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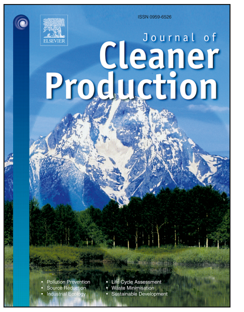

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Environmental and techno-economic assessment on the valorization of vine-side streams to produce resveratrol.

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Abstract

One of the most promising alternatives to face the environmental deterioration and handling of waste is the development of biotechnological processes. In this context, the winemaking process of red grapes gives rise to waste streams whose properties are suitable for the biotechnological production of high-value-added products, such as resveratrol, a polyphenol with functional properties. In this research article, vine pruning residues, grape must and wine lees are valorized through precision fermentation, considering its modeling at a real production scale using the SuperPro Designer tool. Besides, economic and environmental assessments provided valuable information on the potential commercialization of the resveratrol based on the proposed valorization process. The results obtained show that the use of grape must residues to produce resveratrol is the most promising alternative from both the techno-economic and environmental perspectives. In conclusion, it could be stated that the bioproduction of resveratrol by precision fermentation using wine-related waste is both sustainable adequate and economically attractive.

Keywords: winemaking process residues, sustainability assessment, economic analysis, environmental profile, antioxidant, resveratrol.

1. Introduction: wine side-streams as biorefinery feedstocks

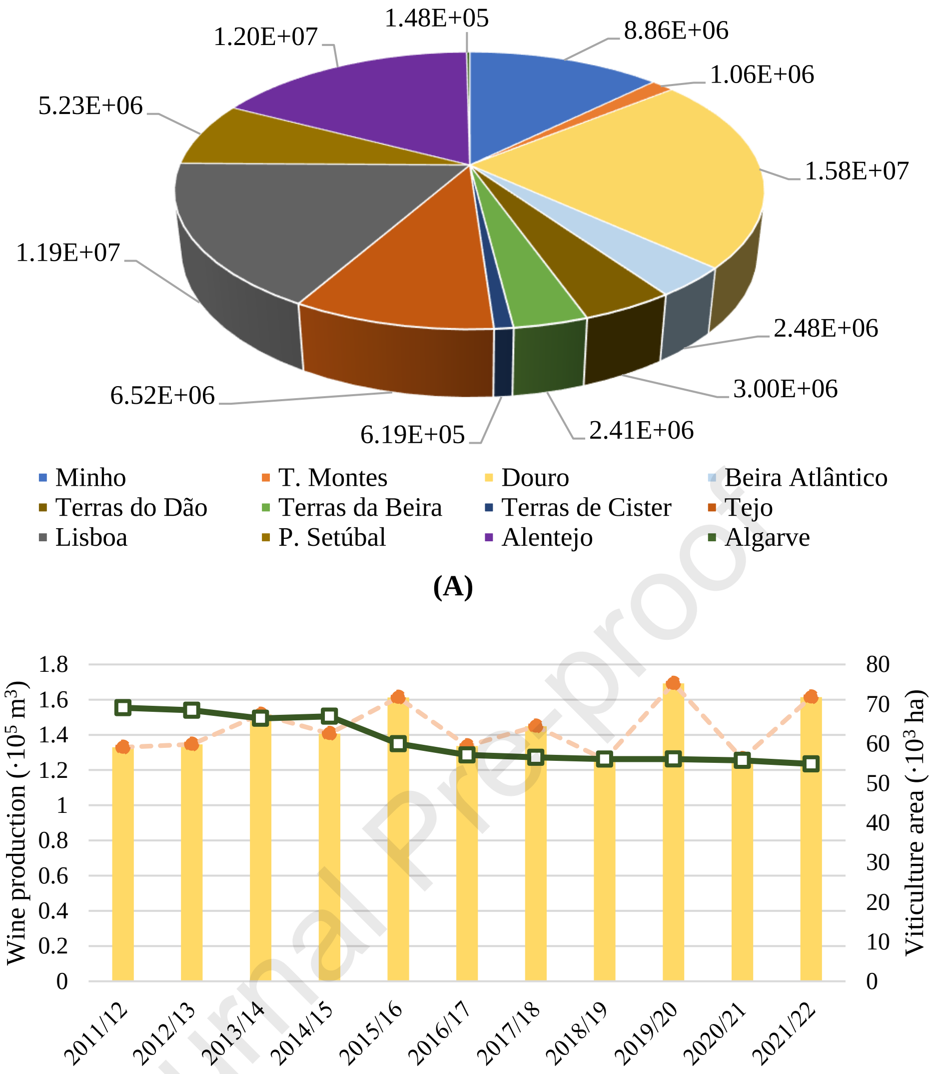
The European Union is the leading producer and exporter of wine, with Italy, France and Spain leading the ranking of countries with annual global production shares of 16.4%, 15.9% and 12.1%, respectively (Zacharof, 2017). The annual production of 75 million tons of wine grapes involves large volumes of secondary streams and waste, as well as on-site emissions, wastewater and biosolids (Bucić-Kojić et al., 2022; Ioannidou et al., 2022).

Wine production is divided into four main stages, starting with destemming and crushing of harvested grapes to remove grape stems, so that crushed grapes and must are obtained before the alcoholic fermentation stage. Once fermentation is complete, the pressing phase removes skins and seeds, so that the broth is sent to an aging and stabilization stage. This part of the process is the most time-dependent and is where the remains of yeast and grape solids settle to the bottom of the equipment and are removed, known “wine lees” residues (Cañón et al., 2014; Ncube et al., 2021; Rodrigues et al., 2022).

In this context, the secondary streams from the wine production process could be considered as a potential lignocellulosic biomass resource that could be used in a biorefinery scheme given their composition in lignocellulosic compounds, fermentable sugars and antioxidants (Filippi et al., 2022; Gonzalez-Garcia et al., 2018; Kucharska et al., 2018). Vine pruning residues are the most significant by-product of viticulture, representing more than 90% by mass. Its usual management is its burning in the agricultural field or its use as compost according to circular economy strategies. Its high availability, together with its low cost and potential composition make this residue a potential feedstock to be used in a biorefinery scheme, but for this it requires a pretreatment to release fermentable sugars that allow the bioconversion of its compounds (Jesus et al., 2022; Wei et al., 2022). In this regard, it has been reported that two main valorization alternatives could be developed: (1) separation and extraction of high valuable compounds, as flavorings, dyes or phenols (Jesus et al., 2020) and (2) bioconversion strategies, in which fermentation procedures stand out, for the production of bio-based chemicals, biofuels, enzymes and/or antioxidants (Berbel and Posadillo, 2018; Jesus et al., 2017; Kalli et al., 2018; Winterhalter et al., 2015; Zacharof, 2017). In the particular case of low quality wine, it is possible to identify processing schemes to produce a number of value-added products such as antioxidants, nutrients, ethanol, microbial oil or even, bioplastics, such as PHB (polyhydroxybutyrate) (Hijosa-Valsero et al., 2021; Kopsahelis et al., 2018; Maina et al., 2017).

The rationale behind the development of new process strategies for the valorization of wine production is related to the significant environmental impacts associated with its management and/or disposal. In fact, some researchers have evaluated the potential impacts of winery side-streams using the Life Cycle Assessment (LCA) methodology. It has been estimated that about 1300 tons of CO_2 equivalents are produced from winemaking waste (Lucarini et al., 2018). In fact, biorefinery approaches for the valorization of wine side streams have also been evaluated considering the environmental approach and following the LCA method, obtaining that energy requirements, both steam and electricity, are the main contributors on the environmental profile, with a total of 2.54 ton of CO_2 eq emitted per ton of wine lees residues processed within the biorefinery scheme (Cortés et al., 2019).

With this in mind, the present research article addresses the valorization of winery side streams produced in companies from the Douro region of Portugal, since it is the region where the largest amount of wine is produced annually, as can be seen in **Figure 1.** Moreover, it is important to mention that Portugal is the 12th country with the highest amount of wine production annually, so a large availability of wine production side streams is expected to be used within a biorefinery process scheme (Gaspar et al., 2019).



(B)

Figure 1. Winery production data. (A): total amount of wine produced in EU countries from 2011 to 2022 (in volume base, hL, yellow columns). (B): wine production (yellow dashed line with orange markers) and viticulture harvesting area (green line with square white markers) of Douro Region.

Specifically, this research focuses on the valorization of winery by-products, both those coming from harvesting activities, i.e., vine pruning residues (VP), and the waste streams obtained by the winery production facilities, known as grape must (GM) and wine lees (WL), for the production of resveratrol (3,4,5-trans-trihydroxystilbine). To develop the conceptual design, the SuperPro Designer software is used for the modeling of the three scenarios. This software allows to model, evaluate and optimize both batch processes, such as the present report, and continuous processes for different types of industries (such as bio-based industries, given the information available for fermentation reactors, which are not addressed in sufficient detail in the Aspen Hysys tool). Techno-economic and environmental assessments have also been developed, following ISO 14040 and ISO 14044 standards, to identify the cost-effectiveness of the process scheme and the main associated environmental burdens, seeking to evaluate the potentiality of alternative scenarios under a sustainable perspective.

2. Materials and methods

2.1 Process description and scale-up modelling

Resveratrol is a polyphenolic compound present in grapes and wine products and in higher quantity than in the other 72 species of plant that are able to synthesize it. It is considered a high-value compound, as it is antioxidant, anti-inflammatory, anti-aging, neuroprotective and anticarcinogenic, so its presence in nutraceutical, pharmaceutical and food markets is expected to be high (Jabbar et al., 2022; Piyaratne et al., 2022; Rabesiaka et al., 2011). The side-streams are analyzed under a conceptual design, techno-economic and environmental analysis perspective. The analysis is based on the experimental results obtained for *de novo* resveratrol production with and engineered industrial *Saccharomyces cerevisiae* strain (Costa et al., 2022b, 2022a, 2021).

The production process scheme differs slightly between the three waste streams used as feedstock, as VP requires a pretreatment step for the release of fermentable sugars, while for GM and WL, no pretreatment is required. **Figure 2** depicts the main stages of each of the process scheme scenarios developed in this research article, also considering the viticultural activities, as well as the background process related to vine pruning and grape production, together with the wine production process itself, since both grape must and non-wine secondary streams are obtained through the development of wine production. Furthermore, it is worth mentioning that the modeling approach is based on the valorization schemes of the secondary streams, not on the wine production process, although, for the development of the environmental assessment through the LCA methodology, the background activities, both the viticulture and the wine production process, are considered. On the other hand, operating conditions, chemicals needed and process efficiency in the production of resveratrol using these residual wine production streams, the results obtained by the research article developed by Costa et al. (2022) and Baptista et al. (2023) have been considered (Baptista et al., 2023; Costa et al., 2022a).

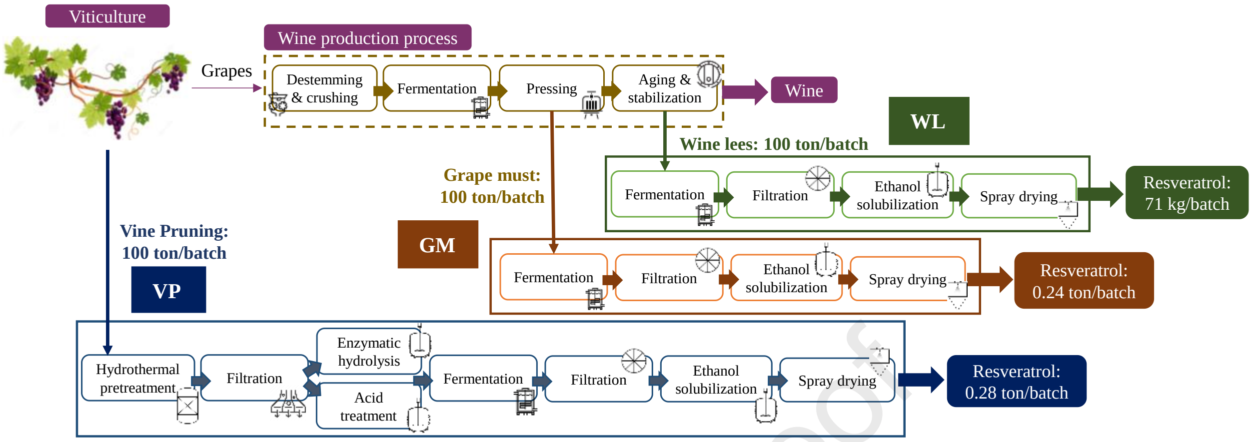


Figure 2. Main stages of the biorefinery approach of wine production side-streams valorization alternatives. Acronyms: VP (Vine Pruning), GM (Grape Must) and WL (Wine Lees).

Table 1 shows a summary of the process parameters and variables considering as input flow 1000 ton/batch of each of the side streams (VP, GM and WL). The characteristic details of each process as a function of the processed stream are presented next. The VP residues requires a hydrothermal pretreatment (liquid-to-solid ratio of 8 kg water/kg VP, and a severity of 3.89 at 215°C) carried out in a plug flow reactor (PFR-101, **Figure 1. SM**). Afterwards, a filtration step is required (BF-101**, Figure 1. SM**), in which the solid fraction undergoes an enzymatic hydrolysis treatment (R-101, **Figure 1. SM**) (using Cellic CTec 2 as enzyme, 626 U/mL, 24 h and 45ºC) and the liquid fraction undertakes a chemical post-hydrolysis (R-103, **Figure 1. SM**) with sulfuric acid (72% w/v at 121ºC). These two processes of both the solid and liquid fractions provide a higher release of fermentable sugars, and therefore an increase in the yield of the process.

After the pretreatment stage, encompassing both liquid and solid fractions, the fermenter of the process (FR-101, **Figure 1. SM**) operates in batch regime with glucose and xylose levels of 40 g/L and 24 g/L, respectively, and 7.5 g/L of yeast extract as nutrient source, room temperature, constant agitation and 96 h. After the fermentation section, the fermentation broth is sent to a filtration stage (RVF-102, **Figure 1. SM**), to remove the biomass to continue with the purification of resveratrol by ethanol extraction (V-103, **Figure 1. SM**). After separation of the remaining components in the product stream (S-134, **Figure 1. SM**), the resveratrol stream is sent to a spray drying stage (SDR-101, **Figure 1. SM**) to obtain resveratrol at 95% purity.

For the case of GM (**Figure 2. SM**) and WL (**Figure 3. SM**), an analogous process scheme is proposed, except for the pretreatment step, the composition of the fermentation medium and the yield in resveratrol production. For GM, its composition on glucose and fructose amounts to 111 g/L and 116 g/L, respectively, directly used in the fermenter, with the addition of 7.5 g/L of yeast extract, while in the case of WL, the C-source used to produce resveratrol is the ethanol, which amounts to 99.3 g/L. Resveratrol *de novo* biosynthesis from glucose/fructose it is accomplished through phenylalanine via the shikimate pathway. Ethanol can also serve as a (sole) carbon source by being converted into acetaldehyde. Resveratrol is formed by condensation of one molecule of *p*-coumaroyl-CoA (derived from phenylalanine) and three molecules of malonyl-CoA (derived from acetyl-CoA).

Table 1. Production capacity of the different scenarios assessed per 100 ton/batch of raw material.

Process	VR	GM	WL
Batch time (h)	327.5	245.3	254.3
Number of batches per year	24.00	31.00	31.00
Batch size (kg resveratrol)	284.4	242.3	71.09
Resveratrol production (kg/year)	6,826	7,494	2,198

2.2 Environmental assessment following the LCA methodology

The main objective is the study of the environmental profile of the valorization process of the waste streams from the wine-making activity. As for the system boundaries, a "cradle to gate" approach has been chosen, which takes into account all the stages between the extraction of the raw materials and the production of resveratrol (Arias et al., 2020). The reason for choosing these study boundaries is that, in this case, the LCA approach focuses on the evaluation of the degree of adequacy of the biorefinery process, for the identification of the potential of the circular economy process model within the concept of environmental sustainability. In addition, opening the system boundaries towards consumer consumption of the product would imply a more extensive level of process data, which, at this stage of development, are not available and do not imply a better understanding of the process.

For gathering the data for constructing the life cycle inventories (LCI) of the process, the modeling stage, based on the development of mass and energy balances, is used as source of data. On the other hand, the functional unit selected is the production of 1 kg of resveratrol, so the focus is on the product, in line with the objective of the environmental assessment.

Regarding the selection of the calculation methodology, two methodologies have been considered: ReCiPe MidPoint (Huijbregts et al., 2017), consisting of 18 impact categories of which 13 have been selected, as they are the most relevant for the environmental analysis of the valorization of wine side-streams, and ReCiPe EndPoint, through which 3 damage categories are integrated in a single score (**Table 2**). This last calculation methodology provides an overall impact value, which is considered a suitable model for comparison between the three production schemes, as it simplifies the analysis of the environmental impact associated with the alternative valorization process. Lastly, with respect to the *software* used to achieve the environmental scores, SimaPro® has been selected.

Table 2. ReCiPe MidPoint and EndPoint categories used for the characterization of the environmental profiles of the wine side-streams valorization scenarios.

Impact category	Acronym	Unit	Impact category	Acronym	Unit
ReCiPe MidPoint methodology					
Global Warming	GW	kg CO_2 eq	Marine Ecotoxicity	MET	kg 1.4-DCB
Stratospheric Ozone Depletion	SOD	kg CFC_{11} eq	Human Carcinogenic Toxicity	HCT	kg 1.4-DCB
Terrestrial Acidification	TA	kg SO_2 eq	Human Non-Carcinogenic Toxicity	HNCT	kg 1.4-DCB
Freshwater Eutrophication	FE	kg P eq	Land Use	LU	m^2a crop eq
Marine Eutrophication	ME	kg N eq	Mineral Resource Scarcity	MRS	kg Cu eq
Terrestrial Ecotoxicity	TET	kg 1.4-DCB	Fossil Resource Scarcity	FRS	kg oil eq
Freshwater Ecotoxicity	FET	kg 1.4-DCB			
ReCiPe EndPoint methodology					
Human Health	HH	Pt	Ecosystems	ES	Pt
Resources	RS	Pt			

The last stage is the interpretation of the results obtained on the environmental profile, as well as the identification of the main critical points, which are defined as the major contributors to the environmental loads of the evaluated impact categories. In addition, it is at this stage where sensitivity evaluations are also developed, with the objective of providing an improvement scenario with reduced environmental loads.

2.3 Techno-economic analysis

Seeking to identify the economic feasibility of the proposed scenarios, an economic evaluation has been developed considering the costs of purchasing equipment, labor, utilities and materials, in order to obtain the values of the total direct and indirect costs of the plan, the annual operating expenses and the expected income obtained from the sale of resveratrol, the target product. In addition, in order to evaluate and compare the three scenarios evaluated, the minimum selling price of resveratrol was calculated, since the yields of the scenarios are different, leading to a significant variation in the minimum selling price of resveratrol that guarantees the economic viability of the process scheme.

Regarding the calculation of the equipment purchase costs, the equations and tables available in Smith and Towler & Sinnot books have been considered (Smith, 2005; Towler and Sinnott, 2021) in order to provide the most accurate empirical estimation possible for the purchase costs, since these equations take into account the actual volume and characteristics of the equipment, data obtained from the modeling of the process using the SuperPro Designer tool. In this regard, **Equation 1** is used to calculate the purchase cost of the equipment according to the Smith methodology, while **Equation 2** is the one related with the Towler and Sinnot recommendations:

$$C_E = C_B \cdot (\frac{Q}{Q_B})^M \qquad \textbf{Equation 1}$$

Where CE is the cost of an equipment with a known Q-capacity ($), CB is the base cost of an equipment with a known QB capacity ($), M is a constant that depends on the type of equipment.

$$C_E = (a + b \cdot S^M) \qquad \textbf{Equation 2}$$

Where CE is the cost of an equipment with capacity S ($), a and b are two constants that vary depending on the type of equipment, n is an exponent that depends on the type of equipment.

Once these equations have been applied, it must be considered that the purchase cost obtained must be updated to 2023. For this purpose, the CEPCI (Chemical Engineering Plant Cost Index) indexes have been taken into account and **Equation 3** has been applied:

$$\frac{C_{E1}}{C_{E2}} = \frac{Index\ 1}{Index\ 2} \qquad \textbf{Equation 3}$$

Where CE_1 refers to the equipment purchase cost in year 1 ($), CE_2 to the equipment cost in year 2 ($), Index 1 to the CEPCI index in year 1 and Index 2 to the CEPCI index in year 2 (2023). To this end, when the Smith equation is used, the values of the parameters are based on the year 2000 estimates, whose CEPCI index is 394.1, and when the Sinnot equation is required, then the parameters are estimated for the year 2006, whose CEPCI index is 478.6.

Some other assumptions and considerations have been made for the development of the techno-economic assessment: a construction period of 30 months and 4 months for the start-up, a project lifetime of 15 years, inflation of 4% and an income tax of 25%. Regarding the plan operation capacity, it operates at 100% of its production capacity for 11 months per year, using 1 month for periodic maintenance of the facilities.

3. Results

3.1 Process modeling

The development of the process modeling using SuperPro Designer allows obtaining the equipment characteristics (i.e., equipment volume, drying capacity, filtration speed, etc.), as well as the number of equipment required to treat a total of 1000 kg/batch of feedstock, either VP, GM or WL wine side-streams. In this regard, the main equipment, its required number and characteristics are depicted in **Table 1 Supplementary Material (Table 1SM)**, for the case of using VP as substrate, **Table 2 Supplementary Material (Table 2SM)**, when using GP as feedstock and **Table 3 Supplementary Material (Table 3SM)** for WL as resource for resveratrol production. It is worth mentioning that the number of equipment required for the valorization of VP is higher than for GP and WL, given the lack of pre-treatment on the last two. However, as shown in **Table 1**, VP is the resource that leads to the highest resveratrol production per batch.

3.2 Environmental evaluation

The three scenarios have also been evaluated under an environmental perspective following the LCA methodology. Vine pruning residues scenario is denoted as S01, grape must valorization scenario as S02, and wine lees residues valorization is the S03.

3.2.1 S01-Vine pruning residues

The summary of the life cycle inventory data used for the development of the environmental assessment of S01- Vine Pruning is included in **Table 3**. All the inputs and outputs related with the process have been estimated from mass and energy balances.

Table 3. Main inventory data for the biotechnological valorization of VP residues.

INPUTS FROM TECHNOSPHERE			OUTPUTS TO TECHNOSPHERE		
Air	54.14	ton	Resveratrol	1	kg
Cellic CTec2	2.17	kg			
Dextrose	0.40	kg			
Ethanol	19.65	kg			
Peptone	0.41	kg	*Emissions to air*		
Sulfuric acid	0.48	kg	Carbon dioxide	1.32	kg
VP	318.98	kg	Acetic acid	3.40	kg
Water	1.41	m^3			
Yeast extract	1.11	kg	*Waste to treatment*		
Electricity/heat			Biomass	13.69	kg
Cooling water	146.7	m^3	Solid waste	187.9	kg
Steam	4.99	ton			
Electricity	478.1	kWh			

Once the LCI has been developed, the ReCiPe Midpoint methodology has been applied to obtain the environmental profile of the valorization process, depicted on **Figure 3.** As can be seen, three main contributors could be identified, being steam the one that entails the highest environmental load on the most impact categories under assessment. The rationale behind this huge impact is derived from the amount of steam required per FU (around 673 kg steam/kg of resveratrol produced), as fossil-based resources are used for its production. On the other hand, it should be mentioned that the steam requirements have been partially reduced by the development of an anaerobic digestion of the remanent lignin and biomass derived from the process, that allows to produce 83.42 kg steam/kg resveratrol produced, about a 12% of reduction on external steam. The electricity also contributes over the toxicity-related impact categories, namely FET, MET, HCT and HNCT, while the use of ethanol as solubilization agent for the purification of resveratrol has an important load over the ME, LU and MRS impact categories. Regarding electricity, its production from fossil-based resources is the main reason of its contribution, while for the case of ethanol, the related background activities required for its production (both materials consumption and energy requirements) are the reason behind its environmental loads over the aforementioned impact categories.

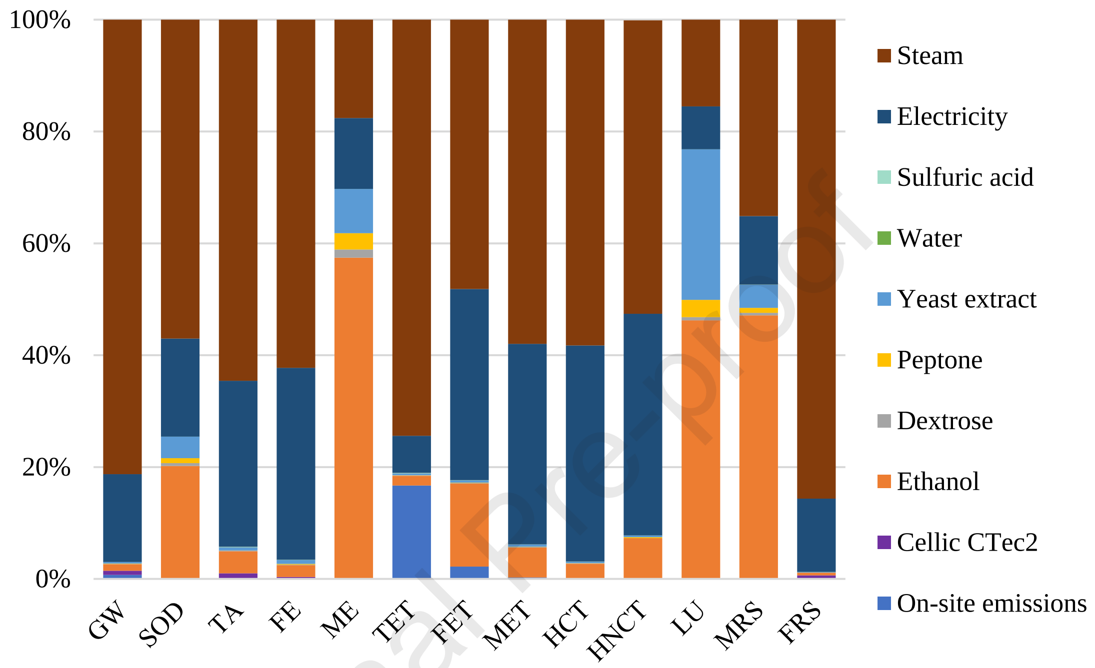


Figure 3. Environmental profile of resveratrol production using VP as substrate

3.2.2 S02- Grape must

The summary of the life cycle inventory data used of S02- Grape Must is included in **Table 4**, considering as functional unit the production of 1 kg of resveratrol.

Table 4. Main inventory data for the biotechnological valorization of GM residues.

INPUTS FROM TECHNOSPHERE			OUTPUTS TO TECHNOSPHERE		
Air	4664	kg	Resveratrol	1	kg
Dextrose	6.98	kg			
Ethanol	0.05	kg			
Peptone	7.14	kg	*Emissions to air*		
GM	374.36	kg	Carbon dioxide	1.66	kg
Water	166.68	kg	Acetic acid	4.03	kg
Yeast extract	4.01	kg	*Waste to treatment*		
Electricity/heat			Biomass	3.22	kg
Cooling water	46.32	m^3	Solid waste	90.27	kg
Steam	36.29	kg			
Energy	268.38	kWh			

The lack of a feedstock pretreatment stage implies a significant decrease in the heat energy requirements of the process, since the hydrothermal liquefaction, enzymatic hydrolysis and post-chemical hydrolysis, that are highly demanding of this utility, are avoided. Therefore, in this scenario, the environmental contribution of steam is irrelevant (**Figure 4**), being in this case electricity the one that carries a higher environmental load in most of the impact categories, with the exception of the SOD, ME, LU and MRS categories, where it is the use of peptone and yeast extract, used as nutritional supplementation in the fermentation processes, that have a higher environmental contribution, being that of peptone slightly higher.

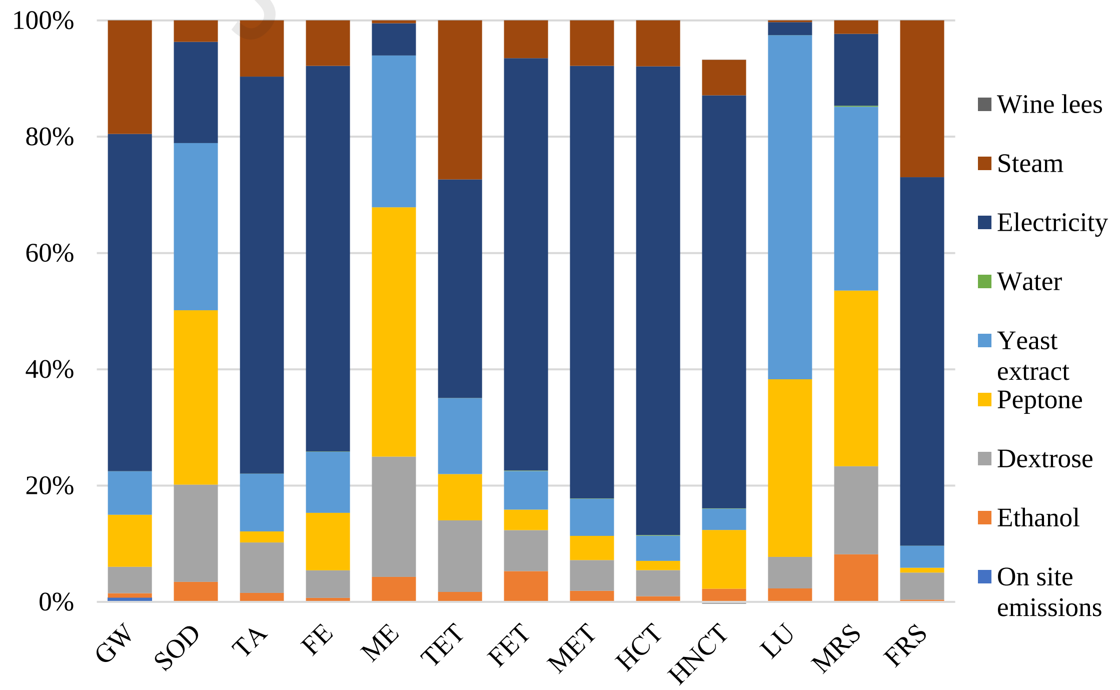


Figure 4. Environmental profile of resveratrol production using GM as substrate

3.2.3 S03- Wine lees

The gathered data of the life cycle inventory data used of S03- Wine lees is included in **Table 5**. All the inputs and outputs associated with the process scheme are included, obtained by the mass and energy balances, together with the related emissions and waste outputs flows to the technosphere.

Table 5. Main inventory data for the biotechnological valorization of WL residues.

INPUTS FROM TECHNOSPHERE			OUTPUTS TO TECHNOSPHERE		
Air	37.65	ton	Resveratrol	1	kg
Dextrose	31.49	kg			
Ethanol	7.74	kg			
Peptone	32.34	kg	*Emissions to air*		
WL	1276	kg	Carbon dioxide	5.71	kg
Water	724.92	kg	P-coumaric acid	0.61	kg
Yeast extract	20.51	kg	*Waste to treatment*		
Electricity/heat			Biomass	16.13	kg
Cooling water	201.8	m^3	Solid waste	76.29	kg
Steam	699.5	kg			
Energy	1172	kWh			

On **Figure 5** is depicted the environmental profile of the scenario S03-WL valorization in which it is observed that there is a greater distribution of impacts among the components that make up the life cycle inventory. However, it is still identified that the electrical requirements are the ones that make the greatest environmental contribution to the profile obtained in most of the impact categories, with the exception of 4, SOD, ME, LU and MRS, analogous to what was observed for the profile of scenario S02. Once again, the supplementation of the fermentative medium is the main cause of the environmental load of these four categories. On the other hand, it can be observed in this profile that the contribution of steam is slightly higher than in the previous section. The reason for this variation, given the analogy between the processes, is due to the reduction in the amount of resveratrol obtained, which implies a greater need for heat energy per unit kg of resveratrol produced.

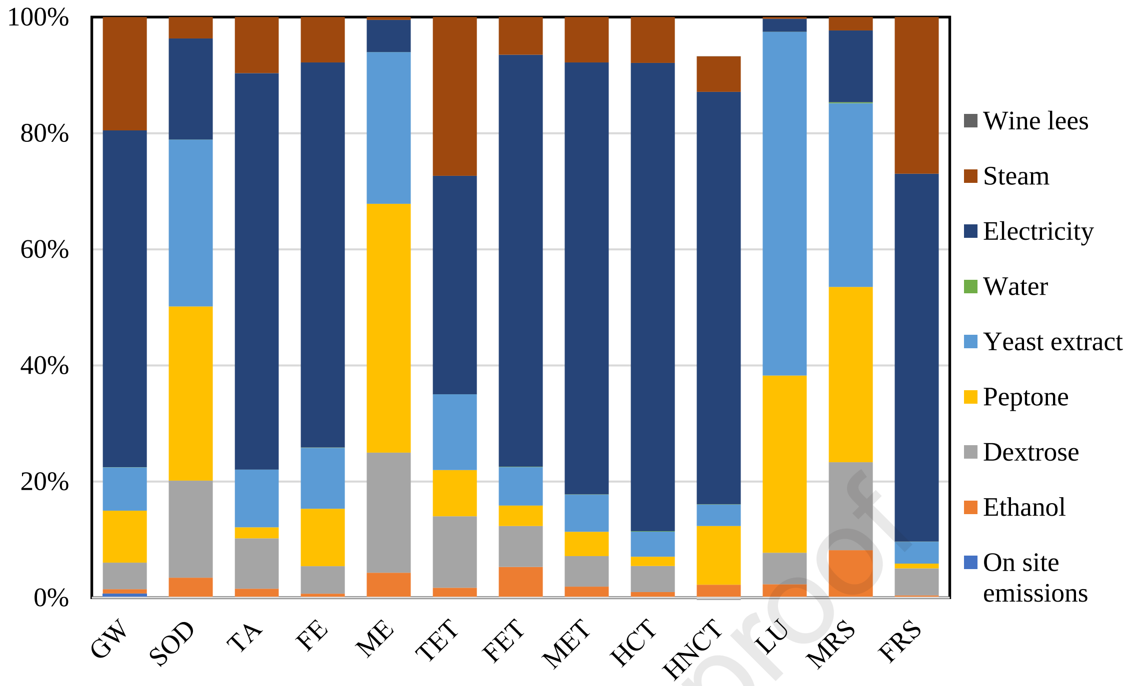


Figure 5. Environmental profile of resveratrol production using WL as substrate

3.3 Sensitivity assessments for reducing the environmental loads

In order to reduce the environmental impact associated with the evaluated scenarios, sensitivity analyses have been performed around the main hotspots identified in the previous section. They have been evaluated separately for each of the valorization schemes.

3.3.1 S01- Vine pruning residues

The main critical points identified for the S01-VP have been steam requirements, firstly, followed by electricity requirements and, in certain impact categories, by ethanol, used for the solubilization of resveratrol for its purification. For this, the sensitivity analysis has been based on the three components, and two approaches have been considered, on the one hand, the possibility of improving the productivity and efficiency of the process, which will lead to a reduction in the need for energy and material requirements, and on the other, by using renewable resources to obtain both steam and electricity, instead of using resources of fossil origin for their production.

In the case of steam and electricity, a 20% reduction in requirements has been estimated, which is expected to be reduced by the increase in production efficiency, which is considered feasible since the results of the laboratory scale approach have been scaled up and the degree of improvement is greater. On the other hand, as far as ethanol is concerned, only a 10% dose reduction has been evaluated, due to the fact that the solubilization of resveratrol in this compound is important to achieve its adequate purification, and it is possible that the reduction of the ethanol dose would change the final quality of the product. With this in mind, **Figure 6** shows that the reduction of steam requirements leads to a better improvement of environmental loads in most of the impact categories evaluated. The only exception is observed for ME and LU where it is the reduction of ethanol that provides the smallest impact. With respect to the reduction in electricity requirements, the reduction in impact is also notable, with the categories related to toxicity and eutrophication showing the greatest reduction.

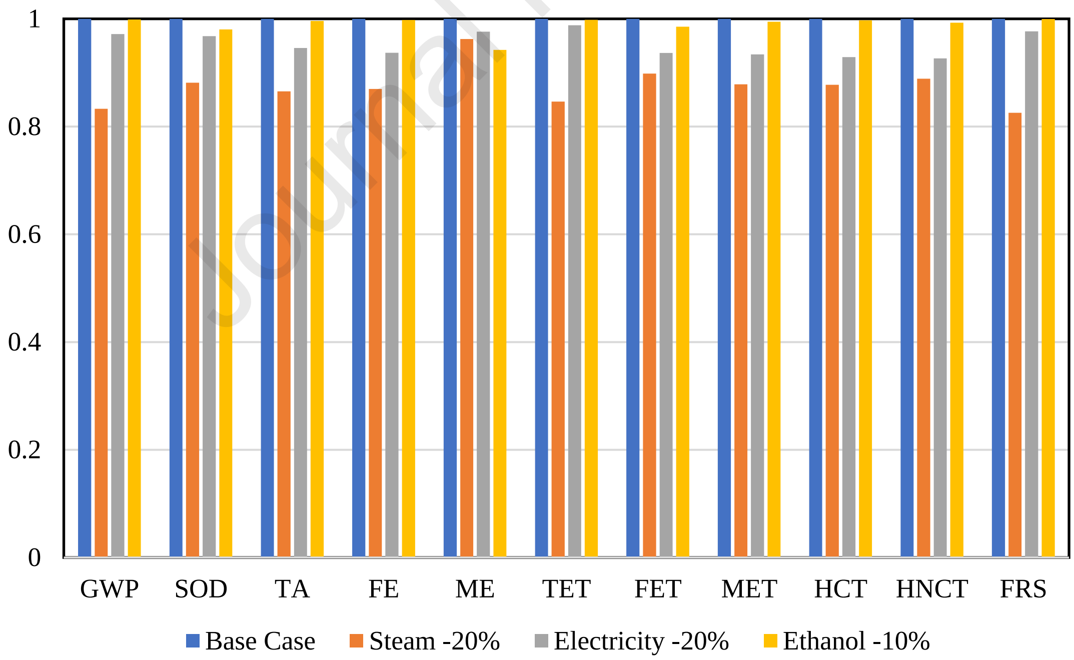


Figure 6. Sensitivity assessment of the environmental profile of VP valorization for resveratrol production considering a reduction on the use of steam and electricity by 20% and on ethanol dose of 10%.

As the contribution of steam and electricity is so high, it has been considered to develop an additional sensitivity assessment, but in this case, based on the use of renewable energy resources. In this sense, **Figure 7** shows that the environmental loads of the process are significantly reduced in most of the impact categories by more than 30% for the GW, TA, FE, HTC and FRS when the resource for steam production is the burning of wood waste in a furnace with a capacity of 5000 kW, while for the case of renewable electricity, reduction on the loads from 5% in the TET category to a maximum of 88% for the MRS, with respect to the base case scenario were estimated. In addition, another sensitivity analysis has been evaluated considering both steam and electricity from renewable resources, instead of considering each scenario separately, for this case, a range of 12% to 98% environmental load reduction is accomplished, which shows the significant improvement of the profile.

On the other hand, it is worth mentioning that in three impact categories, SOD, LU and HNCT, the impact was increased. The fact that renewable resources are used implies a greater impact on land use, given the need to use land to obtain this energy. For example, in the case of steam from hardwood, it implies the use of extensive areas for forest management. In the case of SOD, the production of the equipment necessary for the development of renewable energy production and the derived on-site emission are the ones that influence it most profoundly. Finally, in the case of HNCT, the reason for the impact is only slightly the same as for SOD.

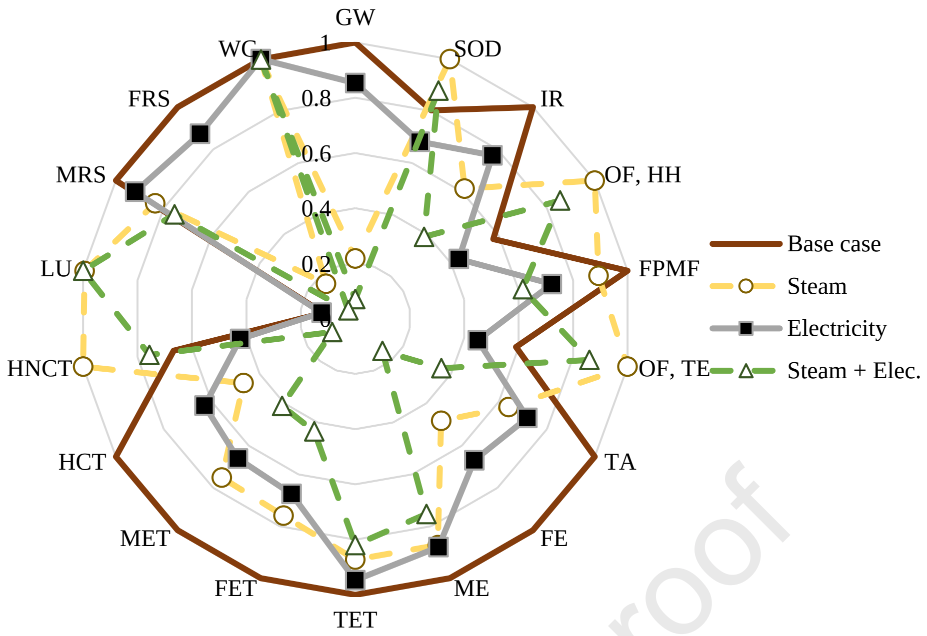


Figure 7. Sensitivity assessment of the environmental profile of VP valorisation for resveratrol production considering the use of renewable resources for electricity and steam production.

3.3.2 S02- Grape must

The hotspots identified for the S02-GM have been the electricity requirements and also some significant contribution of the peptone used as nutritional supplement for the fermentation process. For this, the sensitivity analysis has been based on (1) reduction in the dose of the supplementation, which is expected to be achieved by improving the yield and productivity of the process (the fact that is based on a laboratory scale process really implies a significant improvement when scaled up to an industrial process capacity), (2) the decrease of electricity requirements, by increasing productivity or by improving the equipment used for the process by increasing its efficiency and (3), as in the previous scenario it has been seen that the use of renewable resources for energy production really implies a significant reduction of the environmental load, the use of renewable resources for its production has also been evaluated.

The sensitivity results obtained are depicted in **Figure 8**. As expected, the use of electricity from renewable resources implies the greatest reduction in environmental loads in all impact categories, with the exception of the TET and LU impact categories, where the scores obtained are not as low compared to those of the other categories. The range of reduction on environmental impact goes from 6% for the ME category to 84% for HCT, with respect to the baseline scenario, when renewable resources are used. The percentage decrease is significantly reduced for the case of reducing the amount of electricity required by 20%, from 1% to 18% for the ME and HCT impact categories, respectively. Finally, for the scenario based on reducing the peptone dose, the reduced scores are the lowest, with the highest percentage reduction in the ME impact category, reaching 9%.

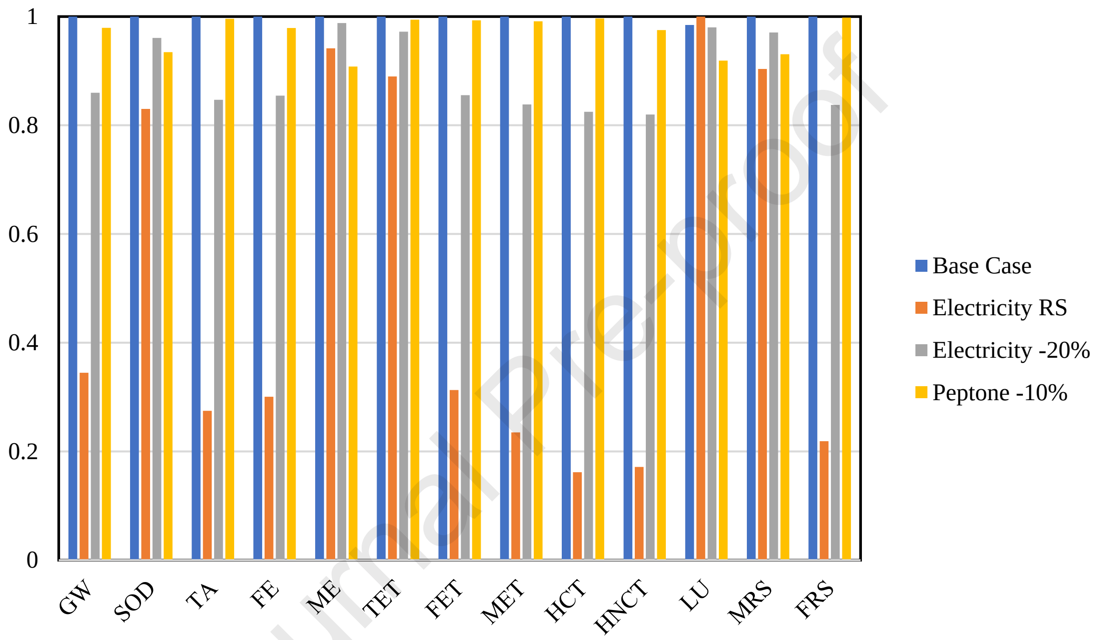


Figure 8. Sensitivity assessment of the environmental profile of GM valorization for resveratrol production considering a reduction on the use of electricity by 20%, the use of renewable resources for electricity production and on peptone dose of 10%.

3.3.3 S03- Wine lees

An analogous trend on the reduction of impact contribution loads is obtained when evaluating the sensitivity analysis of the scenario of wine lees valorization (**Figure 9**), given the similarity between the process scheme of S02-Grape must scenario. The scenarios under evaluation are the same as the previous one, considering renewable resources for electricity production, a reduction on its requirements by 20% and a decrease on the use of peptone supplementation by 10%.

As expected, the renewable nature of the electricity leads to a reduction of environmental loads in the order of 5% to 77%. In this case, the reduction is slightly lower than in the previous case, since a lower amount of resveratrol is obtained. In the case of the 20% reduction in electricity requirements, the load reduction goes from 1% in the ME category to 16% in the impact categories related to human toxicity. As in the previous case, the one with the lowest improvement profile is the reduction of the amount of peptone needed as a supplement, reaching the highest reduction value of 9% in the case of the ME impact category. Therefore, it could be concluded that process improvement actions should focus on opting for the use of renewable energies and improving process efficiency, although a more sustainable production model would be promoted when it is decided to use alternative energy sources.

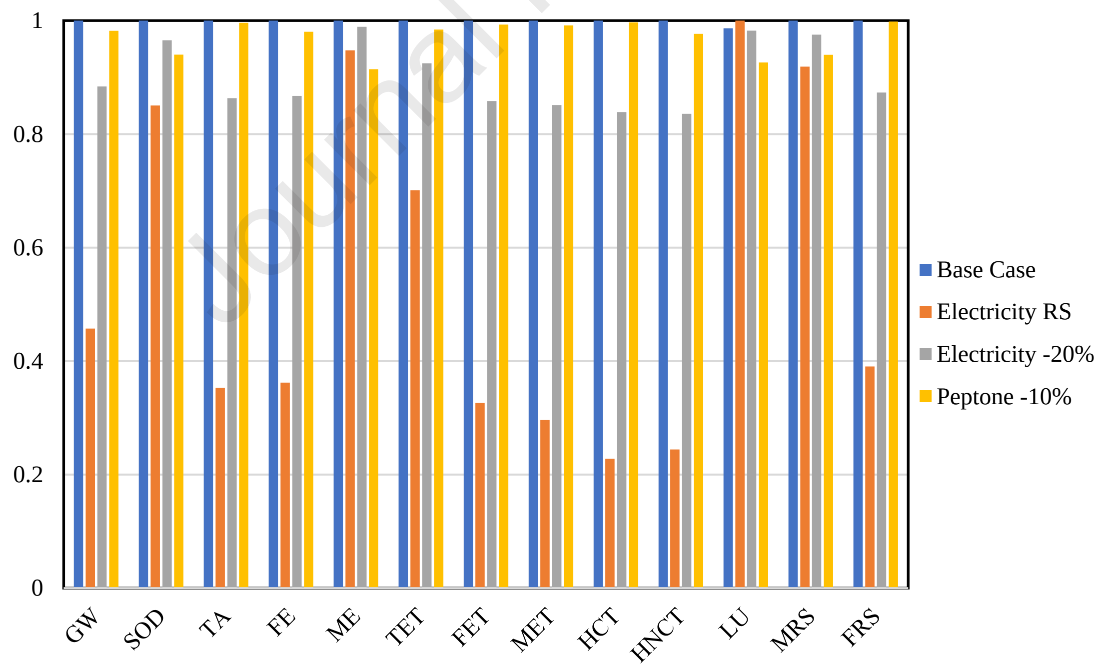


Figure 9. Sensitivity assessment of the environmental profile of GM valorization for resveratrol production considering a reduction on the use of electricity by 20%, the use of renewable resources for electricity production and on peptone dose of 10%.

3.4 Techno-economic analysis

The scores obtained for the economic evaluation of the scenarios evaluated are shown in **Table 6**. As can be seen, vine pruning valorization scenario S01 is the one with the highest total investment, given the high equipment acquisition cost, which is more than double the value obtained in the other two scenarios. This value was to be expected, given the need for pretreatment of pruning residues for their valorization in the fermentation scheme. Although, in the case of the cost of materials and utilities, its value is somewhat lower, the reason for this is due, firstly, to the use of lignin and fermentation biomass for obtaining heat energy, which reduces the external need for steam, which is one of the utilities with the highest cost and, secondly, to the higher resveratrol extraction yield in comparison to the S03-WL scenario, which implies that the costs of materials are also somewhat lower for this first scenario. Therefore, through the economic evaluation, taking into account annual operating costs and expected revenues, the minimum resveratrol selling price for the S01-VP process to be economically viable amounts to 380.28 €/kg, being the intermediate value when comparing the three scenarios.

Table 6. Economic parameters obtained by performing the economic evaluation of the biotechnological production process of resveratrol using VP, GM and WL as substrates.

Economic Parameters	S01-VP	S02-GM	S03-WL
Total Investment [€]	9,537,000	3,131,000	3,380,000
Equipment cost [€]	42,239,000	19,203,000	20,750,000
Fixed Capital [€]	42,239,000	19,203,000	20,750,000
1. Total Plant Direct Cost [€]	23,696,000	10,436,000	11,277,000
2. Total Plant Indirect Cost [€]	13,033,000	6,262,000	6,766,000
Labor Cost [€/year]	1,172,884	1,580,048	2,190,753
Material Cost [€/year]	80,854	1,673,071	2,395,819
Utilities Cost [€/year]	2,055,000	5,837,650	7,665,123
Annual Operation Cost [€/year]	8,328,000	12,740,047	16,190,753
Revenues [€/year]	19,709,313	37,924,464	11,125,097
MSP [€/kg Resveratrol]	380.28	193.82	882.36

In the case of S02-Grape must valorization, it is the best case when comparing between S01 and S03, both in terms of the total investment costs, as these are the lowest ones, and the expected revenues that could be obtained from the sale of the resveratrol obtained, which are the highest. The reason for this fact is based on the fact that this scenario provides an increased resveratrol yield, with the S03 scenario being the one that yields the least amount of resveratrol at the end of the process. Therefore, the minimum selling price for S02 amounts to 193.82 €/kg, while for the case of S03, this value is significantly higher (882.36 €/kg).

On the other hand, in order to evaluate whether these sale prices obtained are within the range of resveratrol sale prices, a bibliographic review of its sale prices for a purity of 95% has been carried out. According to BOCSCI Inc., a custom lab chemical supplier of bulk compounds for the pharmaceutical, agrochemical and biotechnology industries, the purchase price amounts to 9,353 €/kg. Another company, BULK, which is based on the selling of nutritional and supplementation related foods, considers that the selling price of resveratrol should be around 1,500 €/kg, while for Sigma Aldrich is 1,784 €/kg. As these companies provide resveratrol for a laboratory level production for an individual supplementation intake, it has been assumed that the selling price of this compound could be reduced to a half value, thus obtaining a range of selling price between 750-4,677 €/kg, thinking about 750 €/kg the most realistic one to be applied for these case scenarios under development. Bearing this into account, only the scenario S03-Wine lees has a minimum selling price a little bit higher, 18% than the selected one. However, it could be considered that also this price is feasible given the range of selling price values that could be found on the literature.

3.5 Comparison between scenarios

As done for the case of the techno-economic analysis, also a comparison between the three process scenarios has been developed within an environmental perspective, considering the scores obtained by the application of the MidPoint and EndPoint calculation methodologies. It should be mentioned that this comparison is developed for the base case scenarios and not for the improved ones by the sensitivity analysis.

In this regard, **Table 7** includes the environmental scores obtained for each impact category and process scenario, highlighting in bold the process alternative that entails the highest environmental load in each impact category. For all the impact categories scenario S02-GM is the one that entails the lowest environmental load, given its higher resveratrol productivity, entailing thus a reduced impact per kg of product obtained. The reason behind the highest impact of S01-

VP in some impact categories is the energy requirements associated with the pretreatment section, particularly the steam needs. Steam production entails a significant load to the global warming potential, given the release of emissions that affects this category, and because of the use of fossil resources for its production, which implies scarcity on its availability

Table 7. Environmental parameters obtained by performing the ReCiPe MidPoint methodology of the biotechnological production process of resveratrol using VP, GM and WL as substrates.

Impact category	Unit	S01-VP	S02-GM	S03-WL
GW	kg CO_2 eq	**1303**	148.08	781.11
SOD	kg CFC_{11} eq	$4.48\cdot10^{-4}$	$2.04\cdot10^{-4}$	**$1.01\cdot10^{-3}$**
TA	kg SO_2 eq	**3.59**	0.72	3.51
FE	kg P eq	0.21	0.05	**0.24**
ME	kg N eq	0.04	0.04	**0.19**
TET	kg 1.4-DCB	**1978**	483.62	778.22
FET	kg 1.4-DCB	6.22	1.54	**6.84**
MET	kg 1.4-DCB	8.48	1.94	**9.23**
HCT	kg 1.4-DCB	11.82	2.70	**12.80**
HNCT	kg 1.4-DCB	302.14	69.24	**331.69**
LU	m^2a crop eq	48.08	76.84	**383.07**
MRS	kg Cu eq	0.10	$4.46\cdot10^{-2}$	**0.23**
FRS	kg oil eq	**435.99**	35.20	197.11

Given the low productivity of the process in the production of resveratrol, as well as the consumption of energy resources and supplementation for the fermentation and purification phase of the final product, the S03-Wine lees valorization scenario is the one with the highest environmental contribution in mostly all the impact categories studied compared to the other two valorization scenarios evaluate, with the exception of GW, TA, TET and FRS, for which the S01-VP is the most detrimental scenario. In fact, to show the degree of significant increase in impact, the values obtained have been normalized between 0 and 1, shown in **Figure 10**, where it can be seen that the impact caused by the S03 and S01 scenarios are significantly higher in comparison to that of S02-GM, as in all the impact categories its environmental contribution is 60% higher. Therefore, it can be stated that the S03 scenario is the least favorable and that, perhaps, for its better use and lower environmental impact, another valorization strategy should be chosen, such as the production of biofuels or its direct energetic valorization.

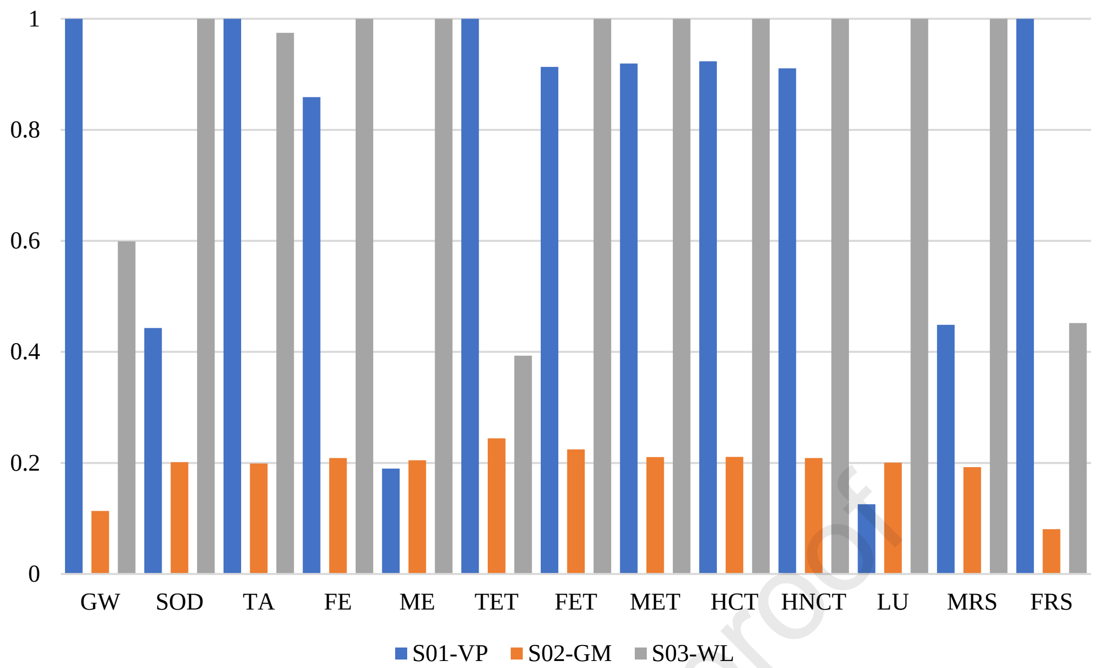


Figure 10. Comparison of the environmental loads of the assessed scenarios using the ReCiPe MidPoint methodology.

Figure 11 represents the sensitivity scenarios for vine pruning using renewable electricity (VP-BC-ER), the renewable electricity scenario for the case of grape must valorization (GM-BC-ER), and the same for the case of wine lees (WL-BC-ER). As can be seen, there is an analogy between the results obtained for the base case with respect to the study under the ReCiPe MidPoint methodology, where the use of GM as raw material is again the one with the lowest environmental contribution and the use of WL the one with the highest impact on the environment. However, it is important to note that the option for renewable energy sources significantly reduces the environmental burdens of the scenarios, with the most significant reduction being observed in the case of the valorization of VP and GM.

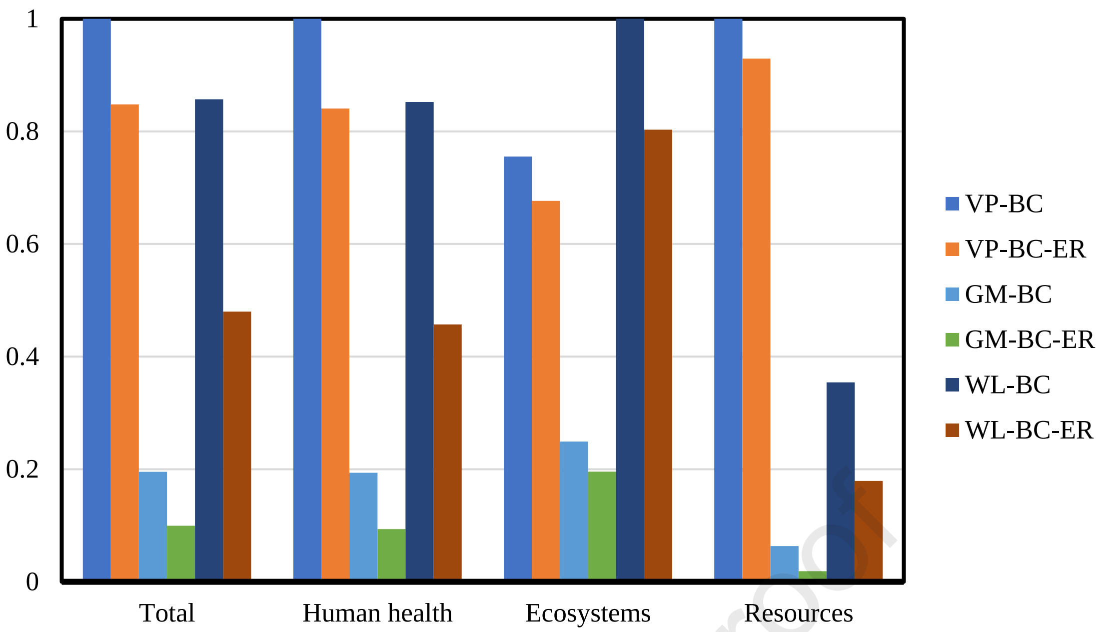


Figure 11. Comparison of the environmental loads of the assessed scenarios using the ReCiPe EndPoint methodology.

4. Discussion and Conclusion

This research article has been based on the development of three scenarios of the biotechnological production of resveratrol by a novel engineered yeast industrial strain using three renewable feedstocks related to the wine production sector. The modeling of the valorization of wine waste streams by means of a modeling software has provided the first data on its potential to be effectively applied in the wine value chain. The development of mass and energy balances, as well as the selection of the most suitable equipment to carry out all the stages of the biotechnological process, have allowed its analysis under an environmental and techno-economic perspective, with the aim of identifying bottlenecks and critical points. However, although this preliminary evaluation could provide an overall view on the effectiveness and adequacy of this valorization strategy, further steps are required before its actual application in real production. Pilot-scale experimentation will be a more representative environment to effectively evaluate whether the obtained modeling results match the robustness of the biotechnological process under conditions closer to the factory model. Once pilot-scale production has demonstrated its feasibility, process optimization should be the next step, also in order to verify once again the cost-effectiveness and environmental suitability of the biotechnological process. Finally, if all the above requirements are met, the technology could be transferred and commercialized, which should be the ultimate goal.

At this stage of development, with the scale-up of the results obtained from laboratory scale experiments, it could be concluded that, both grape must and vine pruning residues are feasible substrates for resveratrol bioproduction. However, for the case of the valorization of wine lees, significant improvements are needed to make this process more profitable and less harmful to the environment, and thus an alternative valorization process should be considered to promote a more beneficial and sustainable process scheme.

CRediT authorship contribution statement

A.A.: Methodology, Formal analysis, Investigation, Writing-original draft, Writing-review & editing. CE.C: Formal analysis, Investigation, Supervision, Writing-review & editing. MT.M: Supervision, Writing-review & edition. G.F.: Supervision, Writing-review & edition. L.D.: Conceptualization, Validation, Supervision, Writing-review & editing.

Declaration of Competing Interest

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

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Highlights

Vine residues has demonstrated that could be used as sources for resveratrol bioproduction.

Grape must and vine pruning residues are the most environmentally friendly and economic feasible.

Wine lees requires optimization to be economically viable and less harmful to the environment.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: