

Environmental Evaluation of Antioxidant Extraction from Hops Using Innovative Extraction Techniques and Natural Eutectic Solvents

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Abstract

The transition to a sustainable bioeconomy requires the development of innovative production strategies aligned with the Safe and Sustainable by Design framework. Utilizing waste as a resource for producing high-value-added products is critical to achieving the Sustainable Development Goals (SDGs) and fostering a circular bioeconomy. In this context, it is vital to assess the potential of emerging processes at an early design stage—ranging from laboratory scale to full-scale implementation—to ensure their feasibility for integration into market value chains. This research study is framed within such an approach, focusing on the valorization of garlic waste to extract organosulfur bioactive compounds. The study also explores more efficient and sustainable extraction methods, including pressurized liquid extraction (PLE) technologies and natural eutectic solvents (NADES). The experimental setup enabled the evaluation of bioactive compounds through various assays to determine their functionality, including analyses of antioxidant activity, anti-inflammatory potential, and neuroprotective properties. Subsequently, the extraction process was modeled at full scale using the SuperPro Designer tool, followed by a Life Cycle Assessment (LCA) to determine the environmental profile and potential damage associated with the proposed extraction model.

Keywords: emerging extraction techniques; NADES; bioactive compounds; sustainability; life cycle assessment.

1. Introduction and process description

This research aims to explore the potential of novel extraction technologies with low environmental impact, leveraging advanced methodologies and alternative bio-based solvents to obtain high-value compounds from garlic residues. To achieve this, pressurized liquid extraction (PLE) technologies are proposed, including subcritical water extraction (SWE) and natural eutectic solvents (NADES) under compressed fluid conditions- a combination with great potential but less explored (Grisales-Mejía et al., 2024).

PLE is regarded as an advanced extraction technique due to its advantages over traditional processes. It operates under high temperature and pressure (≤ 100 bar), always below the critical points of the solvent, ensuring that the solvent remains in the liquid state throughout the extraction process (Álvarez-Rivera et al., 2020). Under these specific pressure and temperature conditions, the physicochemical properties of the solvent are altered. For instance, mass transfer rates are enhanced, solvent surface tension and viscosity are reduced, and analyte solubility increases. These changes enable the solvent to penetrate more easily and deeply into the solid matrix being extracted, resulting in significantly higher extraction yields compared to conventional methods. Moreover, due to its operation modes, this extraction process demonstrates substantial potential for industrial-scale applications.

Moreover, to evaluate the efficacy of the process, the phytochemical profile (HPLC-DAD-QTOF-MS/MS analysis), antioxidant activity (ABTS and ORAC assays), anti-inflammatory potential (inhibition of lipoxygenase enzymes), and neuroprotective potential (inhibition of cholinesterase enzymes) have been applied to determine the bioactive properties of the compounds extracted from the garlic waste.

With respect to the operation conditions, those are depicted on **Table 1**. It should be mentioned that the NADES solvent is composed by a mixture of choline chloride and glucose, in a molar ratio of 2:1 respectively. Besides, in principle, it should be mentioned that any downstream or purification process stage is required, the

extracts could be used directly, only a drying step could be needed for the hydroalcoholic extract, depending on the application. As for the raw material for extraction, hops are used.

Table 1. Operation conditions for UAE and PLE with conventional and NADES solvents

Operational value	UAE	PLE
Process time	30 min	20 min
Pressure	1 atm	100 bar
Solvent used	10 mL/g hop	15 mL/g hop
Solvent 1	<i>H₂O</i>	
Solvent 2	<i>EtOH:H₂O</i> , 70:30 v/v	
Solvent 3	<i>NADES:H₂O</i> , 70:30 w/v	
Agitation	Not required	Not required
Temperature	25 °C	40, 100, 160 °C

2. Methodology. Environmental assessment.

To evaluate the sustainable potential of the new extraction processes proposed, the Life Cycle Analysis (LCA) methodology, standardized in ISO 14040 (2006), have been applied. LCA involves four consecutive stages: identification of objective and scope, gathering process data for the development of the life cycle inventory (LCI), environmental impact assessment and interpretation of the results, and performing an environmental sensitivity analysis. The data included in the LCI is derived from large-scale modeling of the process, using laboratory-scale operational conditions and process productivity while considering a production capacity comparable to the market average. For background processes, the EcoInvent database has been utilized. Regarding system boundaries, a cradle-to-gate approach has been adopted, excluding impacts associated with infrastructure activities and transportation. Three functional units have been considered, based on the functionality of the extracted product: antioxidant activity, anti-inflammatory potential, and neuroprotective potential. In terms of the calculation methodology, both ReCiPe 2016 Midpoint (H) V1.07/World (2010) H and ReCiPe 2016 Endpoint (H) V1.07/World (2010) H/H were considered, allowing for the determination of both midpoint impact values and a single score based on damage categories. The midpoint impact categories and endpoint damage categories are detailed in **Table 2**.

Table 2. ReCiPe Midpoint and EndPoint categories under analysis

Impact category	Acronym	Unit	Impact category	Acronym	Unit
Global warming	GW	kg CO ₂ eq	Terrestrial ecotoxicity	TET	kg 1.4-DCB
Stratospheric ozone depletion	SOD	kg CFC11 eq	Freshwater ecotoxicity	FET	kg 1.4-DCB
Ionizing radiation	IR	kBq Co-60 eq	Marine ecotoxicity	MET	kg 1.4-DCB
Ozone formation, Human health	OF,HH	kg NO _x eq	Human carcinogenic toxicity	HCT	kg 1.4-DCB
Fine particulate matter formation	FPMF	kg PM _{2.5} eq	Human non-carcinogenic toxicity	HNCT	kg 1.4-DCB
Ozone formation, Terrestrial ecosystems	OF,TE	kg NO _x eq	Land use	LU	m ² a crop eq
Terrestrial acidification	TA	kg SO ₂ eq	Mineral resource scarcity	MRS	kg Cu eq
Freshwater eutrophication	FE	kg P eq	Fossil resource scarcity	FRS	kg oil eq
Marine eutrophication	ME	kg N eq	Water consumption	WC	m ³
Ecosystems	Ec	Pt	Resources	Re	Pt
Health	He	Pt			

3. Results

The extraction yields of the 9 scenarios associated with the PLE extraction technology and the 3 ones for the UAE are presented in **Table 3**. In case of the PLE, it could be observed that, increasing temperature implies

an increase on the extraction yield for all the solvents under analysis. On the other hand, when comparing between solvents and same temperature it could be seen that, for 160 °C, using NADES or ethanol implies the same level of extraction, higher than with only water solvent, while for 100 °C the use of ethanol-based solvent significantly increased the yield value and antioxidant potential, from 30.68 mg GA/g hop for NADES: H₂O to 35.38 mg GA/g hop for EtOH: H₂O.

In the case of UAE, all scenarios take place at 25 °C, which is a real advantage in terms of energy requirements and, as a consequence, also in terms of environmental profile and impact loads. When comparing solvents, the use of NADES: H₂O is the one that gives a higher level of antioxidant potential, which is 18.05 mg GA/g hop, while the use of H₂O as a solvent implies a lower potential.

Table 3. Extraction yield per type of technology (PLE or UAE), solvent used and temperature in terms of antioxidant activity (mg GA/g hop)

Scenario	Solvent	Temp	mg GA/g hop	Scenario	Solvent	Temp	mg GA/g hop
PLE-1	H ₂ O	100 °C	22.52	PLE-7	NADES: H ₂ O	40 °C	22.16
PLE-2	H ₂ O	40 °C	13.64	PLE-8	NADES: H ₂ O	100 °C	30.68
PLE-3	EtOH: H ₂ O	160 °C	36.04	PLE-9	H ₂ O	160 °C	27.91
PLE-4	EtOH: H ₂ O	100 °C	35.38	UAE-1	NADES: H ₂ O	25 °C	18.05
PLE-5	NADES: H ₂ O	160 °C	36.06	UAE-2	H ₂ O	25 °C	9.38
PLE-6	EtOH: H ₂ O	40 °C	26.96	UAE-3	EtOH: H ₂ O	25 °C	17.98

On the other hand, **Figure 1** depicts the comparison of environmental loads across all PLE and UAE scenarios, measured per ton of input material of hops and per kilogram of antioxidant obtained (kg GA equivalents). It should be noted that: (1) higher temperatures in PLE result in increased environmental loads due to the higher energy requirements for the process, (2) the need for higher pressure in PLE (10 bar) compared to UAE (1 bar) leads to a greater environmental impact, as pressurizing the reactor requires a compressor unit, (3) the best alternative depends on the functional unit selected, with 1 kg of antioxidant in GA equivalents being the preferred unit. This functional unit allows for a clearer observation of the extraction yield and efficiency of each procedure, (4) NADES involves a solvent mixture of glucose and choline chloride. For the latter, no specific EcoInvent database entry is available, and the proxy “Ammonium chloride {GLO}| market for | Cut-off, U” has been used instead.

For PLE, when considering the production of 1 kg of antioxidant compound, the use of NADES results in a higher environmental load due to the significant contribution of the solvent mixture. However, when comparing environmental loads per ton of input material, the use of ethanol-based solvents also has a high impact. For UAE, for both functional units under analysis, the use of NADES leads to the highest environmental impact, while the use of H₂O as solvent remains as the best alternative.

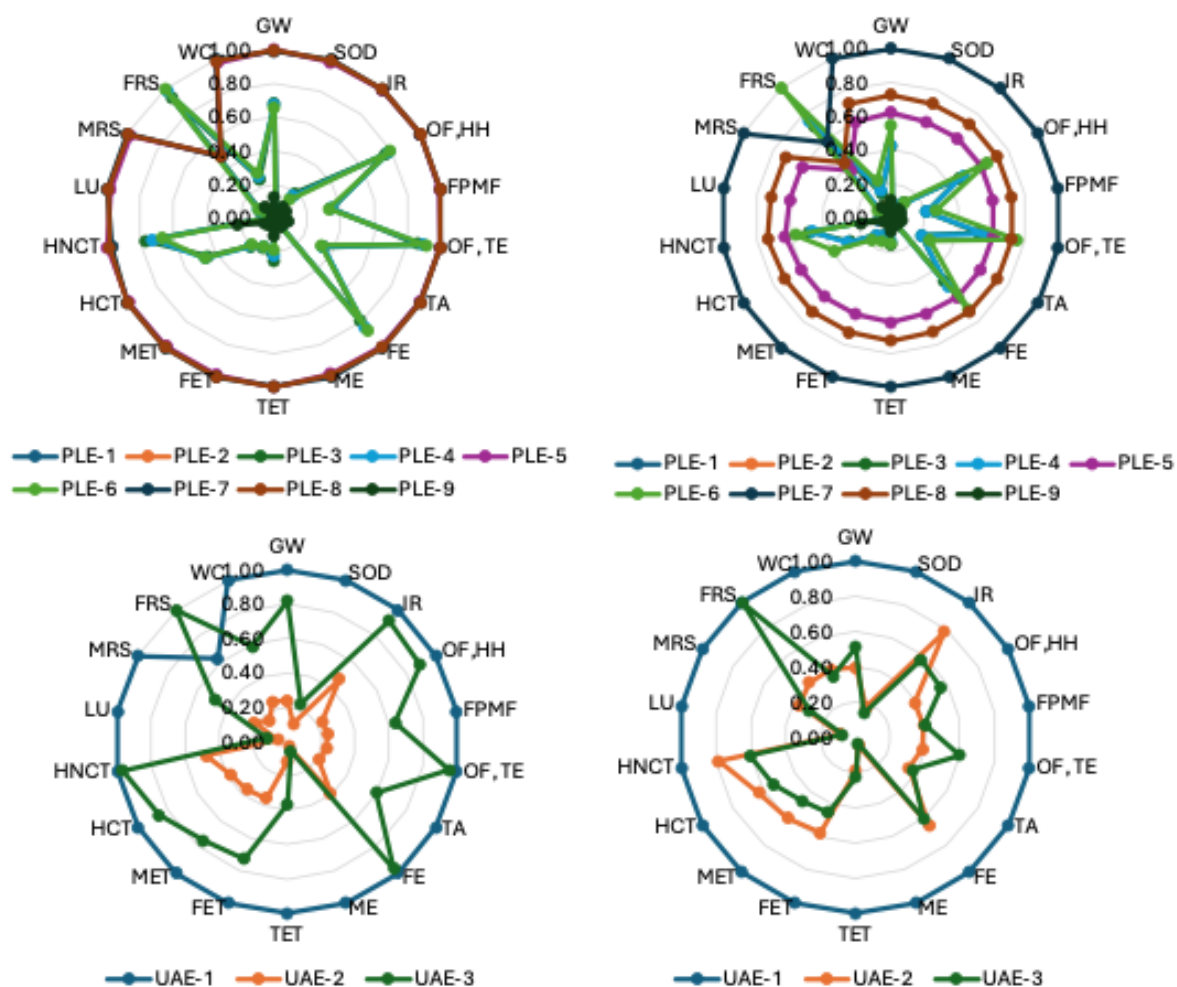


Figure 1. A. PLE - 1 ton of input hops and B. PLE - per kg of antioxidant obtained. C. UAE - 1 ton of input hops and D. UAE- per kg of antioxidant obtained.

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