculated and a contribution analysis is provided with the aim to determine the most relevant steps and their influence on the environmental sustainability of the innovative fish oil and organic fertilizer production.

A sensitivity analysis was thus performed with the aim to test the robustness of the model as well as to evaluate the influence of the assumptions on the final outcomes.

2.1.2. Life cycle inventory (LCI)

The system under scrutiny consists of a sequence of processes aimed

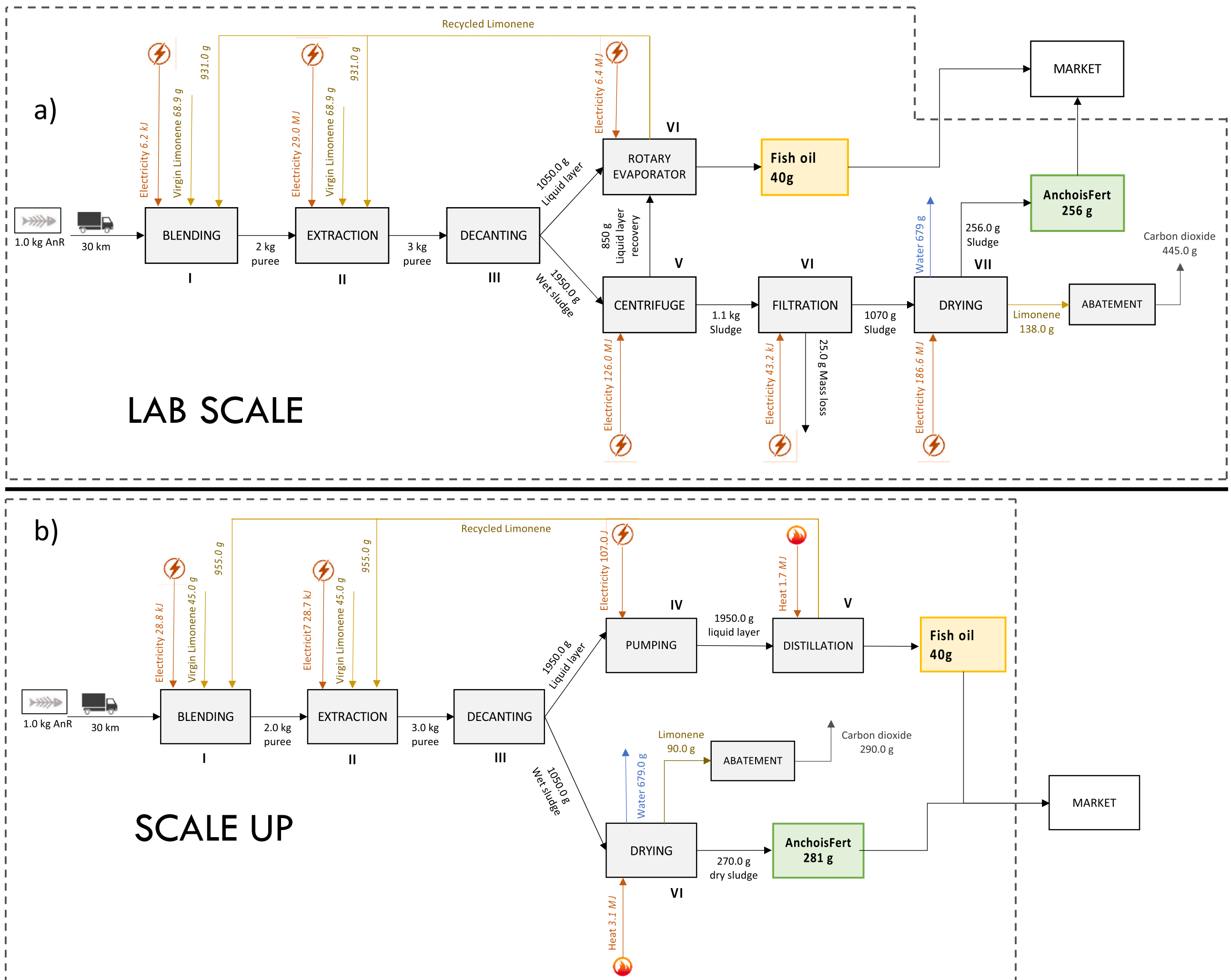

Fig. 1. System boundaries for each model. (a) LabS and (b) SUp.

at extracting the valued AnOil obtaining AnFert as co-product. Primary data from laboratory experiments were collected to fill mass balances in the case of LabS scenario. Dedicated checklists were adopted. On the other hand, for the SUp modelling we had to face one of the most challenging steps of the prospective LCA, that is the unavailability of life cycle inventory data (van der Giesen et al., 2020). In facts, in the field of chemical engineering, SUp is a decisive and integral part and many authors dedicated time and resources to this area, since the upscaling normally consists in several steps before the actual plant is built (Piccinno et al., 2016). According to Piccinno et al. (2016), LabS steps must be converted into larger scale reactors, apparatus and main equipment. It is specified that overall goal of the developed SUp framework is to allow a simulation of the process, following an LCA perspective and referring to logically and systematically compiled data. In this view, some simplifications had to be made to adapt the described framework. For this reason, the inventory was elaborated referring to the methodologies described by Maranghi et al. (2020) and Piccinno et al. (2016) and compiled using secondary (background) data based on the relevant literature in the field or from the ecoinvent 3.7 database (Wernet et al., 2016b). The main adaptation from the articles of Maranghi and Piccinno is related to the choice of reactor/equipment, in replacement of each laboratory phase (see Paragraph 2.1.2). Materials and energy flows involved in each step of the system were reported in Table 1, while proxy processes drawn from ecoinvent are listed in Table 2.

The detailed description of the main assumptions and of the product system is as follows:

Table 1
Data inventory of the LimoFish.

LabS		SUp	
Inbound transportation	30 km	Inbound transportation	30 km
Blending	6.2 kJ_e* 68.9 g of d-limonene	Blending	28.8 kJ_e 45 g of d-limonene
Extraction	29.0 MJ_e 68.9 g of d-limonene	Extraction	28.7 kJ_e 45 g of d-limonene
Decanting	/	Decanting	/
Centrifuge	126.0 MJ_e	Pumping	107.1 J_e
Rotary evaporator	6.4 MJ_e	Distillation	1.7 MJ_t*
Filtration	43.2 kJ_e	/	/
Drying	186.6 MJ_e	Drying	3.1 MJ_t

*Letters e and t refer to electric and thermal energy respectively.

(i) Since the end-of-life management applied to the amount of AnLeft used as primary source for the AnOil is currently not known, it is not possible to provide estimations about the benefits and/or burdens that its management would imply. For this reason, it was decided to apply the conservative *"zero burden"* criterion, and no environmental impacts were attributed to its production. However, since its supply is necessary to allow the functioning of the system, the contribution due to transportation

Table 2
ecoinvent reference processes.

ITEM	Unit	Ecoinvent Process
Water	kg	*Tap water {CH}| market for | APOS, U*
Anaerobic digestion emissions (LabS)	m^3	*Biogas {CH}| anaerobic digestion of manure | APOS, U* *iii
Anaerobic digestion emissions (SUp)	m^3	*Biogas {CH}| anaerobic digestion of manure | APOS, U* *iii
Cogeneration emissions	MJ	*Electricity, high voltage {IT}| heat and power co-generation, biogas, gas engine | APOS, U* *iii
D-Limonene (emission to air)	kg	*d-Limonene to air*
Electricity consumption	MJ	*Electricity, medium voltage, at grid/IT U*
Electricity production	MJ	*Electricity mix/IT U*
Exhausted filter management	kg	*Waste paper, unsorted {CH}| market for | APOS, U*
Fertilizer NPK	kg	*NPK (15–15–15) fertiliser {RER}| market for NPK (15–15–15) fertiliser | APOS, U*
Heat	MJ	*Heat, central or small-scale, natural gas {RER}| market group for | APOS, U*
Transportation (with refrigeration)	tkm	*Transport, freight, lorry with refrigeration machine, 3.5–7.5 ton, EURO5, R134a refrigerant, freezing {GLO}| transport, freight, lorry with refrigeration machine, 3.5–7.5 ton, EURO5, R134a refrigerant, freezing | APOS, U*

of the AnLeft is included in system boundaries, by assuming 30 km distance covered by truck.

(ii) The environmental impacts of the electricity consumption were calculated according to the Italian electrical energy mix in 2020 (IEA, 2022), consistent with the geographical boundaries of the system investigated.

(iii) In LabS case the biogas process of ecoinvent was taken as reference only to estimate the air emissions. Electricity consumption and the production of the waste inflow were excluded, since already included in the model as primary data. The same was done for the SUp scenario. A similar approach was applied to the cogeneration phase, where the impacts associated to the biogas inflow were accordingly substituted from primary data.

(iv) To calculate the potential environmental impacts of the extracting agent (*d*-limonene), the inventory data were adapted from Santiago et al., (2020) considering two production techniques: namely, hydrodistillation and cold pressing from waste orange peel. Hydrodistillation affords a higher production yield (4.5 $kg_{d\text{-}limonene}$/100 kg_{waste}) and lower water consumption (1 g_{water}/100 kg_{waste}). Cold pressing affords a production yield of 3.3 $kg_{d\text{-}limonene}$/100 kg_{waste} and a water consumption of 5.4 kg_{water}/100 kg_{waste}. Cold pressing, however, requires significantly lower energy (101.6 MJ_e and 1.0 MJ_t, respectively) than hydrodistillation (29.3 MJ_e and 1607.9 MJ_t). In light of its high yield, we selected hydrodistillation as the production route of choice. The inventory was modeled accordingly using the LCA Software of SimaPro 9.2 (PRé Consultants, 2021), considering the Italian energy mix for the electricity (IEA, 2022) and natural gas as primary source for heat (Table S1 and S22).

(v) Santiago et al., (2020) simulated the co-production of *d*-limonene from citrus waste, together with biogas and digestate. Therefore, in our simulation an economic allocation of 9.6 % was applied to the total impact to simulate the environmental burdens due to d-limonene production. The influence of biosolvent production technology choice on the final results was consistently tested in the sensitivity analysis. Being derived from biowaste, the aforementioned zero burden criterion was applied to raw source of *d*-limonene, waste orange peel (Santiago et al., 2020).

(vi) The electricity flows were not directly measured during the AnOil extraction. Hence, the electricity consumption (EC) of each step was calculated in accordance with Equation (1):

$$EC\,(MJ) = P\,(kW) \bullet t(h) \bullet 0.3 \bullet 3.6\,(\frac{MJ}{kWh}) \tag{1}$$

where *P* is the equipment power consumption (in kW), when used at its maximum power, *t* is the working time (in h) and 3.6 is the converting factor from kWh to MJ. The correction factor 0.3 is arbitrarily fixed, assuming that each specific equipment operates at the 30 % of its potential due as a consequence of its oversize in relationship to real dimensions of the experiment. The impact of this assumption on the final results is discussed in the paragraph 3.1.

For the SU simulation, electricity and heat consumptions were estimated following the approach of Piccinno et al. (2016). For more details see Table S3 (electricity consumption) and Tables S3, S4, S5, S6, S7, S8, S9 (up-scaling). A detailed description of the whole product-system is reported below. The relevance to LabS and/or SUp is in accordance with Piccinno and co-workers.

O) raw material supply and inbound transportation

Having a biorefinery located within a few km is important for reducing transportation costs and emissions, increasing efficiency, and creating a circular economy by utilizing waste streams. This evidence is particularly crucial for feedstocks with high water content, where transportation can significantly impact both the economy and the environment, especially when considering carbon footprint. Literature suggests that optimal distances for transportation range between 7.5 and 100 km (Giwa et al, 2022). The AnLeft is therefore assumed to be delivered by a freight truck equipped with a refrigerator from a supplier located 30 km away from the LimoFish production plant. The impacts associated to the transportation (tkm) were gathered from the ecoinvent database (Wernet et al., 2016b). The oil:solid:water composition ratio to extract 1 kg of AnLeft was set at 0.04:0.28:0.98, according to the LabS conditions.

I) Blending (LabS and SUp). The process is required to homogenize the AnLeft. In the SUp, the blending step is performed with a rotor–stator homogenizer of 5000 L. The parameters related to dimensions and technology were reported in Piccinno et al. (2016). The stirring time was fixed at 9 h (Ciriminna et al., 2022). The electricity input to allow a proper mixing of 1 kg of AnLeft is 6.2 kJ_e/FU in the LabS while the SUp value of 28.8 kJ_e/FU is calculated according to Equation (1), in which the type of the impeller (N_p), the diameter of the impeller (*d*), the stirring rotational velocity (*N*), the density of the reaction mixture (ρ_{mix}), the reaction time (*t*) and an efficiency value (η_{stir}) were considered (Piccinno et al., 2016). The virgin (68.8 g/FU on LabS and 45 g/FU on SUp) and recycled *d*-limonene (1.9 kg/FU on LabS and 1.2 kg/FU on SUp) were entered at 50 % rate in the blending step and at 50 % rate in the extraction step. For both the LabS and SUp the output of the blending process was 2 kg/FU of anchovy leftovers puree at low grade.

Equation (1): SUp blending.

$$E_{stir}[J] = \frac{N_p{}^*\rho_{mix}(kg/m^2)^*N^3(1/s)^*d^5(m)^*t(s)}{\eta_{stir}}$$

II) Extraction (LabS and SUp). This step was applied to extract the fish oil fraction from the AnLeft puree (3 kg) and separate it from the other components (e.g., d-limonene, water, impurities). Also in this case the process was performed in a rotor–stator homogenizer of 5000 L, assuming the same conditions of the blending step but with a reaction time of 12 h (Ciriminna et al., 2022). The electricity consumption of the process is 29.0 MJ_e/FU for the LabS and 28.8 kJ_e/FU for the SUp (Equation (1)). For both LabS and SUp steps of the process, the mix of puree and *d*-limonene output was sent to decanting. For the SUp process, the extract (d-limonene + AnOil) was then directed to centrifuge and the wet sludge to the drying phase.

III) Decanting (LabS and SUp). The aim of this process is to separate the two different fractions present in the 3 kg of puree deriving from the extraction step. The decantation step does not require energy or external chemical inputs. The liquid fraction (1.05 kg/FU for LabS and 1.95 kg/FU for SUp), contains the fish oil dissolved in d-limonene. The wet sludge (1.95 kg/FU for LabS and 1.05 kg/FU for SUp), contains water and d-limonene. The d-limonene:water ratio in the drying stage was calculated at 1:4.9 in the LabS and 1:7.5 in the SUp, due the higher efficiency of the recovery at the industrial scale.

IV) Rotary evaporation (LabS) and Pumping (SUp). The liquid fraction separated by decantation and centrifugation constitutes the input to rotary evaporation. This stage enables to recover fish oil (40 g/FU) and d-limonene. The purity of the recovered *d*-limonene was monitored by using an off-line gas chromatograph (Agilent 6890 N) equipped with a wide-bore capillary column (CP-WAX 52CB, 60 m, i.d. 0.53 mm) and a flame ionization detector (FID). A purity higher than 95 % was always detected, aligning our findings with similar results in the literature (Liu and Mamidipally, 2005). In addition, in the laboratory tests the recovered d-limonene via evaporation under reduced pressure was reused up to 4 times without a significant loss in its extraction ability. Concerning the SUp, a pumping stage is needed to convey 1.95 kg of liquid fraction from the decanting step to the distillation step. The electricity consumption, calculated according to Equation 2 (Piccinno et al., 2016), resulted in 10.7 J_e/FU. The expression considers the transferred mass (m), the gravity acceleration (g), an assumed height difference (Δh) and the pumping efficiency (η_{pump}):

Equation 2: SUp pumping.

$$E_{pump}[J] = \frac{m(kg)^*g(m/s)^*\Delta h(m)}{\eta_{pump}}$$

In agreement with what was observed when extracting rice bran oil from rice bran (Liu, 2005) when the amount of oxidized d-limonene remained less than 1 % even after several extraction and biosolvent recovery cycles, in the LimoFish process, the d-limonene recovered via evaporation under reduced pressure (40 mbar) at relatively high temperature (90 °C) remained pure at reusable at length, as confirmed by GC–MS analysis.

V) Centrifuge (LabS) and Distillation (SUp). The goal of this step is to separate the liquid and the solid fractions of the wet sludge deriving from the decanting step. On a lab-scale, centrifuge requires an electricity input of 126 J_e/FU and affords a liquid layer (850.0 g, sent to the rotary evaporator) and a sludge that in its turn was directed to the filtration step. In the SUp process, rotary evaporation is replaced by a distillation reactor that is one of the most energy demanding steps in the overall fish oil and organic fertilizer production route (with 1.7 MJ_t/FU energy demand). The energy consumption was estimated by Equation 3 and 4 (Piccinno et al., 2016). The parameters considered are: the heat capacity (C_p), the mass of reaction mixture (m_{mix}), the d-limonene boiling temperature (T_{boil}), the ambient temperature (T_0), the enthalpy of vaporization of d-limonene (Δh_{vap}), the mass of the liquid that needs to be evaporated (m_{dist}), the reflux ration (R_{min}, calculated by Equation 4) and the heating element's efficiency (η_{heat}). On the other hand, R_{min} was calculated by knowing the relative volatility of the solvents (α), the target purity of distillate (X_{LD}) and the molar fraction of the target compound in feed (X_{LF}) (Piccinno et al., 2016). Distillation enables to obtain the final 40.0 g/FU of AnOil and to recycle 1.2 kg/FU of the solvent.

Equation 3: SUp distillation.

$$Q_{dist}[J] = \frac{C_p(J/kg^*K)^*m_{mix}(kg)^*(T_{boil}(K) - T_0(K)) + \Delta h_{vap}(J/T)^*m_{dist}(kg)^*(1.2^*R_{min} + 1)}{\eta_{heat} - 0.1}$$

Equation 4: SUp, calculation of R_{min} for Q_{dist} estimation.

$$R_{min} = \frac{1}{\alpha - 1}^*[\frac{X_{LD}}{X_{LF}} - \frac{\alpha^*(1 - X_{LD})}{1 - X_{LF}}$$

On large scale, the milled anchovy leftover solid residue after extraction readily precipitates on the vessel of the extraction vessel when stirring is stopped. No centrifugation and/or filtration is required to separate the liquid phase comprised of d-limonene and the extracted lipids and vitamins from the solid phase. The liquid phase is then simply recovered by decantation.

VI) Filtration (LabS). The sludge obtained from the centrifuge on LabS is filtered, with an energy consumption of 43.2 J_e/FU. The waste stream of this step, comprised of the exhausted filter and vapor mass loss (30.0 g), was assumed to be managed as "hazardous waste", with emissions modelling from the ecoinvent database (Wernet et al., 2016b).

VII) Drying (LabS). This step is required to evaporate the water fraction and the residual d-limonene (not recycled) in the sludge from the pumping step. The heat consumption of the oven was estimated to be 3.1 MJ_t/FU. The amount of evaporated d-limonene, estimated to be 90.0 g/FU trough the mass balance, was burned and assumed to be totally converted in CO_2 (stoichiometric approach) emitted in the atmosphere.

VIII) Drying (SUp). As in the previous stage, it is required to remove water and d-limonene traces from the sludge. In the SUp process, the drying phase can remove the majority of d-limonene. The amount of evaporated biosolvent, estimated to be 137.8 g/FU, was also burned converted in CO_2. The electricity consumption of the oven was estimated with Equation 5 (Piccinno et al., 2016) and evaluated equal to 186 MJ_e/FU. The parameters considered in the equation were the specific heat capacity of water ($C_{p,liq}$), the mass of water to be evaporated (m_{liq}), the boiling temperature of water (T_{boil}), the ambient temperature (T_0) and the enthalpy of vaporization (Δh_{vap}). After this step, the dried AnFert can be directly employed as an organic fertilizer without any further treatment replacing a commercial inorganic fertilizer (NPK, 20:10:10) (Muscolo et al., 2022).

Equation 5: SUp drying.

$$Q_{dry}(J) = \frac{C_{p,liq}(J/kg^*K)^*m_{liq}(kg)^*(T_{boil}(K) - T_0(K)) + \Delta h_{vap}(J/kg)^*m_{vap}(kg)}{\eta_{dry}}$$

2.1.3. Life cycle impact assessment (LCIA)

The LCIA phase includes quantitative assessment of the potential environmental impacts resulting from the system under scrutiny. To this aim, environmental mechanisms and characterization models were applied to relate the LCI results to selected categories. The IMPACT World + method (Bulle et al., 2019), one of the most comprehensive and recent LCA methods for environmental impact evaluations (García-Velásquez and van der Meer, 2023; Rotthong et al., 2023; Zhou et al., 2023), was selected for the study. The LCIA method is employed to determine the impacts associated to the LimoFish process (LabS and LU).

2.1.4. Allocation criteria

Due to the absence of economic information related to AnOil fish oil and AnFert organic fertilizer new products obtained through the LimoFish process, no allocation criteria can be applied to the two products obtained. For this reason, the preferred choice is the attribution of the total impacts according to system expansion. That is, the FishOil was set as the main co-product of the LimoFish and a credit was given for avoiding the production of a traditional fertilizer with the same characteristics. Specifically, according to Muscolo et al. (2022), 1 kg of AnFert was assumed to replace 1 kg of NPK (ratio 1:1).

2.2. Sensitivity and uncertainty analysis

The sensitivity analysis was performed to test the robustness of the model created, enabling identification and quantification of the influence of certain parameters onto the environmental impact of the entire system (Weidema and Wesnæs, 1996). Uncertainty evaluation was performed for the midpoint impact categories results. As discussed above, the LCA model for the LabS was filled with primary data to ensure greater reliability of the outcomes. On the other hand, data of the SUp scenario were retrieved by the literature (Piccinno et al., 2016). For this reason, for a quantitative determination of uncertainties associated to each LCI parameter we referred to the data quality pedigree matrix (Weidema and Wesnæs, 1996), a method which is widely employed (Arfelli et al., 2022; De Menna et al., 2016; Laurent et al., 2014; Neri et al., 2018; Passarini et al., 2014) and recommended by the European Commission to assess the quality data in the Product Environmental Footprint and Organization Environmental Footprint (Zampori and Pant, 2019). The pedigree matrix (Table S14) lists the indicator scores assigned to each parameter and the related geometric standard deviation used in uncertainty analysis. In general, the scores associated to temporal, geographical and technological correlation were always estimated as the most optimistic situation (U value in the pedigree matrix = 1), while for reliability and completeness more severe scores were selected given the application of more assumptions (especially in case of SUp). A Monte Carlo simulation with 10,000 runs was also carried out to determine how the intrinsic variability of the parameters and the quality of the data used in the modelling may affect the outcomes.

3. Results and discussion

3.1. IMPACT World+: Midpoint analysis

Results are reported in form of contribution analysis for all the examined categories (Fig. 2) and in form of environmental comparison for only climate change, long term (CChl) and freshwater eutrophication (FEu) categories (Fig. 3). The selection is due to two main reasons. First, aligning to recent findings (Arfelli et al., 2022; Bulle et al., 2019), CChl turned out to be the more contributing category to the single score. Second, the contribution analysis performed for all the impact categories (Fig. 2) showed very similar outcomes for all the categories with the exception of the FEu. In particular, the contribution analysis proposed in Fig. 2 allows to determine the specific contribution of each flow (e.g., electricity) or process (e.g., extraction) with respect to a specific environmental category by its colour intensity (the more intense is the color, the higher is the contribution of the flow or process on the impact category). CChl was accordingly assumed to be representative for the "CChl cluster", consisting of 17 categories (CChl, CChs, FAc, FEc, FNeu, HCT, HnCT, IRa, MEu, LOb, LTb, MRu, OLd, PMF, POf, TAc, WSc).

Fig. 3 shows the results for climate change, long term (CChl) and freshwater eutrophication (FEu) categories. For the sake of completeness, the outcomes of full and detailed LCIA were reported in the Table S11, S12, S13, S14.

Results are reported in two forms: elementary flow perspective (histograms on the left), and process perspective (histograms on the right). This visualization allows to extrapolate, to better split the information and to get the relevance and importance of each phase of the system. Error bars plotting uncertainty ranges are included.

The LabS results show that the main contributor to the environmental impacts is the electricity consumption (93 %, CChl). Indeed, this represents the most relevant flow also for the other categories (except FEu), going from a minimum of 90.0 % (LOb) to a maximum of 99.8 % (WSc). Concerning the FEu, electricity contributes "only" for 54.8 % of the total impact. The assumption of working at the 30 % of the maximum equipment power is therefore highly relevant for the calculation of the absolute impact values. In case of CChl, for instance, enhancing the power consumption to 50 % of the maximum, translates into an enhanced impact value from 29.3 kg of CO_2 eq to 47.6 kg of CO_2 eq. However, this assumption does not significantly affect the contribution evaluation, since the electricity remains the main contributing process. The avoided production of NPK compensates the direct impacts for ≥ 10 % only for the FEu (98.0 %) and LOb (22.5 %) categories. A very weak contribution is identified for the AnLeft transportation (always less than 1 % in the CChl cluster and 5.7 % in FEu) and for the use of virgin *d*-limonene, in agreement with its higher contribution to the FEu (24.7 %), while for the other categories it never reaches the 10 % of the direct impacts.

The process perspective identifies as main contributors the steps characterized by a high electricity consumption (drying). Quite different trends are observed in SUp, since optimizations associated with the upscaling of the process dramatically reduce the estimated impacts (by about 25 times in case of CChl). Such a reduction is justified also by the different power supply between the LabS and SUp equipment: while LabS is fed with electricity, which is, in the drying step, totally converted into heat, in SUp the most impacting steps (*i.e.*, drying and distillation) are fed with heat directly generated from natural gas combustion. The reduced electricity requirement was reflected by a substantial decrease for both the CChl cluster and FEu values. The main contributor for all the examined categories, going from a minimum of 59.1 % (FEu) to a maximum of 74.3 % (IRa), turns out to be the input *d*-limonene, with nearly all (99 %) of the impacts associated to the virgin solvent production due to energy consumption. The CO_2 directly emitted into the atmosphere after the combustion of d-limonene affects only the CChs and CChl categories, participating to the direct impacts for the 16.5 % and 17.9 %, respectively. Inbound transportation, instead, generally contributes for less than 10 %, with the only exception of FEu, where it is responsible for 20.8 % of the total impact.

Electricity consumption, the main contributor in the LabS process, has its highest impacts in the water scarcity (11.1 %). The employment of the dry sludge as a fertilizer credits the overall process for the avoided production of an equivalent amount of NPK and it results in a net-diminution of the environmental impacts for 5 of the 18 analyzed categories (*i.e.*, MRu, FEu, MEu, PMF and LOb). Concerning the process perspective, the highest contribution was observed for blending and extraction, due to the virgin d-limonene input.

3.2. Sup sensitivity and uncertainty analysis

The contribution analysis highlighted two main variables which significantly affect the SUp results: the by-product valorization with AnFert replacing NPK, and the solvent production technology. A sensitivity analysis is applied to the *i*) by-product valorization: the solid residue is converted onsite via anaerobic digestion (AD) into biogas (Ciriminna et al., 2022; Sánchez, 2022), and the latter gas is integrated with a cogeneration unit (CHP) able to convert the resulted biogas into electricity; and *ii*) the production technology of *d*-limonene in order to determine how results are impacted by theses variables.

Fig. 4 displays the new valorization process. In this scenario (named SUp_{AD+CHP}), the drying process was not carried out to remove the presence of water, but principally to evaporate the impurities of non-recycled d-limonene flow, which may inhibit the bacterial growth and compromise the biogas production (Han et al., 2020). The heat consumption and the environmental impacts associated to the AD are calculated through the "ecoinvent" database (Wernet et al., 2016b). The environmental impacts of digestate produced in this phase (185 g/FU) were estimated according to the allocation proposed by Santiago et al. (2020), in order to maintain the same evaluation criteria (90.3 %, TS1) of the rest of the study. This approach is considered very conservative, since the avoided production of material presumably replaceable by digestate is not considered. The volume of biogas produced (0.080 Nm^3/FU), conversely, is directed to the co-generator which theoretically allows the production of electricity (0.53 MJ_e/FU) and heat (0.76 MJ_t/FU). A 100 % capture efficiency was assumed, with a 37 %η_e and 53 %η_t

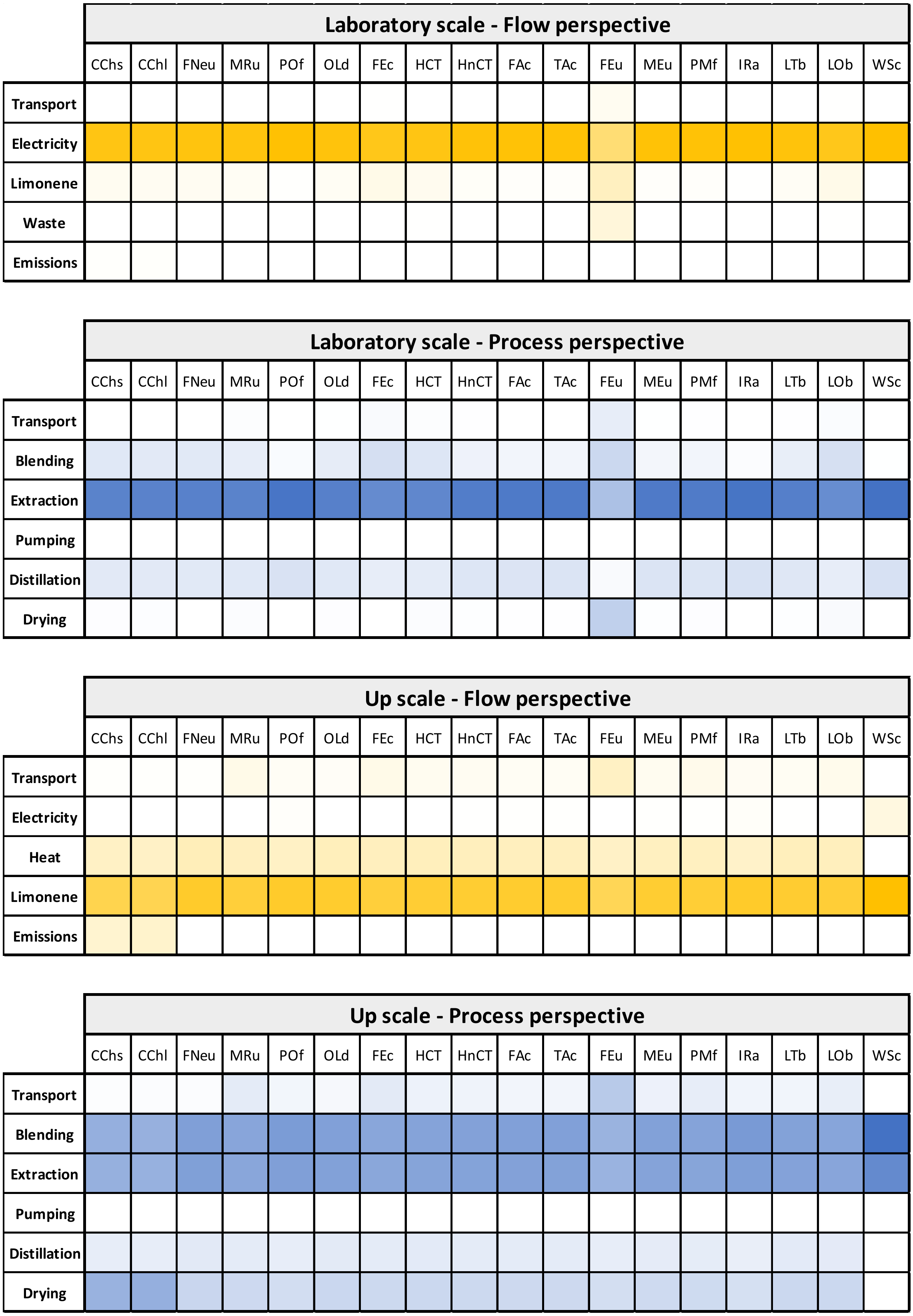

Fig. 2. Contribution analysis of the LimoFish process referred to all the 18 impact categories examined representing (a) LabS, flow perspective; (b) LabS, process perspective; (c) SUp, flow perspective; (d) SUp, process perspective.

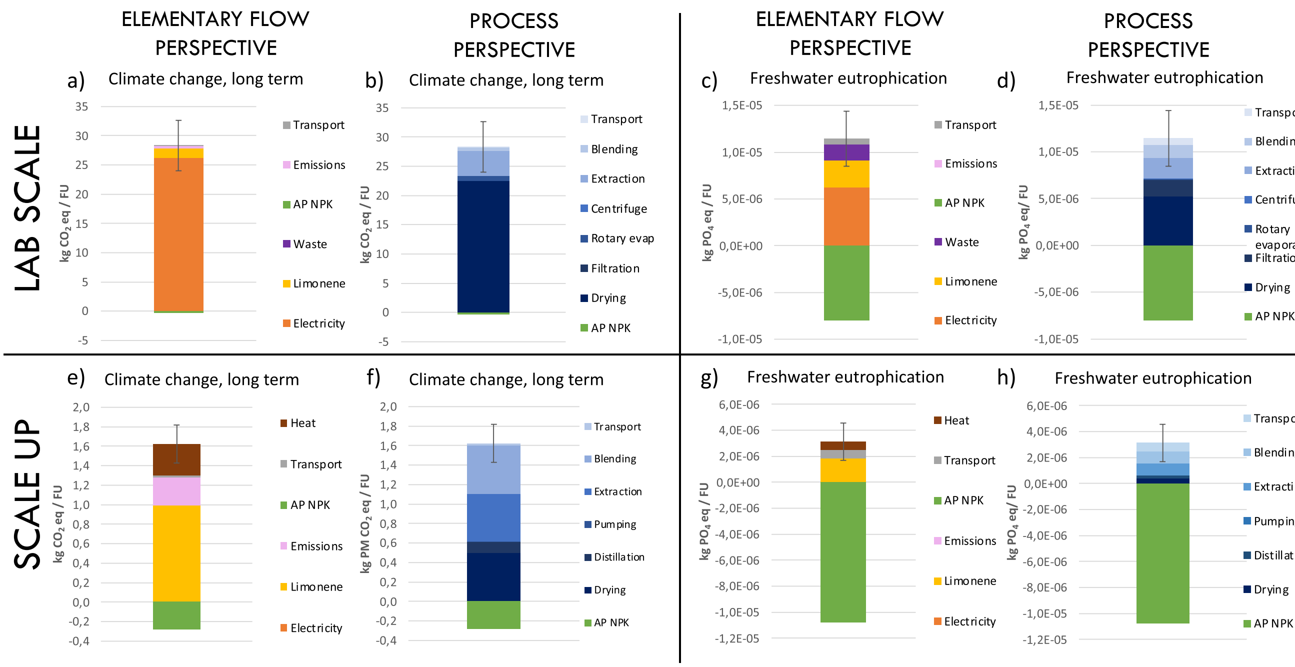

Fig. 3. Environmental impacts of the production system. (a) CChl, LabS, elementary flow perspective; (b) CChl, LabS, process perspective; (c) FEu, LabS, elementary flow perspective; (d) FEu, LabS, process perspective; (e) CChl, SUp, elementary flow perspective.

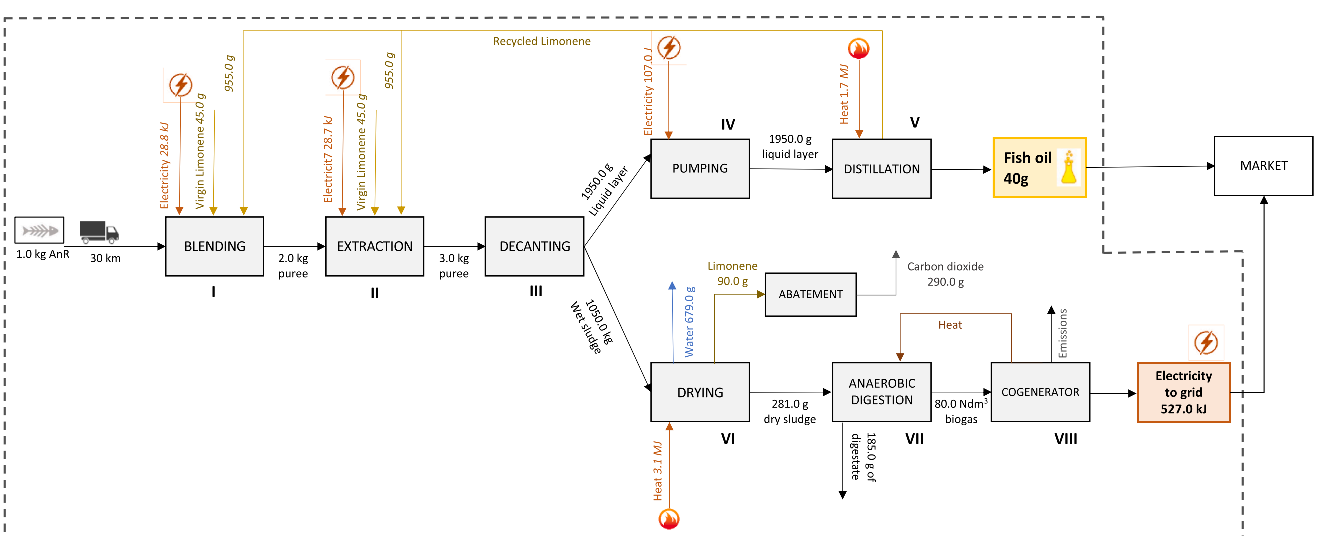

Fig. 4. Alternative scenario. Anaerobic digestion and cogeneration processes as alternative to fertilizer production.

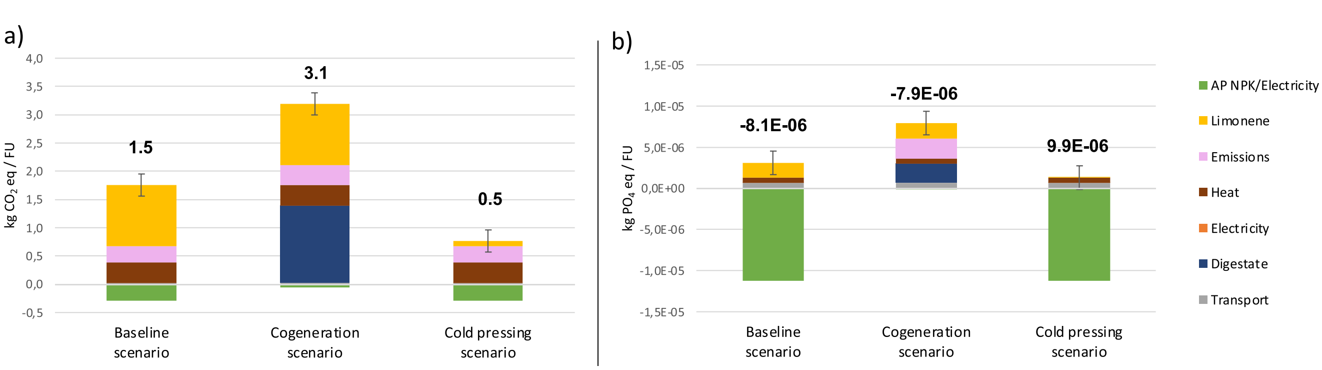

Fig. 5. Sensitivity analysis considering baseline scenario (SUp) compared to SUp_{AD+CHP} and SUp_{CP}.

(Wernet et al., 2016b). The electricity, assumed to be sold to the national grid, is included in the model as an avoided product. The heat produced was instead conveyed to the AD for self-consumption within the system. The heat excess was assumed to be dissipated into the atmosphere as heat waste. Environmental impacts of the co-generation and the credits associated to the avoided production of electricity are measured according to the ecoinvent database.

The environmental burdens associated to d-limonene utilization are calculated according to the inventory proposed by Santiago and coworkers (2020). However, as anticipated in the LCIA, the production techniques may significantly alter the impacts associated to the solvent production. For this reason, the model was accordingly modified substituting the d-limonene produced via hydrodistillation, with that extracted via cold pressing. Since the prices of the three main products (limonene, biogas and digestate) of the process described by Santiago and coworkers (2020) are very susceptible to market price volatility, the allocation criteria should also be considered as an interesting variable to investigate. Amid the two, the production technique (so-called SUp_{CP} scenario) was selected to perform the sensitivity analysis on the raw materials.

The analysis unveiled (Fig. 5a and 5b) significant differences between the baseline scenario and the alternatives. Comparing the SUp with the SUp_{AD+CHP} scenario, for the CClh category the impacts increase of 1.6 kg of CO_2 eq/FU. Such an increase is due to the different by-products management: the advantages originating from electricity production (-60.0 g of CO_2 eq/FU) are lower to those achievable by using the dry sludge as fertilizer (AnFert, −310.0 g of CO_2 eq/FU). For this reason, despite the production of electricity from biogas obtained via AD is generally promoted by feed-in tariffs, in this case the alternative valorization route to organic fertilizer is shown to be advantageous from the environmental point of view. The SUp_{AD+CHP} scenario, furthermore, does not include the digestate management despite its appropriate valorization (e.g., amendment or fertilizer production) could credit the overall environmental performances. However, even supposing to apply a *"zero burden"* criteria to digestate, assuming that a proper valorization would compensate the environmental emissions, the final value of CChl would be 1.8 kg of CO_2 eq/FU, still higher than the 1.5 of the baseline scenario. The conversion of biogas into different energy carries, such as biomethane (Ardolino and Arena, 2019), could be in future investigated as further alternative.

On the other hand, the SUp_{CP} scenario shows higher impact reduction when compared with SUp. Employing the cold pressing technique instead of hydrodistillation, the impact associated to the raw material decreases from 1.1 (73 % of the direct impact) to 0.5 kg of CO_2 eq/FU (18 %) thanks to the lower heat consumption during virgin d-limonene manufacturing.

Lastly, the uncertainty analysis confirmed the good data quality, showing a relative standard deviation of 9 % in CChl and 10 % in FEu (uncertainty bars in Fig. 3 and Fig. 5) with respect to the absolute values. In general, the relative standard deviation associated to the results is lower or equal to 10 % in 8/18 categories (CChs, CChl, POf, FAc, Tac, FEu, MEu, PMf), lower or equal to 20 % in FNeu and higher than 20 % in the resting categories.

4. Conclusions

The LCA methodology was applied to evaluate the environmental performances of an innovative route to produce a new, whole fish oil (AnchoiOil) and a new organic fertilizer (AnchoiFert) starting from the anchovy fillet leftovers and citrus-derived *d*-limonene as a solvent and a stabilizing (antimicrobial) agent. The analysis of the process was carried out on laboratory and industrial scale (*i.e.*, prospective LCA). The quantitative assessment enabled to evaluate the environmental impacts associated to each single flow and production step. The analysis suggests four clear outcomes.

First, the environmental impacts for the process at the laboratory scale are about 25 times higher than those estimated for the industrial process. Second, whereas on a laboratory scale electricity consumption is the dominant flow in terms of contribution to the final impacts, in the scaled up process the production of virgin d-limonene is the most impactful stage. Third, the use of the solid by-product of AnchoiOil extraction as an organic fertilizer is significantly more sustainable than its employment as an input to anaerobic digestion with electricity production from biomethane combustion. Fourth, the production technology of d-limonene plays an essential role in the overall environmental impact evaluation, with cold pressing extraction leading to approximately 70 % lower direct environmental impacts. Clearly, electric power utilization (chiefly for d-limonene extraction but also for d-limonene recovery via evaporation under vacuum) is a crucial contributor to the environmental impact of the LimoFish process at both laboratory as well as at scale-up scale. In this respect, it is relevant the fact that a large fraction (or even all, in case of energy storage in Li-ion batteries) of the electricity required can be self-produced by bioeconomy companies willing to apply the process via roof-integrated solar photovoltaic (PV) modules. Today's, high-power, low-cost PV modules reliably produce on-site large amounts of electrical energy for over 30 years allowing companies to dramatically lower their environmental footprint, especially in sunny areas of the world such as those where anchovies are caught and processed.

While this study focused specifically on anchovies processing and valorization, the LimoFish process is general and can be applied to any fishery industry by-products. For instance, leftovers of the shrimp industry treated with *d*-limonene afford a valued marine oil rich in natural astaxanthin and a solid residue rich in chitin (Scurria et al., 2020b). The approach of this study can thus be extended to the latter seafood leftovers to evaluate the overall environmental sustainability of the LimoFish process also in this case. LCA, in conclusion, confirms its value to evaluate the potential uptake of a new process and thus to support policy makers and stakeholders engaged with bioeconomy, including local communities for planning innovative and circular economy strategies for the valorization of biological residues.

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

Data will be made available on request.

Acknowledgments

This article is dedicated in memory of Emeritus Professor Rosario Pietropaolo that unexpectedly passed away on July 1st 2022. Rector of Università degli Studi Mediterranea di Reggio Calabria, first Dean of the Faculty of Engineering as well as a visionary of science, master of ethics, integrity and dedication and inspiration for many colleagues and students.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2023.06.002.

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