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Environmental and economic assessment of food additive production from mushroom bio-residues

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ABSTRACT

This work analysed the environmental and economic impacts of extracts enriched in ergosterol and vitamin D_2 production from bio-residues of *Agaricus Bisporus* mushrooms. The main hotspots of the extractive processes in environmental terms were identified through the standardized methodology Life Cycle Assessment. In respect to the economic analysis, the Material Flow Cost Accounting approach was applied to identify production costs, distinguishing between valued and wasted costs. The LCA results showed that electricity consumption is a key factor in environmental aspects, having the highest contribution to the process's environmental impact. In general, the most electricity-consuming unit processes analysed were lyophilization, extraction and evaporation. Despite evaporation being a process with great energy consumption, it also mitigates environmental impacts, since it allows solvent recovery through the extraction and reutilization phases in new iterations. The production costs for the identified extracts were determined: extract enriched in ergosterol (2.43€/1 g of extract) and extract enriched in vitamin D_2 (8.28€/1 g of extract). Recovered solvent incorporation in posterior iterations resulted in a total cost decrease of about 47% for ergosterol-extract and about 39% for vitamin D_2 extract. Solvent's recovery and reutilization allowed to not only reduce the environmental impacts but also the production costs.

1. Introduction

The properties of food such as shapes, colours, tastes, smells and textures are improved to satisfy the consumer's expectations (Aggett, 2018). The interest in the use of food additives has increased exponentially in recent years, partly motivated by an industry that needs to improve the shelf life of its products, as well as make them more appealing to an increasingly demanding consumer. A food additive is defined as "any substance not normally consumed as the food itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value" by Codex Alimentarius (Codex Alimentarius, 2021). Currently, there are more than 2500 food additives, mostly used as preservatives, and the use of synthetic additives is a common practice in the food sector. Yet, the harm to human health associated with the consumption of these additives is increasingly known (toxicological or allergenic effects) and supported by several studies (Carocho et al., 2015)– (Martins et al., 2016). As a result, the current consumer, characterized by being more conscious and knowledgeable, has been giving preference to foods free of additives of synthetic origin.

The growing association of negative effects on food consumption with artificial additives has driven the pursuit of new food additives of natural origin, both by the food industry and by consumers. Synthetic additives lead to harmful effects, both for humans (such as dermatitis and fatal anaphylaxis) (Carocho et al., 2014) and for the environment (since these additives are made up of chemical substances, which can lead to contamination of waters and soils and the loss of biodiversity). The expectations from current consumers combined with the detrimental effects of synthetic additives on health and the environment generated a great interest in developing additives based on natural products, which are less detrimental to human health and the environment. Therefore, it is essential to ensure that the production of new natural ingredients is competitively more sustainable.

To this end, the waste resulting from mushroom production presents itself as a potential candidate for the manufacture of additives of natural origin, since its chemical composition includes several bioactive compounds conferring it the capacity to exert beneficial effects at different levels, such as immunomodulating, antitumor, anti-hypercholesterolemia, antibacterial and antifungal, anti-inflammatory,

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antiviral, anti-diabetic, and cardiovascular beneficial effects (Reis et al., 2017). The production of mushrooms generates a large number of by-products (20% of production volume) that hold added-value components containing a high level of nutrients (Uma ñ a et al., 2020). In this context, mushrooms have a high content of biologically active compounds and there is currently ongoing work on future applications based on the valorisation of their by-products into high-value compounds.

Agaricus Bisporus, the most consumed mushroom in the world, is quite rich in ergosterol (Corr ê a et al., 2017), which can be converted into vitamin D_2 by UV radiation (Taofiq et al., 2017). Ergosterol acts as a hypocholesterolemic agent, having the potential to be incorporated into lipophilic foods, such as cheese, and its integration into hydrophilic matrices after stabilization by encapsulation techniques is also possible. Vitamin D_2 can be extracted and mainstreamed into flour for bakery and pastry products.

This work aims to assess the extraction of ergosterol from the *Agaricus Bisporus* species, at an environmental and economic level, to produce extracts enriched in ergosterol and vitamin D_2, using lab-scale data. The environmental and economic evaluation will be assessed through Life Cycle Assessment (LCA) (“ISO 14040, 2006), (ISO 14044: 2006b, 2006) and Material Flow Cost Accounting (MFCA) (N. E. I. 14051:2011, 2011) methodologies. These two methodologies were selected as they are both based on a similar understanding of material flows, with LCA identifying key product/system hotspots and MFCA visualizing resource scarcity flows and inefficient resource use, both contributing to potential product/system improvements. The MFCA methodology was applied instead of LCC, as this analysis follows a gate-to-gate approach, including only the production of the extract. In this sense, MFCA was identified as a more expedient and less complex tool to treat economic data through flow analysis. In short, by combining these two methodologies it is possible to identify potential improvement measures for an optimized resource efficiency.

1.1. Life Cycle Assessment

A life cycle approach implies an overview of a product or process to determine the potential environmental impacts that occur from each life phase, that is, from the extraction of raw materials, through production and use, to the reintroduction of the product into the environment after its final phase, thus closing the cycle.

The Life Cycle Assessment (LCA) is a standardized methodology by ISO 14040/44 (“ISO 14040, 2006; ISO 14044: 2006b, 2006) which comprises four main iterative phases: Goal & Scope definition (i.e. goal, scope, functional unit, product systems, system boundaries and data quality); Life Cycle Inventory (compilation of the quantified material and energy flows within the defined system boundaries, for each life cycle stage, as well as alternative scenarios for sensitivity analysis and/or comparison purposes – related to a pre-determined functional unit); Life Cycle Impact Assessment (assess the environmental impacts using scientifically recognized methodologies); and Interpretation (result analysis, identification of the main hotspots and recommendations) (“ISO 14040, 2006; ISO 14044: 2006b, 2006). The main goal of LCA is to support decision-makers regarding the improvement of the environmental performance of the system or product in analysis.

Although the topic of LCA of food additives production is still at an embryonic stage, there are some studies dedicated to this subject. Forte et al. (Forte et al., Ferreira) evaluated the environmental impacts of bacterial cellulose production to be used as a food additive. The results showed that the production of wastewater treatment, materials products, as well as cooling and heating agents are responsible for major impacts in various categories. Pérez et al. (P é rez-L ó pez et al., 2014) investigated the environmental impacts of the production of bioactive compounds from the microalgae *Tetraselmis suecica*. According to the results, the stage with major contributions to the environmental impacts was the inoculation and culturing stage, due to the use of nitrogen required for algae growth. Some alternatives to nitrogen were proposed, such as inorganic and organic fertilizers. Tedesco et al. (2019) explored the environmental impacts of the bioconversion of fruit and vegetable waste into earthworm meals to be used as a feed source. The main process hotspots are the emissions of methane, dinitrogen monoxide and ammonia (vermicomposting), as well as material transport and energy consumption (earthworm processing). Another study focused on the optimization of the extraction processes of the active compounds while performing an environmental analysis in parallel, namely extraction with ultrasound to obtain the antioxidant polyphenol produced from chicory (Vauchel et al., 2018), in which the use of the ethanol as a solvent implied a large increase in impacts. This study concluded that the use of ultrasound-assisted extraction reduced the environmental impacts. Another work (Rodr í guez-Meizoso et al., 2012) combined water extraction and particle formation on-line (WEPO) with pressurized hot water extraction and on-line drying of the extracts in one single step. This combination of processes demonstrated lower environmental impacts and energy consumption when compared with the conventional supercritical fluid and pressurized hot water extraction processes.

In the literature, it was not possible to find LCA studies of ergosterol and vitamin D_2 production. As such, the scientific contribution of this environmental characterization study is of high relevancy, adding new knowledge to the literature on the environmental and economic impacts of the production processes and providing the industrial players with supporting information for action plans and other decision-making activities.

1.2. Material Flow Cost Accounting

Organizations need to remain competitive in the global market, which requires high production standards (Christ and Burritt, 2015). Thus, the industry is facing the challenge of integrating ecological targets with economic objectives (Sygulla et al., 2014). To this end, Material Flow Cost Accounting (MFCA) has been suggested by several authors as a tool that can support companies in decision making for economic and environmental improvements (Kokubu and Tachikawa, 2013).

The first and foremost concept of MFCA is mass balance. The main purpose of MFCA is to quantify the waste and energy losses related to a product or a system by providing a detailed cost description of the analysed boundaries for the improvement of resource consumption. This methodology is standardized (N. E. I. 14051:2011, 2011) and characterized as a flow-oriented accounting method that traces and quantifies all material and energy flows in physical and monetary units. Furthermore, it compares the cost associated with the products and material losses (Kokubu and Tachikawa, 2013). MFCA is considered to be one of the most powerful tools in environmental accounting and management and is an effective approach to meet the need for increased productivity while reducing the costs and environmental impacts of production, as it enables the transparent identification of material and resource resources (N. E. I. 14051:2011, 2011) along a production chain. In manufacturing industries, all stages of the manufacturing process can be sources of waste and losses. The subsequent decrease in unwanted results implies a reduction in demand for input materials, leading to positive economic and ecological effects, increasing productivity and strengthening the competitiveness of product development (Sygulla et al., 2014).

The MFCA analysis splits the process into quality centres (QC). The quantity centre is a part of the process where inputs and outputs are quantified, and where materials are generally processed. Material flow data collected by QC is translated into monetary units to support decision making (Bierer and G ö tze, 2012). Desired and undesired flows do not only carry the material cost, since each process requires the input of raw material, but also consumables, energy, transportation, among others. MFCA adds all available cost information to the quantitative data of material flows. Thus, the economic loss can be analysed not only through the cost of the material lost but also by including all production

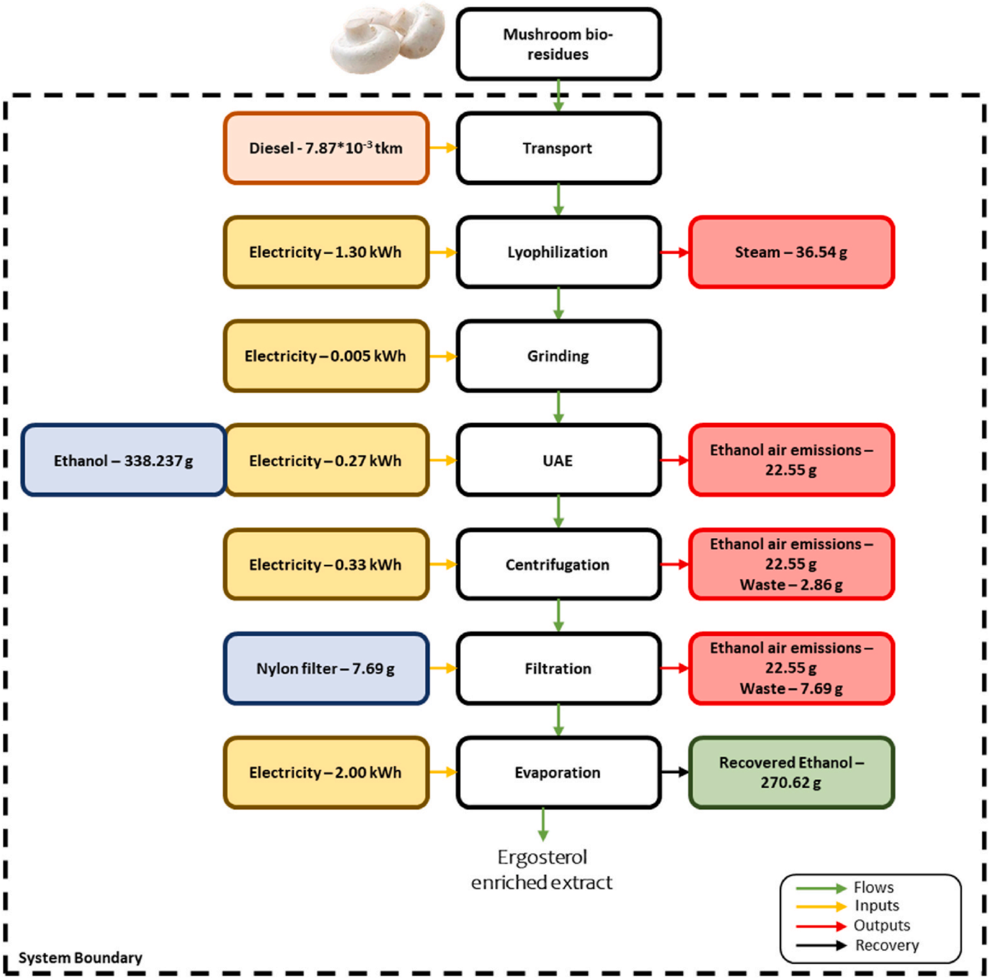

Fig. 1. Production of the ergosterol enriched extract - Map of the flow of materials.

costs (Fakoya and Van Der Poll, 2013). Consequently, it leads to a reduction in the demand for resources, while reducing environmental and economic impacts (Nakajima, 2006). In addition, they can be analysed individually in more detail to identify root causes for inefficiencies as well as the main causes that drive up the costs (N. E. I. 14051:2011, 2011).

Fakoya and Poll (Fakoya and Van Der Poll, 2013) analyzed the integration of an Enterprise Resource Planning (ERP) system with the MFCA methodology in a South African brewery to obtain information on existing waste costs. From the information collected it was concluded that it was necessary to integrate databases from the various divisions into a single system to facilitate the exchange of information and that data on waste are not integrated between divisions. The accessibility of this information, both between the production divisions and for the accounting department will facilitate the integration of MFCA into the database system created and enable an adequate analysis of existing waste for further reduction or reuse. Christ and Burritt (2017) examined the application of the MFCA to improve waste management in the catering industry to improve restaurant performance, both financially and environmentally. It was concluded that MFCA can significantly assist in reducing food waste by identifying direct and indirect costs that crucially contribute to decision support for greater resource savings and better financial performance.

The manuscripts described herein focus mainly on food waste management and not on food additives production. After extensive bibliographic research, it was concluded that there is no diverse literature on the integration of this methodology in the food industry. This sets a precedent for the results obtained in this work to substantially enrich the literature on the integration of MFCA in the food industry, specifically in the analysis of natural additives.

2. Material and methods

2.1. Goal and scope

The main objective of this study is to evaluate the environmental and economic performance of ergosterol extraction for the production of two extracts – ergosterol enriched extract and vitamin D_2 enriched extract - from bio-residues of *Agaricus Bisporus* mushrooms available in the north of Portugal (Mogadouro). The environmental analysis presented in this work follows the principles of an attributional LCA approach and the economic analysis follows the principles of the MFCA methodology. The data presented were obtained from information provided by an experimental laboratory unit, being the results in a Technology Readiness Level (TRL) 2–3.

For this assessment, a gate-to-gate approach was considered, which includes all impacts obtained from the moment the raw material is processed in the laboratory until the desired extracts are obtained. Thus, all stages related to agricultural production and/or provision of raw materials, as well as the stages of incorporation of extracts into food products are excluded from the analysis, since these productions present high uncertainty.

The functional unit (FU), which allows the fair comparison of environmental and economic performance between the various study

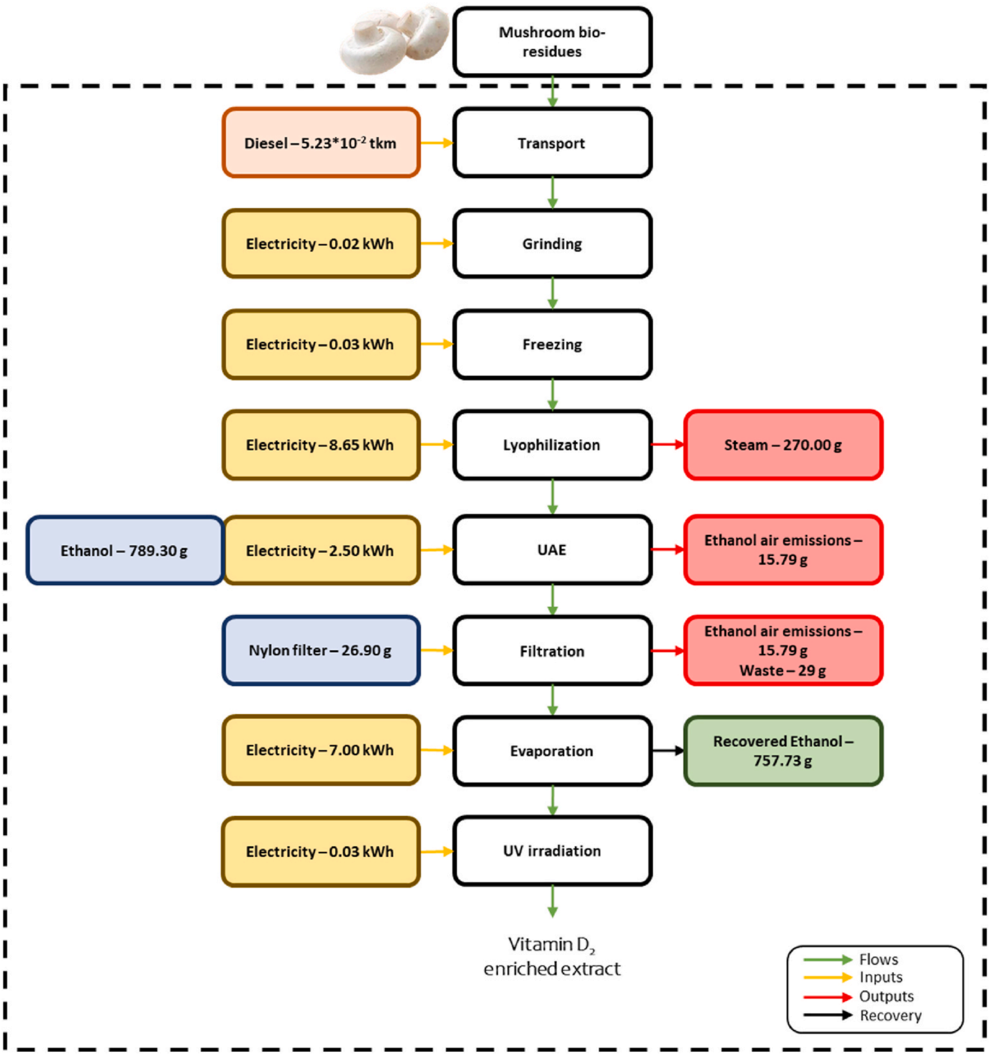

Fig. 2. Production of the vitamin D_2 enriched extract - Map of the flow of materials.

Table 1
Life cycle inventory for ergosterol enriched extract's production. FU/DU: 1g of the extract.

Life Cycle Stage	Inputs	Amount	Unit
Transport	Transport by lorry with refrigeration machine	7.87×10^{-3}	tkm
Lyophilization	Electricity	1.30	kWh
Grinding	Electricity	0.005	kWh
Ultrasound-assisted extraction	Electricity	0.27	kWh
	Ethanol	338.27	g
Centrifugation	Electricity	0.33	kWh
	Bio-waste	2.86	g
	Ethanol air emissions	22.55	g
Filtration	Nylon filter	7.69	g
Evaporation	Electricity	2.00	kWh
Life Cycle Stage	**Outputs**	**Amount**	**Unit**
Lyophilization	Steam	36.54	g
Ultrasound-assisted extraction	Ethanol air emissions	22.55	g
Centrifugation	Ethanol air emissions	22.55	g
Filtration	Bio-waste	4.71	g
	Ethanol air emissions	22.55	g
Evaporation	Recovered ethanol	270.62	g

Table 2
Life cycle inventory for vitamin D2 enriched extract's production. FU/DU: 1g of the extract.

Life Cycle Stage	Inputs	Amount	Unit
Transport	Transport by lorry with refrigeration machine	5.23×10^{-2}	tkm
Grinding	Electricity	0.02	kWh
Freezing	Electricity	0.03	kWh
Lyophilization	Electricity	8.65	kWh
Ultrasound-assisted extraction	Electricity	2.50	kWh
	Ethanol	789.30	g
Filtration	Nylon filter	26.90	g
Evaporation	Electricity	7.00	kWh
UV irradiation	Electricity	0.50	kWh
Life Cycle Stage	**Outputs**	**Amount**	**Unit**
Lyophilization	Steam	270.00	g
Ultrasound-assisted extraction	Ethanol air emissions	15.79	g
Filtration	Bio-waste	29.00	g
	Ethanol air emissions	15.79	g
Evaporation	Recovered ethanol	757.73	g

scenarios. In this specific case, the function of the system is to produce a specific quantity of enriched extracts, over the course of a chain of different processes. A more descriptive method for simplifying the

Table 3
Costs of each quantity centre – Ergosterol enriched extract's production. FU/DU: 1g of the extract.

Life Cycle Stage	Input	Amount	Units	Costs
Transport	Diesel for transport	7.87×10^{-3}	tkm	0,012 €
Lyophilization	Electricity	1.30	kWh	0,182 €
Grinding	Electricity	5.05×10^{-3}	kWh	0,001 €
Ultrasound-assisted extraction	Electricity	0.27	kWh	0,038 €
	Ethanol	338.27	g	1444 €
Centrifugation	Electricity	0.33	kWh	0,047 €
	Bio-waste	2.86	g	0,535 €
Filtration	Nylon filter	7.69	g	0,203 €
	Bio-waste	4.71	g	1050 €
Evaporation	Electricity	2.00	kWh	0,280 €
	Recovered ethanol	270.62	g	1156 €

Table 4
Costs of each quantity centre – Vitamin D_2 enriched extract's production. FU/DU: 1g of the extract.

Life Cycle Stage	Input/Output	Amount	Units	Costs
Transport	Diesel for transport	5.23×10^{-2}	tkm	0,083 €
Grinding	Electricity	0.02	kWh	0,002 €
Freezing	Electricity	0.03	kWh	0,004 €
Lyophilization	Electricity	8.65	kWh	1211 €
Ultrasound-assisted extraction	Electricity	2.50	kWh	0,350 €
	Ethanol	789.30	g	3370 €
Filtration	Nylon filter	26.90	g	0,710 €
	Bio-waste	29.00	g	4842 €
Evaporation	Electricity	7.00	kWh	0,980 €
	Recovered ethanol	757.73	g	3235 €
UV irradiation	Electricity	0.50	kWh	0,070 €

Table 5
Global environmental impact - Ergosterol enriched extract's production. FU/DU: 1g of the extract.

Impact categories	Units	Amount
Global Warming (GW)	kg CO_2 eq	1.49E-04
Stratospheric Ozone Depletion (SOD)	kg CFC_{11} eq	1.40E-05
Ozone Formation (OF)	kg NO_x eq	1.44E-03
Terrestrial Acidification (TA)	kg SO_2 eq	1.56E-04
Freshwater Eutrophication (FE)	kg P eq	3.45E-04
Marine Eutrophication (ME)	kg N eq	9.15E-06
Human Toxicity (HT)	kg 1,4-DCB	5.68E-03
Land Use (LU)	m^2a crop eq	1.12E-04
Mineral Resource Scarcity (MRS)	kg Cu eq	1.29E-08
Fossil Resource Scarcity (FRS)	kg oil eq	5.18E-04
Water Consumption (WC)	m^3	1.96E-04

analysis is used by selecting a declared unit (DU) such as the mass quantity required for the additive to achieve its food functionality. Therefore, the selected unit for the analysis, was defined as the production of 1 g of the extract by the system and its process chain, and is used for the basis of the interpretation of results.

Despite the existence of some studies in the literature related to the environmental impact of mushroom production (Robinson et al., 2019; Leiva et al., 2015a, bLeiva et al., 2017), they were considered out of the scope, since mushrooms are usually produced to be sold as a food product, and this study aims to evaluate the extraction of ergosterol to produce extracts from mushroom residues (with no market value), at the environmental and economic level. Additionally, the main purpose of the LCA and MFCA studies is to evaluate and optimize the technologies for processing, extraction and stabilization of food additives, summarizing the alternatives with less environmental and economic impact. In this study, the environmental and economic performance assessment considers the inclusion of impacts that occurred from the reception of raw materials, including their processing, to the production of the extracts, comprising material inputs and outputs, energy and consumables. For the production of both extracts, mushroom bio residues (mushrooms that esthetically do not meet the expected requirements for sale) were used and transported to a processing unit (laboratory) in a refrigerated truck.

2.1.1. *Ergosterol enriched extract*

In this case study, the ergosterol enriched extract is obtained from the mushroom bio-residues. The production begins with lyophilization, a dehydration process that freezes the bio-residues and removes the water content by sublimation, with approximately 90% of the water removed. Subsequently, in the grinding technique, the freeze-dried mushroom bio-residues are grinded, and a part is stored in a freezer (to be processed later). Then, in the extraction phase, the powder sample is mixed with ethanol and submitted to ultrasound-assisted extraction (UAE). After UAE, the mixture is centrifuged and most of the mushroom bio-residue is removed. Finally, the mixture is filtered (using nylon filters) and enters into the evaporator where the solvent is recovered, thus generating the dry extract. At the end of the production, about 80% of the ethanol used is recovered. As there is no information available on the amount of ethanol lost in each process, it was considered that the volume of lost ethanol (20%) would be equally divided by each process (UAE, centrifugation and filtration). Fig. 1 illustrates the material flow map developed based on the production system of the ergosterol enriched extract.

2.1.2. *Vitamin D_2 enriched extract*

For the production of this extract, the grinding technique was initially used in which the mushroom bio-residues are grinded and then frozen, to later go through the lyophilization process to remove the water content. Subsequently, a UAE is performed, where the powdered sample is mixed with ethanol. Consequently, the sample is filtered (using a nylon filter). After, in the evaporation phase, the mixture from the previous stage enters the evaporator, where the solvent is recovered and generates the dry extract. An ultraviolet irradiation technique was applied to convert the dry extract enriched in ergosterol into Vitamin D_2. About 96% of the ethanol used in this production is recovered. However, since again there is no information about the amount of ethanol lost in each process, the lost volume (4%) was equally divided for each process (UAE and filtration). Fig. 2 illustrates the material flow map developed based on the production system of the vitamin D_2 enriched extract.

2.2. *Life cycle inventory*

The life cycle inventory (LCI) considers all the relevant inputs and outputs for each process that occurs during the product or system life cycle. Of note, both the environmental and economic data were reported by the Centro de Investigação de Montanha (CIMO). To complete the inventory data for the production process of these extracts, primary data made available by the laboratory was considered whenever available (Table 1 and Table 2). The gathered inventory data contains information about the operation and the quantities of each flow (raw material, electricity and consumables). The equipment used in the processes

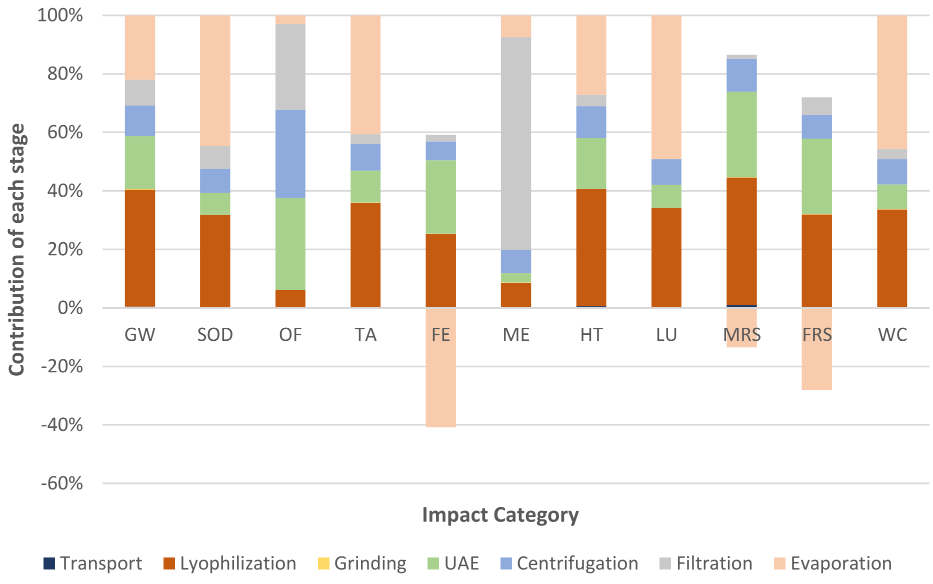

Fig. 3. Environmental impacts of the production process - Ergosterol enriched extract's production. FU/DU: 1g of the extract.

Table 6
Comparison of overall environmental impacts - Ergosterol enriched extract's production. FU/DU: 1g of the extract.

Impact categories	First iteration	Second iteration
Global Warming (GW)	1.49E-04 kg CO_2 eq	1.07E-04 kg CO_2 eq
Stratospheric Ozone Depletion (SOD)	1.40E-05 kg CFC_{11} eq	1.34E-05 kg CFC_{11} eq
Ozone Formation (OF)	1.44E-03 kg NO_x eq	1.36E-03 kg NO_x eq
Terrestrial Acidification (TA)	1.56E-04 kg SO_2 eq	1.37E-04 kg SO_2 eq
Freshwater Eutrophication (FE)	3.45E-04 kg P eq	4.56E-05 kg P eq
Marine Eutrophication (ME)	9.15E-06 kg N eq	8.66E-06 kg N eq
Human Toxicity (HT)	5.68E-03 kg 1.4-DCB	4.23E-03 kg 1.4-DCB
Land Use (LU)	1.12E-04 m^2a crop eq	1.08E-04 m^2a crop eq
Mineral Resource Scarcity (MRS)	1.29E-08 kg Cu eq	5.63E-09 kg Cu eq
Fossil Resource Scarcity (FRS)	5.18E-04 kg oil eq	1.53E-04 kg oil eq
Water Consumption (WC)	1.96E-04 m^3	1.84E-04 m^3

operates on electricity, except for the filtration process, since it is carried out by gravity.

To quantify the environmental impacts related to electricity consumption, the Portuguese energy mix of 2020 was considered. The process for electricity consumption in Portugal that is available in the Ecoinvent v3.7.1 database provides the energy mix in Portugal from 2017. Since then, the Portuguese energy mix has changed significantly with the transition to using more renewable energy on the grid. Using the information available from the Portuguese Renewable Energy Association (APREN) (APREN, 2020), the process was adapted for the timeline between August 2019 and August 2020. The self-consumption estimates were based on the energy production mix from the year 2018, using the information provided by the Directorate General of Energy and Geology (DGEG) (DGEG, 2019). The environmental impact assessment was conducted through the LCA software SimaPro v9.2.0.1, using the life cycle database Ecoinvent v3.7.1 and the impact quantification method ReCiPe 2016, in the hierarchical perspective (H), v1.1. After identifying the main production flows of each of the extracts, it is necessary to define the quantity centers (QC) for the application of the MFCA methodology. Thus, it was defined that each step involved in the production of each extract would be a QC. This analysis results in a flow chart presenting the economic flow based on the resources consumed in each QC. The main objective is to map the real value of waste and the economic flow of production.

Material flows between QCs in the process are first quantified in physical units and then costs are assigned to them. As for physical units, these are related to the material in terms of mass and energy. Regarding monetary units, these are divided into material cost and energy cost. These costs are identified in Table 3 and Table 4. The equipment used in each process was not accounted for in the economic analysis, since no information was made available about the equipment's useful life. At the same time, there is no information about the difference in scale between the laboratory equipment used and the industrial equipment. For the same reason, labor costs were not considered, since laboratory labor time would not be representative of the labor required at a commercial production scale. For the inclusion of this cost to have any industrial value and scientific meaning, it would be necessary to have information about how many products each equipment would be used for, the percentage of use for each product, and the percentage of work of each employee. Furthermore, as most industrial production of extracts is carried out through similar processes as the case studies, the type of equipment would be the same. As such, the exclusion of equipment costs would not have a great impact on the final results, as it is assumed that this equipment would already be owned by the industrial manufacturer. Additionally, the externalities of extract production were not taken into account due to difficulties in collecting information for the identification and characterization of their monetary value. However, since the raw material for these productions comes from mushrooms bio-residues, possible positive externalities can be found in generated market value for the sale of these extracts in comparison with the conventional synthetic alternatives. This new approach to the recovery of bio-residues can potentiate the reduction of waste, creating a better valorization for these residues through an upcycling process.

During the production of these extracts, it is possible to recover the used solvent, and as such, there are two iterations to the production process. The first iteration of the process uses virgin solvent, which is recovered and used in the second iteration, where a small amount of virgin solvent is added to compensate for process losses. The collected inventory was provided through the production of several amounts of extract and then normalized for the selected unit. For each extract, the following general costs were considered:

- Electricity: 0.14 €/kWh
- Diesel (for transport): 1.464 €/L

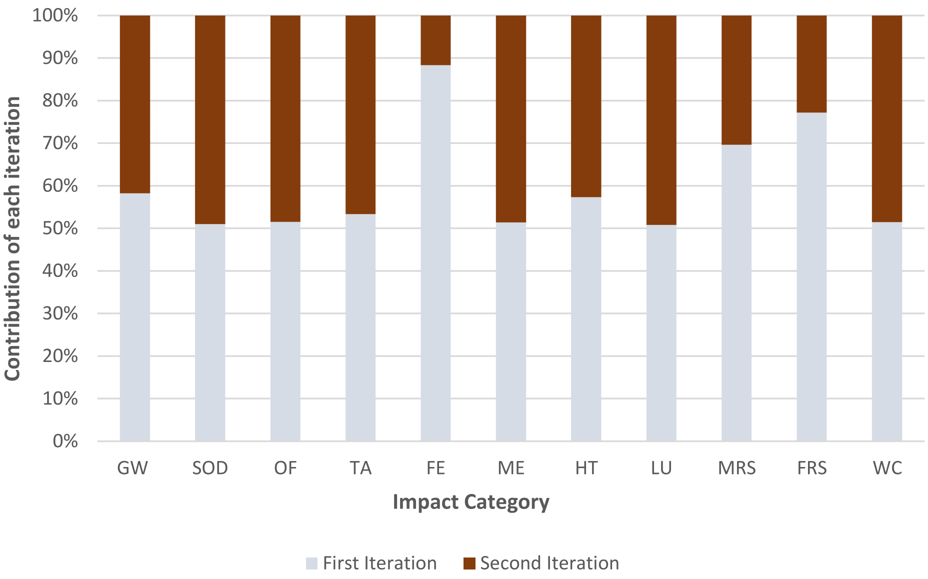

Fig. 4. Comparison of the environmental impacts regarding first and second iteration - Ergosterol enriched extract's production. FU/DU: 1g of the extract.

Table 7
MFCA application - Ergosterol enriched extract's production. FU/DU: 1 g of the extract.

Life Cycle Stages	Inputs	Amount	Unit	Total Cost	Cost (€)/unit	Outputs	Amount	Unit	Total Cost	Cost (€)/unit	Valued Cost (€)	Wasted Cost (€)
Transport	**MBW mass**	45.11	g	0.23 €	0.005 €	**MBW mass**	45.11	g	0.24 €	0.01 €	0.24 €	0.00 €
Transport	**Diesel**	174.40	km	0.01 €	-							
Lyophilization	**MBW mass**	45.11	g	0.24 €	0.01 €	**Lyophilized MBW mass**	8.57	g	0.42 €	0.01 €	0.07 €	0.32 €
Lyophilization	**Electricity**	1.30	kWh	0.18 €	0.14 €							
Lyophilization	**Steam**	36.54	g	-	-							
Grinding	**Lyophilized MBW mass**	8.57	g	0.08 €	0.01 €	**Grinded MBW mass**	8.57	g	0.08 €	0.01 €	0.08 €	0.00 €
Grinding	**Electricity**	0.01	kWh	0.001 €	0.14 €							
UAE	**Grinded MBW mass**	8.57	g	0.08 €	0.01 €	**Macerated MBW mass**	8.57	g	1.56 €	0.18 €	1.56 €	0.00 €
UAE	**Electricity**	0.27	kWh	0.04 €	0.14 €							
UAE	**Ethanol**	338.27	g	1.44 €	0.004 €	**Ethanol**	315.72	g				
Centrifugation	**Macerated MBW mass**	8.57	g	1.56 €	0.18 €	**Centrifuged MBW mass**	5.71	g	1.60 €	0.19 €	1.07 €	0.53 €
Centrifugation	**Electricity**	0.33	kWh	0.05 €	0.14 €							
Centrifugation	**Ethanol**	315.72	g	-	-	**Ethanol**	293.17	g				
Filtration	**Centrifuged MBW mass**	5.71	g	1.07 €	0.19 €	**Filtered MBW mass**	1.00	g	1.27 €	0.22 €	0.22 €	1.05 €
Filtration	**Nylon filter**	7.69	g	0.20 €	0.03 €							
Filtration	**Ethanol**	293.17	g	-	-	**Ethanol**	270.62	g				
Evaporation	**Filtered MBW mass**	1.00	g	0.22 €	0.22 €	**Ergosterol enriched extract**	1.00	g	0.50 €	0.50 €	0.50 €	0.00 €
Evaporation	**Electricity**	2.00	kWh	0.28 €	0.14 €							
Evaporation	**Ethanol**	270.62	g	-	-	**Recovered ethanol**	**270.62**	g	**1.16 €**			

Valued Cost	**0.50 €**
Wasted Cost	**1.93 €**
Total Cost per 1g	**2.43 €**

- Ethanol: 3.36 €/L

3. Results

This section presents the results obtained for the environmental and economic impacts occurring in the life cycle stages for the production of 1g of ergosterol and vitamin D_2 enriched extracts. Through Simapro V9.1.0.7 it was possible to match the information provided by the laboratory unit with the available life cycle data in Ecoinvent v3.7.1, thus obtaining information inherent to the extractive and transformative processes of the material and energy flows. To apply the MFCA methodology, an MS Excel™ sheet was developed which organizes all the information and calculates the costs associated with the production process of each extract.

For this methodology, costs were divided into two types: valued costs and wasted costs. Valued costs are here defined as costs that add value to the final product and thus are incorporated in the output mass. On the other hand, although wasted costs can be necessary and add value to the final product, they can also represent inefficiencies in the processes. Either way, they represent the mass and energy losses throughout the processes and are not incorporated into the final mass. Accounting for these costs is extremely important, as it allows identifying where they occur and assessing whether they are unavoidable consequences of the process itself or whether they can be eliminated. At the same time, if they are unavoidable, their identification allows the search for alternative processes that may result in their mitigation. This is accounted on MFCA once the costs are associated with mass balances. When the output mass is lower than the input mass, that means that was a loss of mass. This lost mass has energy and materials incorporated, which are accounted in the MFCA tool as wasted value due to its non-incorporation in the final mass of the product.

Table 8
MFCA application - Solvent recovery - Ergosterol enriched extract's production. FU/DU: 1 g of the extract.

Life Cycle Stages	Inputs	Amount	Unit	Total Cost	Cost (€)/unit	Outputs	Amount	Unit	Total Cost	Cost (€)/unit	Valued Cost (€)	Wasted Cost (€)
Transport	**MBW mass**	45.11	g	0.23 €	0.005 €	**MBW mass**	45.11	g	0.24 €	0.01 €	0.24 €	0.00 €
Transport	**Diesel**	174.40	km	0.01 €	-							
Lyophilization	**MBW mass**	45.11	g	0.24 €	0.01 €	**Lyophilized MBW mass**	8.57	g	0.42 €	0.01 €	0.07 €	0.32 €
Lyophilization	**Electricity**	1.30	kWh	0.18 €	0.14 €							
Lyophilization	**Steam**	36.54	g	-	-							
Grinding	**Lyophilized MBW mass**	8.57	g	0.07 €	0.01 €	**Grinded MBW mass**	8.57	g	0.08 €	0.01 €	0.08 €	0.00 €
Grinding	**Electricity**	0.01	kWh	0.001 €	0.14 €							
UAE	**Grinded MBW mass**	8.57	g	0.08 €	0.01 €	**Macerated MBW mass**	8.57	g	0.41 €	0.05 €	0.39 €	0.00 €
UAE	**Electricity**	0.27	kWh	0.04 €	0.14 €							
UAE	**Ethanol**	338.27	g	0.29 €	0.004 €	**Ethanol**	315.72	g				
Centrifugation	**Macerated MBW mass**	8.57	g	0.41 €	0.05 €	**Centrifuged MBW mass**	5.71	g	0.45 €	0.05 €	0.29 €	0.15 €
Centrifugation	**Electricity**	0.33	kWh	0.05 €	0.14 €							
Centrifugation	**Ethanol**	315.72	g	-	-	**Ethanol**	293.17	g				
Filtration	**Centrifuged MBW mass**	5.71	g	0.30 €	0.05 €	**Filtered MBW mass**	1.00	g	0.51 €	0.09 €	0.09 €	0.41 €
Filtration	**Nylon filter**	7.69	g	0.20 €	0.03 €							
Filtration	**Ethanol**	293.17	g	-	-	**Ethanol**	270.62	g				
Evaporation	**Filtered MBW mass**	1.00	g	0.09 €	0.09 €	**Ergosterol enriched extract**	1.00	g	0.37 €	0.37 €	0.37 €	0.00 €
Evaporation	**Electricity**	2.00	kWh	0.28 €	0.14 €							
Evaporation	**Ethanol**	270.62	g	-	-	**Recovered ethanol**	**270.62**	g	**1.16 €**			

Valued Cost	**0.37 €**
Wasted Cost	**0.91 €**
Total Cost per 1g	**1.28 €**

Table 9
Global environmental impact - Vitamin D_2 enriched extract's production. FU/DU: 1g of the extract.

Impact categories	Units	Amount
Global Warming (GW)	kg CO_2 eq	1.08E+01
Stratospheric Ozone Depletion (SOD)	kg CFC_{11} eq	8.06E-06
Ozone Formation (OF)	kg NO_x eq	5.93E-02
Terrestrial Acidification (TA)	kg SO_2 eq	6.24E-02
Freshwater Eutrophication (FE)	kg P eq	1.42E-03
Marine Eutrophication (ME)	kg N eq	2.08E-04
Human Toxicity (HT)	kg 1,4-DCB	5.40E+00
Land Use (LU)	m^2a crop eq	7.02E+00
Mineral Resource Scarcity (MRS)	kg Cu eq	1.27E-02
Fossil Resource Scarcity (FRS)	kg oil eq	3.59E+00
Water Consumption (WC)	m^3	5.16E-01

3.1. Ergosterol enriched extract

3.1.1. Life Cycle Assessment

Table 5 presents the overall environmental impact results for the process of obtaining the ergosterol enriched extract, covering all the impact categories considered. Subsequently, Fig. 3 represents the contributions of each stage to each of the analyzed categories.

The overall production processes and analysis of the main phases reveals that ultrasound-assisted extraction (36%), lyophilization (24%) and evaporation (14%) are the processes with the highest impact in almost all categories.

The UAE process is the one that demonstrates the highest contribution to the total production impacts. It reveals a higher contribution of environmental impacts for the GW, OF, FE, HT, MRS and FRS categories. This contribution can be justified by the use of ethanol as a solvent in this process. In the lyophilization and evaporation processes, there is a high energy consumption by the equipment, and the related impacts are

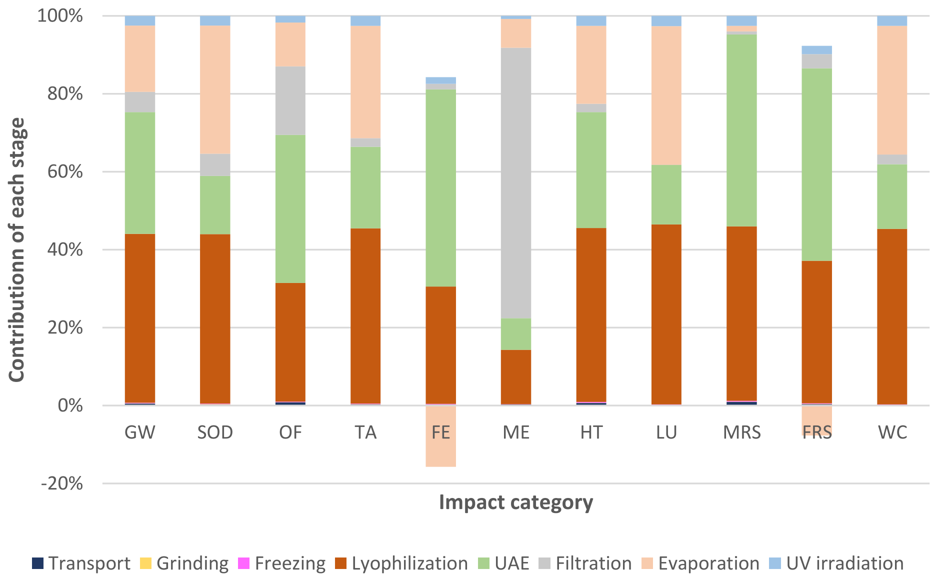

Fig. 5. Environmental impacts of the production process - Vitamin D_2 enriched extract's production. FU/DU: 1g of the extract.

Table 10
Comparison of overall environmental impacts – Vitamin D2 enriched extract's production. FU/DU: 1g of the extract.

Impact categories	First iteration	Second iteration
Global Warming (GW)	6.57E-04 kg CO_2 eq	5.39E-04 kg CO_2 eq
Stratospheric Ozone Depletion (SOD)	6.49E-05 kg CFC_{11} eq	6.34E-05 kg CFC_{11} eq
Ozone Formation (OF)	1.78E-03 kg NO_x eq	1.25E-03 kg NO_x eq
Terrestrial Acidification (TA)	7.22E-04 kg SO_2 eq	3.38E-04 kg SO_2 eq
Freshwater Eutrophication (FE)	1.16E-03 kg P eq	8.87E-04 kg P eq
Marine Eutrophication (ME)	3.50E-05 kg N eq	6.67E-04 kg N eq
Human Toxicity (HT)	2.52E-02 kg 1.4-DCB	4.25E-04 kg 1.4-DCB
Land Use (LU)	5.32E-04 m^2a crop eq	8.81E-04 m^2a crop eq
Mineral Resource Scarcity (MRS)	5.00E-08 kg Cu eq	5.97E-03 kg Cu eq
Fossil Resource Scarcity (FRS)	1.85E-03 kg oil eq	4.61E-03 kg oil eq
Water Consumption (WC)	9.16E-04 m^3	2.11E-02 m^3

mainly due to the production and energy distribution. However, Fig. 3 demonstrates a negative percentage in the evaporation process in the FE (−29%), MRS (−8%) and FRS (−19%) categories. This can be explained by the fact that there is a recovery of the used solvent (ethanol) at the end of this process, since it returns to the technological sphere to be reused, allowing the reduction of the impacts. The filtration process evidences a large contribution of the total impacts of the ME category, due to the use of nylon filters. Moreover, the stages with less relevance are transport and grinding, with a contribution lower than 1%, in all the analyzed impact categories.

As mentioned herein, the solvent used for the production of this extract is reused in the second iteration of this production. The notable difference in the environmental impacts between the first and second iteration is at the stage where the solvent enters, i.e., the EAU stage. Thus, Table 6 presents the comparison of totals between the first and second iterations for the selected impact categories. Fig. 4 presents the contribution of each iteration for the different impact categories.

Fig. 4 shows that the recovery of the solvent (ethanol) reduces the total environmental impacts in all selected categories. In the previous analysis, the use of ethanol presented contributions in the environmental impacts of the FE, HT, MRS and FRS categories, however, with the reuse of ethanol there is a reduction of impacts in these categories. Thus, the action of recovering the solvent can be considered as an improvement measure in these productions to reduce the environmental impacts.

3.1.2. *Material Flow Cost Accounting*

For the production of this extract, 45.11 g of mushroom bio-waste (MBW) were transported. As shown in Table 7, the production of 1 g of ergosterol enriched extract has a total cost of 2.43 € (1.93€ being wasted). With ethanol recovery, in a second iteration, only 0.29€ of virgin ethanol will be required to produce this extract again. Thus, as shown in Table 8, with solvent re-use, producing 1 g of ergosterol enriched extract costs 1.28 € (0.91 € being wasted).

As verified in the environmental and economic results, a potential cost reduction is observed when using solvent leftover from previous iterations. With this reuse, a difference of €1.15 can be identified in the total cost of producing this extract, which translates into a total reduction of around 47%.

3.2. *Vitamin D_2 enriched extract*

3.2.1. *Life Cycle Assessment*

Concerning the Vitamin D_2 enriched extract, Table 9 shows the global environmental impacts obtained for its extraction process. Fig. 5 represents the contributions of each unit process in all of the analyzed impact categories.

The process with the highest contribution to the total environmental impacts is lyophilization, representing about 39%, followed by extraction (31%) and evaporation (15%). It should be noted that most of these impacts are due to the energy consumption of the equipment used throughout the production process. In the extraction, the highest percentage of impact is in the category of FE (60%), due to the use of ethanol as solvent. Looking at Fig. 5, it is noted that filtration shows a high percentage (69%) in the ME category due to the nylon filters, as in the ergosterol case study. Finally, the processes with the lowest contribution to the total impacts of this production are transport, crushing, freezing and UV irradiation with contributions of less than 2%.

As analysed for the ergosterol-enriched extract, the environmental impacts of the Vitamin D2-enrich extract production process were assessed taking into account the solvent recovered in the first iteration. As expected, there are clear differences between production using virgin solvent and production using a recovered solvent. Thus, Table 10 presents the comparison of environmental impacts between the first and second iterations. Fig. 6 represents the contributions of each iteration in

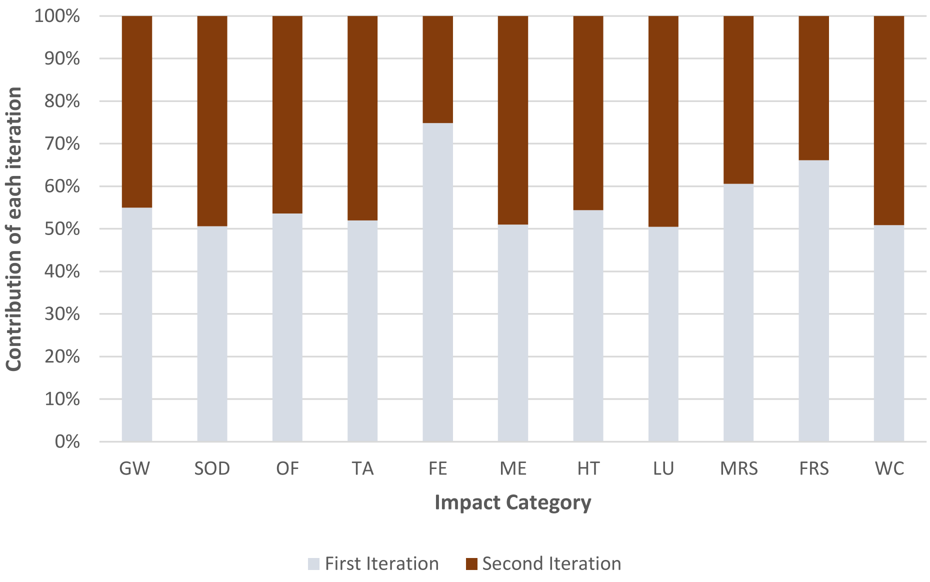

Fig. 6. Comparison of the environmental impacts regarding first and second iteration – Vitamin D_2 enriched extract's production. FU/DU: 1g of the extract.

Table 11

MFCA application - Vitamin D2 enriched extract's production. FU/DU: 1 g of the extract.

Life Cycle Stages	Inputs	Amount	Unit	Total Cost	Cost (€)/unit	Outputs	Amount	Unit	Total Cost	Cost (€)/unit	Valued Cost (€)	Wasted Cost (€)
Transport	**MBW mass**	300.00	g	1.50 €	0.004990 €	**MBW mass**	300.00	g	1.58€	0.01€	1.58€	0.00€
Transport	**Diesel**	174.40	km	0.08 €	-							
Grinding	**MBW mass**	300.00	g	1.58 €	0.005265 €	**Grinded MBW mass**	300.00	g	1.58€	0.01€	1.58€	0.00€
Grinding	**Electricity**	0.02	kWh	0.002 €	0.14 €							
Freezing	**Grinded MBW mass**	300.00	g	1.58 €	0.005273 €	**Frozen MBW mass**	300.00	g	1.59€	0.01€	1.59€	0.00€
Freezing	**Electricity**	0.03	kWh	0.004 €	0.14 €							
Lyophilization	**Frozen MBW mass**	300.00	g	1.59 €	0.01 €	**Lyophilized MBW mass**	30.00	g	1.59€	0.01€	0.28€	2.52€
Lyophilization	**Electricity**	30.00	kWh	4.20 €	0.14 €							
Lyophilization	**Steam**	270.00	g	-	-							
UAE	**Lyophilized MBW mass**	30.00	g	0.58 €	0.02 €	**Macerated MBW mass**	30.00	g	4.00€	0.13€	4.00€	0.00€
UAE	**Electricity**	2.50	kWh	0.35 €	0.14 €							
UAE	**Ethanol**	789.30	g	3.37 €	0.0043 €	**Ethanol**	773.51	g	-	-		
Filtration	**Macerated MBW mass**	30.00	g	4.00 €	0.14 €	**Filtered MBW mass**	1.00	g	4.71€	0.16€	0.17€	4.55€
Filtration	**Nylon Filter**	26.90	g	0.71 €	0.03 €							
Filtration	**Ethanol**	773.51	g	-	-	**Ethanol**	757.73					
Evaporation	**Filtered MBW mass**	1.00	g	0.16 €	0.17 €	**Dry MBW mass**	1.00	g	1.14€	1.14€	1.14€	0.00€
Evaporation	**Electricity**	7.00	kWh	0.98 €	0.14 €							
Evaporation	**Ethanol**	757.73	g	-	-	**Ethanol**	**757.73**	g	**3.24€**	-		
UV irradiation	**Dry MBW mass**	1.00	g	1.14 €	1.15 €	**Vitamin D₂ enriched extract**	1.00	g	1.21€	1.21€	1.21€	0.00€
UV irradiation	**Electricity**	0.50	kWh	0.07 €	0.14 €							

Valued Cost	**1.21 €**
Wasted Cost	**7.07 €**
Total Cost per 1g	**8.28 €**

Table 12

MFCA application - Solvent recovery - Vitamin D_2 enriched extract's production. FU/DU: 1 g of the extract.

Life Cycle Stages	Inputs	Amount	Unit	Total Cost	Cost (€)/unit	Outputs	Amount	Unit	Total Cost	Cost (€)/unit	Valued Cost (€)	Wasted Cost (€)
Transport	**MBW mass**	300.00	g	1.50 €	0.005€	**MBW mass**	300.00	g	1.58€	0.01€	1.58€	0.00€
Transport	**Diesel**	174.40	km	0.08 €	-							
Grinding	**MBW mass**	300.00	g	1.58 €	0.005 €	**Grinded MBW mass**	300.00	g	1.58€	0.01€	1.58€	0.00€
Grinding	**Electricity**	0.02	kWh	0.002 €	0.14 €							
Freezing	**Grinded MBW mass**	300.00	g	1.58 €	0.01 €	**Frozen MBW mass**	300.00	g	1.59€	0.01€	1.59€	0.00€
Freezing	**Electricity**	0.03	kWh	0.004 €	0.14 €							
Lyophilization	**Frozen MBW mass**	300.00	g	1.59 €	0.01 €	**Lyophilized MBW mass**	30.00	g	2.80€	0.01€	0.28€	2.52€
Lyophilization	**Electricity**	8.65	kWh	1.21 €	0.14 €							
Lyophilization	**Steam**	270.00	g	-	-							
UAE	**Lyophilized MBW mass**	30.00	g	0.28 €	0.01 €	**Macerated MBW mass**	30.00	g	0.76€	0.03€	0.76€	0.00€
UAE	**Electricity**	2.50	kWh	0.35 €	0.14 €							
UAE	**Ethanol**	789.30	g	0.13 €	0.004 €	**Ethanol**	773.51	g	-	-		
Filtration	**Macerated MBW mass**	30.00	g	0.76 €	0.03 €	**Filtered MBW mass**	1.00	g	1.47€	0.05€	0.05€	1.42€
Filtration	**Nylon Filter**	26.90	g	0.71 €	0.03 €							
Filtration	**Ethanol**	773.51	g	-	-	**Ethanol**	757.73					
Evaporation	**Filtered MBW mass**	1.00	g	0.05 €	0.05 €	**Dry MBW mass**	1.00	g	1.03€	1.03€	1.03€	0.00€
Evaporation	**Electricity**	7.00	kWh	0.98 €	0.14 €							
Evaporation	**Ethanol**	757.73	g	-	-	**Ethanol**	**757.73**	g	**3.24€**	-		
UV irradiation	**Dry MBW mass**	1.00	g	1.03 €	1.03 €	**Vitamin D₂ enriched extract**	1.00	g	1.10€	1.10€	1.10€	0.00€
UV irradiation	**Electricity**	0.50	kWh	0.07 €	0.14 €							

Valued Cost	**1.10 €**
Wasted Cost	**3.94 €**
Total Cost per 1g	**5.04 €**

the different selected categories.

As observed in the previous analysis, the use of ethanol is reflected in some impact categories, namely in the category of FE. Analysing the graphical representation, we verify a decrease in the contribution of the second iteration in this category. As reported for the extract enriched in ergosterol, the reuse of the solvent presents itself as a potential improvement to be implemented in the laboratory production processes of vitamin D_2.

3.2.2. *Material Flow Cost Accounting*

To produce this extract, 300 g of mushroom bio-waste (MBW) were required. As shown in Table 11, the production of 1 g vitamin D2 enriched extract costs 8.28 € (7.07 € being wasted). The ethanol recovered at the evaporation stage is reused in a second iteration, where only 0.13 € of virgin ethanol is required to produce this extract again. As can be seen in Table 12, producing 1g of vitamin D_2 enriched extract with the addition of recovered solvent has a cost of 5.04 € (5.04 € being wasted).

As with the ergosterol-enriched extract, solvent recovery also has a significant impact on the total cost of this production. With this, a decrease of 3.24€ is observed, which represents a reduction of around 39%.

4. Conclusions

The main purpose of this study was to provide a better understanding of the main environmental and economic impacts of the studied food additives. This work presented a gate-to-gate environmental and economic analysis of the production of extracts enriched in ergosterol and vitamin D_2 from bio-residues of *Agaricus Bisporus* mushrooms, based on data from an experimental laboratory unit.

Analyzing the results obtained in the environmental analysis, it can be seen that the highest contribution to the environmental impacts is mainly due to the energy consumption used for each production process, namely in the lyophilization, extraction and evaporation processes. The extraction process reveals significant impacts in some of the categories due to the use of ethanol as a solvent; however, these can be mitigated by the evaporation process, where the solvent is recovered allowing its use in the next batches. Filtration, as it is a gravity filtration, presents its greatest contribution in the marine eutrophication category due to the use of nylon filters. Finally, the transport, grinding, freezing and UV irradiation processes have a minimum impact on the selected categories. Within this, it is concluded that energy consumption is the main factor in the total impacts generated by these productions. Thus, the use of energy from renewable sources can be an improvement measure to mitigate the impacts from these extract's production. Nonetheless, the optimization of the equipment capacity may demonstrate a better environmental performance concerning these productions, something to be expected in large-scale industrial production.

The MFCA methodology was used in this study to perform an economic evaluation. This method differs from the vast majority of economic assessment tools because it aims at bringing together environmental and economic aspects and thus gaining a deeper insight into the manufacturing processes of novel food additives. For this specific case, each of the processes involved in the production of each extract was defined as quantity centers. The production costs for the identified food additives were determined for each case study: extract enriched in ergosterol (2.43€/1 g of extract) and extract enriched in vitamin D_2 (8.28€/1 g of extract). In the scenario considering the incorporation of the reused solvent, there is a decrease in the total cost of production: about 47% for ergosterol-enriched extract (€1.28/1g extract) and about 39% for vitamin D2-enriched extract (€5.04/1g extract). In this work, MFCA was used on a laboratory scale, so the results presented were also normalized on the same scale and to the defined unit (1 g of extract produced). According to MFCA, the mass losses of raw material, extract and solvents throughout the process have associated cumulative waste costs that should be minimized. However, for the ingredients understudy, decreasing waste generation (wastage) is often not feasible as there are fundamental mass losses to the process (e.g., water vapor in the lyophilization process and raw material lost in filtration). However, using values referring to an industrial scale, different MFCA results can be obtained. Since the analysis focuses on laboratory production at a TRL 2–3 level, i.e., at an experimental stage, the environmental and cost impacts can be inflated.

Considering reuse as a decision support parameter in these productions allows improvement actions to reduce environmental and economic impacts, ensuring an optimized sustainability performance for recovery and reintegration of materials in the value chain. Thus, considering the reuse of the solvent there is a potential for reduction of both the environmental impacts and the total cost of both productions. Nevertheless, future work should also cover and support the increasing TRL transition of laboratory produced extracts to an industrial production scale.

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.

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