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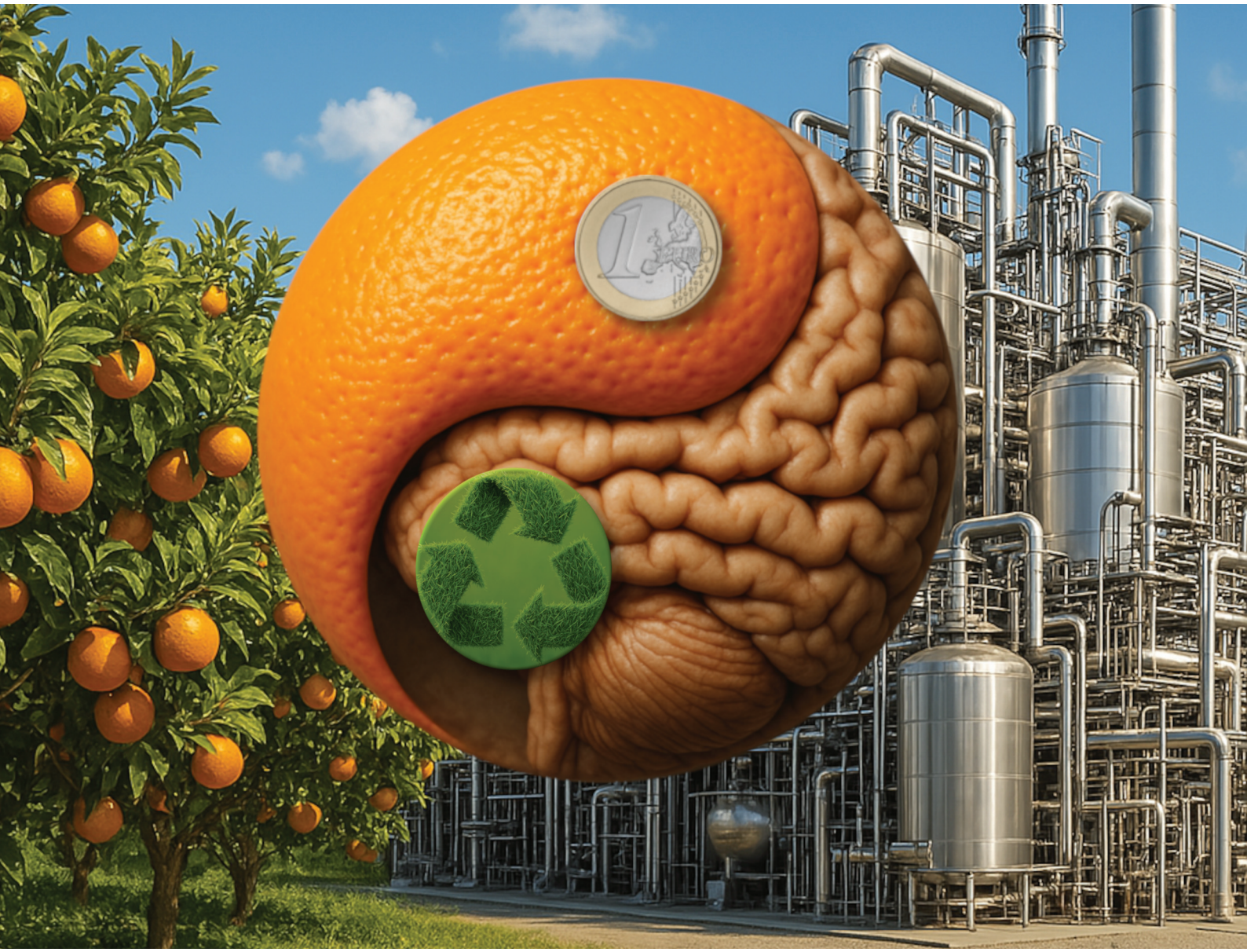

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Transforming orange by-products into high-value neuroprotective products: environmental and economic assessment of advanced green extraction methods

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This study explores the valorization of orange by-products for the production of neuroprotective fractions using three extraction methods: maceration, gas expanded liquid (GXL) extraction, and pressurized liquid extraction (PLE). The objective was to optimize solvent use while ensuring high bioactivity and minimal environmental impact. Initial tests with greener solvents like water and ethanol were unsuccessful in extracting neuroprotective fractions, leading to the implementation of GXL (CO_2 : ethyl acetate 1 : 1, 50 °C, 10 MPa), which effectively minimized ethyl acetate use while maintaining bioactivity. Life cycle assessment (LCA), greenness assessment (AGREEprep) and economic analysis were performed to evaluate each method. LCA and greenness assessment presented concordant results, revealing that GXL had the lowest environmental impact, while maceration had the highest environmental impact. Economic analysis showed that PLE had the best economic performance, with the lowest costs, highest ROI, and shortest payback time, making it the most cost-effective option. Despite GXL's slightly higher costs compared to PLE, it achieved substantial environmental benefits. These findings confirm that optimizing advanced extraction methods like PLE and GXL can transform citrus waste into profitable, high-value neuroprotective extracts while promoting sustainability in the food processing industry. This approach supports the development of a circular bioeconomy and eco-friendly extraction practices.

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Green foundation

1. This work advances green chemistry by optimizing environmentally friendly extraction methods for recovering neuroprotective compounds from orange by-products, integrating solvent minimization, energy reduction, and comprehensive environmental and economic assessments to promote sustainable biorefinery practices.
2. We reduced solvent usage by over 95% and energy consumption by up to 90% using gas expanded liquid extraction compared to maceration, while maintaining bioactivity, thus achieving a greener and more efficient process for high-value compound recovery.
3. Future work could incorporate renewable energy sources, biodegradable solvents, and real industrial-scale validation. Integrating real-time process monitoring and further refining LCA-AGREEprep synergies would also enhance sustainability and operational scalability in green extraction systems.

1. Introduction

The global orange juice industry produces millions of tons of juice annually, resulting in significant quantities of waste, primarily in the form of peels and pulp, which constitute about 50% of the fruit's weight. Improper disposal of these residues can lead to environmental challenges, including soil and water pollution, as well as greenhouse gas emissions from decomposition.[1] In fact, they represented 10 million tons only in China in 2016,[2] while in the European Union, they were around 5 million tons per

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year in the period 2008–2024.[3] However, these by-products present an opportunity for sustainable valorization, aligning with the food industry's shift towards circular economy models. Citrus peels and pulp are rich in valuable bioactive compounds such as flavonoids, terpenoids and essential oils, among others, which possess health-promoting properties.[1] For instance, they exhibit significant antioxidant activity, which can help combat oxidative stress—a contributor to various chronic diseases.[1] Additionally, some citrus-derived compounds inhibit acetylcholinesterase, potentially offering neuroprotective benefits relevant to conditions like Alzheimer's disease.[4]

On the other hand, the growing emphasis on sustainability has led to the development of environmentally friendly extraction methods for recovering bioactive compounds from citrus residues. Current extraction methods often face challenges, such as inefficiencies, high costs, and the environmental impact of toxic organic solvents. In contrast, green extraction methods such as pressurized liquid extraction (PLE) and gas expanded liquid (GXL) extraction can be seen as sustainable alternatives; in this sense, PLE uses very low amounts of subcritical organic solvents or water as an extraction solvent, minimizing hazardous emissions and reducing energy consumption.[5] Similarly, GXL employs environmentally benign gases like CO_2, enhancing the extraction efficiency while avoiding toxic solvents. These approaches align with green chemistry principles by reducing environmental impact and improving the overall sustainability of the extraction process.[6] Recent advances, such as the method developed by Sánchez-Martínez *et al.* (2022),[7] have shown promise in extracting terpenoid-rich extracts with neuroprotective potential using pressurized liquid extraction (PLE). However, there remains a need to further enhance the environmental performance of the process (by minimizing environmental impact and maximizing efficiency) while maintaining the bioactivity of the extracts.

Thus, the specific objectives of this research are to optimize the best extraction conditions from the work of Sánchez-Martínez *et al.* (2022)[7] using greener solvents (water and carbon dioxide) to enhance environmental performance while maintaining the bioactivity of the extracts. The present study also aims to evaluate the bioactivity of these optimized extracts, focusing specifically on antioxidant properties and acetylcholinesterase inhibition. Additionally, a life cycle assessment (LCA) will be conducted to compare the environmental impact of the improved extraction methods against traditional solvent-based approaches and the method proposed. Finally, an economic analysis was performed to assess the scalability and feasibility of these optimized green extraction processes.

By addressing these objectives, the study contributes to sustainable food processing and waste valorization. It aligns with circular economy principles, advancing the development of eco-friendly technologies for bioactive compound recovery, with potential applications in functional foods and nutraceuticals.

2. Materials and methods

2.1. Biomass and chemicals

Orange juice by-products (*Citrus sinensis*, *Navel Late* variety) were provided by J. García Carrión, S. L. (Huelva, Spain) and consisted of peels and pulp (leaves and seeds were discarded). The resulting biomass was freeze dried, ground, vacuum-sealed and stored at −18 °C.

Ethanol (EtOH) and ethyl acetate (ETAC), technical quality, were sourced from VWR Chemicals (Barcelona, Spain). Acetylcholinesterase (AChE) type VI-S from *Electrophorus electricus*, butyrylcholinesterase (BChE) from equine serum, acetylthiocholine iodide (ATCI), linoleic acid (LA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trizma hydrochloride (Tris-HCl), disodium phosphate (Na_2HPO_4), monopotassium phosphate (KH_2PO_4), gallic acid, ascorbic acid, quercetin, and lipoxidase from *Glycine max* (soybean) were obtained from Sigma-Aldrich (Madrid, Spain). 4-(Amino-sulfonyl)-7-fluoro-2,1,3-benzoxadiazole (ABD-F), galantamine hydrobromide, and 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) were procured from TCI Chemicals (Tokyo, Japan). Ultrapure water was produced using a Millipore system (Billerica, MA, USA). All 96-well microplate assays were conducted using a spectrophotometer and a fluorescence reader (Synergy HT, BioTek Instruments, Winooski, VT, USA).

2.2. Maceration extraction

The solid–liquid extraction experiments were done using the Sánchez-Martínez *et al.* (2021) protocol.[4] Briefly, an orange by-product (5 g) was extracted with ethyl acetate (45 mL) using an orbital shaker (Thermo Scientific) at 200 rpm for 24 hours at room temperature, shielding from light. The resulting extract was filtered (0.45 μm nylon filter, Agilent Technologies) and concentrated to dryness under a nitrogen flow (TurboVap® LV Biotage). Dried extracts were stored at −20 °C until analysis. All extractions were performed in triplicate.

2.3. Pressurized liquid extraction (PLE)

For the pressurized liquid extraction (PLE) experiments, two different setups were utilized. A lab-scale apparatus, as described in the work of Sánchez-Martínez *et al.* (2022),[7] was employed for initial extractions to replicate the previously optimized conditions and to optimize water extraction conditions. Additionally, a semi-pilot scale PLE system, Helix (Applied Separations, Allentown, Pennsylvania, USA), customized to use solvents, was used to scale up the process; scheme shown in Fig. 1. The Helix system was equipped with a 300 mL extraction cell, allowing for larger-scale extractions while maintaining controlled parameters. Extraction conditions in this scale were sample mass (50 g), sand mass (100 g), pressure (10 MPa), cell volume (300 ml), static extraction, temperature (100 °C), solvent (ethyl acetate) and extraction time (30 min). Experiments were performed as described in the work of Gilbert-López *et al.* (2015).[8] After extraction, solvents were evaporated using nitrogen stream to calculate the yield and perform further analysis. Dry extracts were stored at −20 °C protected from light. This dual approach enabled a com-

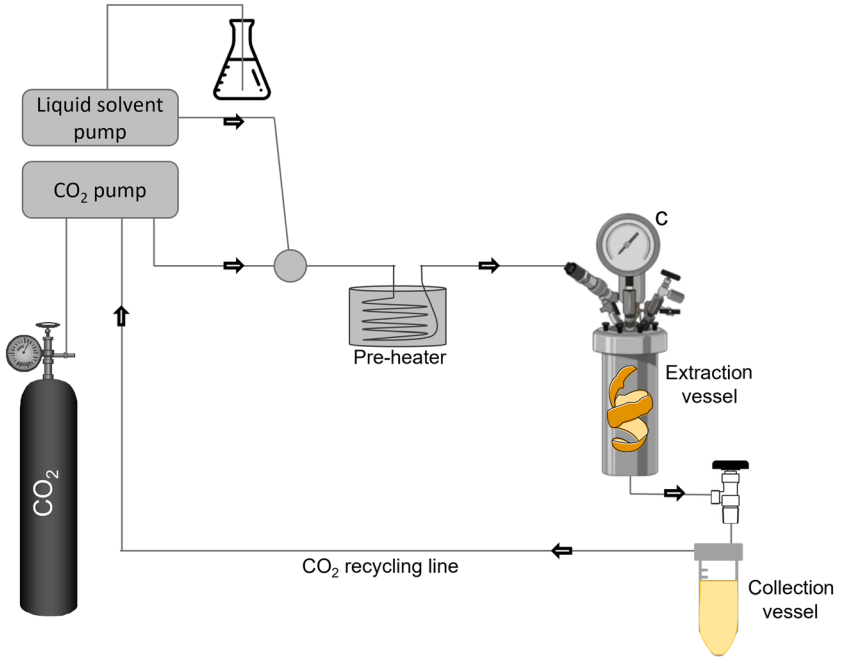

Fig. 1 Scheme of the instrumental setup used in pressurized liquid extraction (PLE) and gas expanded liquid (GXL) extraction.

parison between lab-scale and semi-pilot scale operations, providing insights into the scalability and practical application of the PLE method.

2.4. Gas expanded liquid (GXL) extraction

The GXL experiments were performed on the same Helix apparatus used in the semi-pilot scale PLE experiments; see Fig. 1. The extraction solvents used were carbon dioxide and ethyl acetate. A factorial experimental design was used to evaluate the suitability of changing from PLE to GXL. This design included two factors (extraction temperature and solvent flow rate), each with three levels (3^2) and three replicates at the center. The parameters evaluated are listed in Table 1. Other experimental conditions such as sample mass (50 g), sand mass (100 g), pressure (10 MPa), cell volume (300 ml), static extraction and extraction time (30 min) were kept constant.

Table 1 Inventory of the extraction process of orange by-product-based extracts by means of maceration, PLE, and GXL methods

Material	Maceration	PLE	GXL
INPUTS			
Orange by-product (g)	200	1.37	31.19
Ethyl acetate (g)	1623.60	47.50	2.35
N_2 (g)	96	0.12	0
Electricity (kWh)	47.28	10.70	4.73
CO_2 (g)	0	0	33.52
OUTPUTS			
Extract (g)	1.02	1	1.21
Solid residue (g)	198.80	1.64	17.42
Ethyl acetate (recuperated) (g)	1623.60	47.50	2.35
CO_2 (recuperated) (g)	0	0	33.52
N_2 (g)	96	0.12	0

2.5. Experimental designs used for extraction optimization

A multilevel factorial design using response surface methodology (RSM) was employed to optimize the PLE temperature (40–180 °C), ethanol percentage (0–100%), and formic acid (2.5–5%) percentage in the solvent mixture. Response variables selected were total phenolic content (mg GAE per g extract), antioxidant capacity measured using ABTS (mM TE per g extract), global yield (%) and neuroprotective potential measured using AChE (inhibition %).

On the other hand, the optimization of GXL extraction conditions was done using a full factorial 3^2 experimental design. The experimental factors of the design were temperature (50, 75 and 100 °C) and percentage of CO_2 (10, 30 and 50%) to be mixed with ETAC as the extraction solvent. In this case, the studied responses were global yield (%) and neuroprotective potential measured using AChE (inhibition %).

All the experiments were run in replicate and carried out randomly. Statgraphics Centurion XVIII software (Statgraphics Technologies, Inc., The Plains, VA, USA) was used to analyze data. The confidence level was considered 95% for all the variables.

2.6. Functional characterization of extracts

2.6.1. Total phenolic content (TPC). TPC was determined using the Folin–Ciocalteu colorimetric method[9] with modifications from the work of Montero *et al.* (2013).[10] Fresh extract (10 μL) was mixed with 600 μL of ultrapure water, 50 μL of Folin–Ciocalteu reagent, and 150 μL of 20% sodium carbonate, adjusting the volume to 1 mL. After incubating for 2 hours at 25 °C in darkness, absorbance was measured at 760 nm using a microplate spectrophotometer. The results are expressed as

mg of gallic acid equivalents per g of dried extract (mg GAE per g). Analyses were performed in triplicate.

2.6.2. ABTS radical cation decolorization assay. Antioxidant activity was measured using the ABTS assay.[11] ABTS radicals were generated and adjusted to an absorbance of 0.7 at 734 nm. Samples (10 μL) were mixed with 1 mL of ABTS solution, incubated for 45 minutes, and measured at 734 nm. Trolox was used as the standard and the results are expressed as TEAC (Trolox equivalent antioxidant capacity) values (mM Trolox equivalents per g extract). Analyses were performed in triplicate.

2.6.3. Inhibition of acetylcholinesterase activity. AChE inhibition was assessed using a fluorescent assay[4] adapted from the classical UV-Vis assay. Extracts (200–2000 μg mL^{-1}) were incubated with AChE, buffer, and ABD-F, and fluorescence was measured every minute for 10 minutes at 37 °C (λ_{ex} = 389 nm, λ_{em} = 513 nm). The inhibition degree (ID%) was calculated using enzyme velocity (V_{mean}). Galantamine hydrobromide was used as the reference inhibitor and the results are presented at 666 μg mL^{-1} when 50% inhibition was not reached. Analyses were performed in triplicate.

2.7. Environmental life cycle assessment

2.7.1. Goal and scope definition. The environmental assessment was performed according to LCA principles (ISO 14040/44) using SimaPro V9.3.03 software. The goal was to evaluate the potential environmental impacts of the production of extracts from orange by-products (pulp and peels) extracted with three different extraction methods. The product consisted of an extract with neuroprotective and antioxidant potential. The functional unit was based on the value of acetylcholinesterase inhibition capacity (IC_{50}) of the orange by-product extracts. It was defined as a gate-to-gate system and the boundaries are presented in Fig. 2. This figure shows that the system boundaries include the extraction phase, involving the extraction process step and the drying step. The geographical location selected for this study is Madrid, Spain, in which the Institute of Food Science Research (CIAL) is located, that is, the location where the experimental extraction processes of high value compounds, such as neuroprotective compounds from orange by-products, were done.

2.7.2. Life cycle inventory. The inventory data were based on previous works for maceration experiments.[4,7] Besides, PLE and GXL data from the current research were used. The inventory data can be seen in Table 1.

2.7.3. Life cycle impact assessment. Once the life cycle inventory was registered, the inventory data were analyzed using SimaPro V9.3.03 software to quantify the environmental impacts. The database used was Ecoinvent V3.8 and the impact assessment method employed was CML-IA non-baseline. The impact categories evaluated were acidification potential (kg SO_2 eq.), global warming (kg CO_2 eq.), abiotic depletion (kg Sb eq.), eutrophication potential (kg PO_4 eq.), freshwater aquatic ecotoxicity (kg 1.4-DB eq.), marine aquatic ecotoxicity (kg 1.4-DB eq.), freshwater sediment ecotoxicity (kg 1.4-DB eq.), marine sediment ecotoxicity (kg 1.4-DB eq.), photochemical oxidation (kg C_2H_4 eq.), terrestrial ecotoxicity (kg 1.4-DB eq.), human toxicity (kg 1.4-DB eq.), land competition ($m^2 \times a$), ionizing radiation (DALYs), ozone layer depletion (kg CFC-11 eq.), and maladorous air (m^3 air).

2.8. Greenness assessment

Due to recent awareness in developing and using green analytical methods,[12–14] an easy sample preparation greenness assessment was performed to compare with LCA. Analytical greenness metric for sample preparation (AGREEprep)[15] was developed based on categories related to the twelve greenness analytical chemistry (GAC) principles,[13,14] evaluating reagents

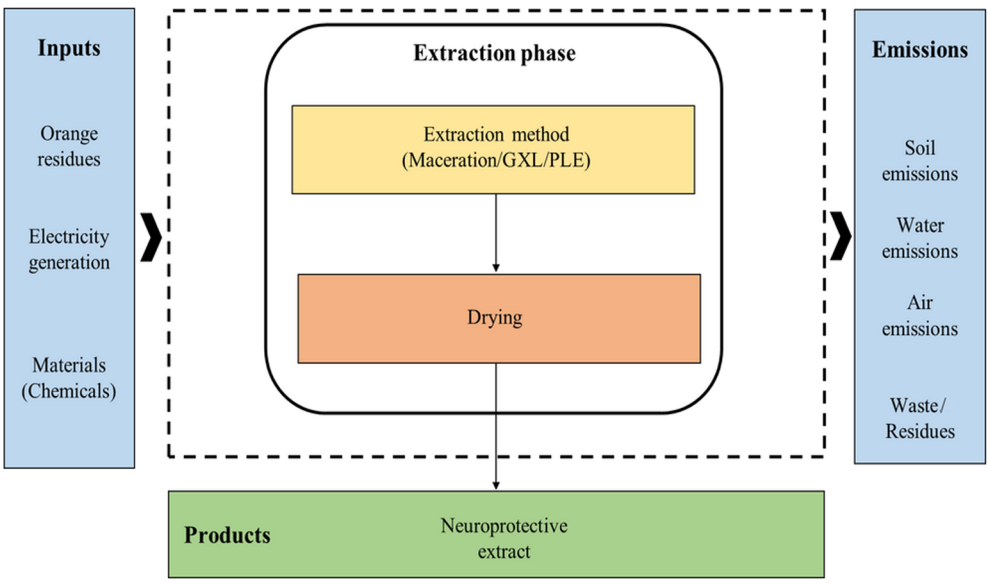

Fig. 2 System boundaries for the life cycle assessment (LCA) of the production process of extracts from orange by-products.

and materials, energy consumption, waste generation and solvents used in a method, among others, and granting a final score between 0 and 1, 1 being the most sustainable and 0 the least sustainable. Additionally, compared to its former version, the analytical greenness metric (AGREE),[16] AGREEprep pays more attention to sample preparation steps that were previously overlooked. This tool provides a pictogram indicating the method's compliance to GAC principles on each evaluated criterion.

2.9. Economic assessment

The economic assessment refers to the calculation of the parameters that determine the economic viability of a system. The goal of this economic analysis is comparing technically and economically the three extraction processes (maceration, PLE, and GXL) based on orange residues and determining their economic performance and feasibility. The economic assessment of the three extraction systems of orange residue extracts was performed by means of CAPCOST software[17] (version 2017, available in ref. 18). The analysis includes four stages: (1) calculation of the total investment, (2) operating cost, (3) revenues, and (4) profitability assessment, which are described as follows (details of the analysis are given in Supplementary information).

I. **Total investment:** In the first stage, the total investment was estimated, which includes expenses for purchasing and installing the equipment, and other general expenses.

II. **Operating cost:** In the second stage, the operational costs were estimated considering the costs of feedstock and raw materials, waste treatment, operating labor, utilities, maintenance and repairs, and general expenses. The data used were as follows: ethyl acetate (ETAC) costs 1.70 USD per kg, nitrogen (N_2) costs 6.5 USD per kg, carbon dioxide (CO_2) costs 2.8 USD per kg, and orange residue costs 0.0378 USD per kg. Electricity and steam costs were quantified using CAPCOST software used for the economic assessment.

III. **Revenues:** During the third stage, revenues were calculated, considering the potential sales of the product.

IV. **Profitability:** In the fourth stage, the profitability assessment was performed by calculating the economic indicator of return of investment (ROI) (eqn (1) and (2)) and payback time (eqn (3)),[19–21] which are explained as follows:

a. *Return on investment* (ROI) describes the rate of return on money invested in the extraction system. A positive ROI means that the investment gains compare favorably to the costs; hence, the larger the ROI, the better.[20]

b. *Payback time* refers to the length of time that the project will take to recover the invested capital. In other words, it measures the time it takes for an investment to pay for itself. The smaller the payback time, the better.[20] Finally, the economic feasibility is reached when revenues and ROI are positive, and the payback time is shorter than the plant lifetime.

$$\mathrm{ROI}\,(\%) = \frac{\text{cashflow}}{\text{total investment}} \quad (1)$$

$$\mathrm{ROI}\,(\%) = \frac{1}{\text{payback time}} \times 100 \quad (2)$$

$$\text{Payback time (years)} = \frac{\text{total investment}}{\text{cashflow}} \quad (3)$$

The main assumptions considered in equipment sizing and scaling are the following:

- The same performance is obtained at the laboratory and industrial scales.
- The operation conditions of extraction processes are the same at the laboratory and industrial scales.
- Cost of raw materials (ethyl acetate, and CO_2) considers recovery of 90%; therefore, 10% is considered in the cost.
- Ethyl acetate density = 0.902 g ml^{-1}.
- Orange peel density = 625 kg m^{-3}.
- Cost of the land is not considered.
- Working time = 8321.16 h (346.71 days per year).
- Production of extract = 1 kg h^{-1} or 8321.16 kg per year.
- Selling price orange extract = 100–1000 USD per kg.
- Depreciation with 35% of interest for 7 years in 10 years plant lifetime was considered.

It is important to acknowledge that the 90% recovery assumption for ethyl acetate and CO_2 was based on laboratory-scale extrapolations and the previous literature.[22] However, actual recovery efficiencies may vary at industrial scale equipment design.

3. Results and discussion

3.1. Greening extraction process

The primary objective of this work was to develop an environmentally sustainable extraction process based on the optimized PLE process identified by Sánchez-Martínez *et al.* (2022)[7] to obtain neuroprotective fractions from orange juice by-products. The published procedure involves the use of ethyl acetate (ETAC) as an extraction solvent under PLE conditions (100 °C, 30 minutes, and 10 MPa). In the present work, we aimed to further optimize these conditions by incorporating greener solvents (specifically water and carbon dioxide) to reduce environmental impact while maintaining extract bioactivity. The previous study evaluated a broader range of neuroprotective mechanisms, including butyrylcholinesterase inhibition and anti-inflammatory activity. However, for the purpose of process optimization, we focused on two key endpoints: antioxidant capacity and acetylcholinesterase (AChE) inhibition. These were selected as representative and sensitive indicators of neuroprotective potential, allowing us to assess the functional performance of the extracts in parallel with environmental and economic metrics. Bioactivities achieved previously provided significant antioxidant activity (ABTS IC_{50} = 13.5 μg mL^{-1}) and AChE inhibition (IC_{50} = 137.1 μg mL^{-1}),[7] with an overall extraction yield of 2.1%. This study seeks not only to maintain these levels of bioactivity but also to contribute to sustainable and efficient valorization of citrus by-products through greener extraction strategies.

3.1.1. PLE employing greener solvents. Building on these initial findings, greener solvents were tested under pressurized liquid conditions, including water, ethanol, a 50% (v/v) ethanol–water mixture, and an acidified mixture of water, ethanol, and formic acid. These solvents were selected based on their suitability for use in food processing and as potential ingredients, as well as their proven efficacy in our previous research for extracting bioactive compounds from fruit by-products.[23] Initial tests employed classical maceration (experiments 1-M, 2-M, and 3-M, Table 2). Among them, pure water proved to be the least effective, while the acidified ethanol–water mixture (50 : 45 : 5 ratio) yielded the best results (Table 2). Given that the outcomes were similar across solvent types, the remaining experimental design focused on combinations of these three solvents (experiments 4–21). Additionally, to assess the impact of formic acid, experiments without adding the acid (experiments 22–30) were conducted and compared to their acidified counterparts. All extracts were functionally characterized by measuring total phenolic content using the Folin–Ciocalteu method, antioxidant activity *via* ABTS radical scavenging, and neuroprotective activity through acetylcholinesterase (AChE) inhibition assays. Normally, AChE inhibition results are expressed as IC_{50} values (concentration required to inhibit 50% of enzyme activity). In this study, several concentrations were tested, but none of the extracts obtained with greener solvents reached 50% inhibition. Therefore, the concentration was increased up to 666 μg mL^{-1} (well above physiologically relevant levels) yet most extracts still failed to achieve IC_{50}. For this reason, Table 2 reports the inhibition percentage at 666 μg mL^{-1} for comparative purposes. This approach provides valuable information about the compound's inhibitory potential, despite the lack of a precise IC_{50} determination.

The experimental results indicate that extraction yield, total phenolic content (TPC), antioxidant activity (ABTS TEAC), and acetylcholinesterase (AChE) inhibition vary significantly based on the extraction conditions. Higher temperatures (110–180 °C) and ethanol–water mixtures generally led to improved extraction yields, with the highest yield (2.16%) observed at 110 °C with 50% ethanol and no formic acid (Expt. #26). Conversely, lower temperatures and 100% water resulted in the lowest yields, as seen in Expt. #9 (0.22%). TPC was the highest (92.99 mg GAE per g) at 180 °C with 100% ethanol and 5% formic acid (Expt. #21), while the lowest values were found at lower temperatures, particularly with pure water extractions.

Table 2 Pressurized liquid extraction (PLE) of orange by-product optimization and the results obtained: global extraction yield, total phenol content (TPC), ABTS antioxidant capacity (TEAC), and neuroprotective activity (AChE)

	Experimental factors				Responses		
Expt. #	Temperature (°C)	% Ethanol in water	% Formic acid	Yield (%)	TPC (mg GAE per g extract)	ABTS TEAC (mM TE per g extract)	AChE[a] (ID %)
1-M	25	100	0	0.86 ± 0.01	10.48 ± 0.91	0.410 ± 0.031	8.54 ± 0.21
2-M	25	0	0	0.81 ± 0.06	0.58 ± 0.02	0.162 ± 0.008	48.12 ± 3.04
3-M	25	50	5	0.93 ± 0.04	10.83 ± 0.03	0.057 ± 0.005	16.07 ± 0.23
4	40	0	2.5	0.74 ± 0.06	16.43 ± 0.06	0.144 ± 0.006	43.01 ± 3.84
5	40	0	5	0.71 ± 0.04	16.79 ± 0.98	0.056 ± 0.005	51.58 ± 1.35
6	40	50	2.5	1.07 ± 0.09	14.85 ± 1.38	0.415 ± 0.016	45.31 ± 4.10
7	40	50	5	1.13 ± 0.04	14.95 ± 0.83	0.102 ± 0.009	51.12 ± 4.53
8	40	100	2.5	0.34 ± 0.05	24.65 ± 1.82	0.528 ± 0.050	37.65 ± 3.49
9	40	100	5	0.22 ± 0.07	11.76 ± 0.04	0.216 ± 0.007	38.91 ± 2.34
10	110	0	2.5	0.93 ± 0.09	13.37 ± 0.28	0.06 ± 0.001	42.17 ± 3.19
11	110	0	5	0.64 ± 0.04	11.57 ± 0.31	0.067 ± 0.005	44.23 ± 2.41
12	110	50	2.5	1.83 ± 0.01	19.37 ± 1.13	0.128 ± 0.013	47.95 ± 0.76
13	110	50	5	1.46 ± 0.00	15.74 ± 1.54	0.107 ± 0.009	34.16 ± 0.01
14	110	100	2.5	1.15 ± 0.07	35.87 ± 2.07	0.505 ± 0.008	47.36 ± 0.59
15	110	100	5	0.75 ± 0.02	22.36 ± 0.50	0.242 ± 0.020	45.19 ± 2.16
16	180	0	2.5	1.29 ± 0.04	54.18 ± 1.11	0.197 ± 0.017	41.2 ± 2.11
17	180	0	5	1.23 ± 0.04	51.29 ± 0.47	0.241 ± 0.009	18.56 ± 1.23
18	180	50	2.5	1.92 ± 0.07	19.95 ± 0.63	0.141 ± 0.009	18.95 ± 1.25
19	180	50	5	1.79 ± 0.03	28.21 ± 0.89	0.125 ± 0.011	36.65 ± 1.35
20	180	100	2.5	1.3 ± 0.06	57.53 ± 2.62	1.133 ± 0.023	24.56 ± 0.41
21	180	100	5	1.29 ± 0.10	92.99 ± 0.86	1.618 ± 0.088	22.43 ± 1.26
22	40	0	0	0.72 ± 0.03	5.17 ± 0.22	0.267 ± 0.022	29.23 ± 2.54
23	40	50	0	0.66 ± 0.07	16.47 ± 1.33	0.673 ± 0.057	41.38 ± 2.02
24	180	0	0	1.14 ± 0.02	4.4 ± 0.01	0.872 ± 0.063	15.58 ± 1.00
25	110	100	0	0.9 ± 0.04	4.11 ± 0.08	0.392 ± 0.034	43.1 ± 1.36
26	110	50	0	2.16 ± 0.06	14.04 ± 0.94	0.219 ± 0.011	32.65 ± 0.99
27	180	100	0	1.29 ± 0.01	10.04 ± 0.91	0.157 ± 0.010	16.57 ± 0.02
28	180	50	0	1.24 ± 0.04	67.08 ± 1.61	1.001 ± 0.083	19.89 ± 1.86
29	40	100	0	0.29 ± 0.01	2.75 ± 0.20	0.332 ± 0.004	47.91 ± 3.99
30	110	0	0	0.52 ± 0.05	22.55 ± 0.58	0.674 ± 0.064	38.63 ± 0.43

[a] AChE ID% column shows the inhibition corresponding to 666 μg ml^{-1}.

Antioxidant activity, measured as ABTS TEAC, reached its peak (1.618 mM TE per g) under conditions of 180 °C, 100% ethanol, and 5% formic acid (Expt. #21). On the other hand, water-only extractions consistently exhibited low antioxidant capacities. For AChE inhibition, the most effective results (51.58%) were observed at 40 °C with 0% ethanol and 5% formic acid (Expt. #5). Higher ethanol concentrations or the absence of formic acid generally led to lower inhibition values. Overall, these results suggest that optimal conditions for maximizing the yield, phenolic content, and antioxidant activity involve higher temperatures with ethanol–water mixtures and formic acid, while lower ethanol levels and higher acid content are more favorable for AChE inhibition. Based on the results obtained in this section (Table 2), none of the extraction conditions using greener solvents such as ethanol and water under PLE led to improvements in extraction yield or antioxidant activity compared to the previously optimized method. More importantly, none of the extracts achieved 50% inhibition of acetylcholinesterase activity, preventing the determination of IC_{50} values and indicating a lack of neuroprotective potential. As a result, none of these conditions were selected as the final options. Given the inability of these greener solvents to extract bioactive neuroprotective compounds effectively, further experimentation along this line was deemed scientifically unjustified.

Comparing the results from Table 2 with those previously published by Sánchez-Martínez *et al.* (2022)[7] (extraction yield = 2.1%; ABTS IC_{50} = 13.5 μg mL^{-1} (or 0.371 mM TE per g extract); AChE IC_{50} = 137.1 μg mL^{-1}), it can be seen that similar results were obtained and therefore no improvements in the extraction process were achieved, despite that water and ethanol are greener solvents. The comparison of the current results with findings from other published studies reveals several insights into acetylcholinesterase (AChE) inhibition using citrus extracts. For example, a study by Abd El-Aziz *et al.* (2022)[24] reported an AChE IC_{50} value of 180 μg mL^{-1} for orange peel extracts, which, although effective, did not match the efficacy of the previous method[7] with an IC_{50} value of 137.1 μg mL^{-1}. Similarly, another study by Sharma *et al.* (2022)[25] indicated that orange peel extracts could inhibit AChE but did not reach IC_{50} levels comparable to Sánchez-Martínez's work, showing the challenge in achieving high neuroprotective potential together with the use of green solvents. Nevertheless, Sharma *et al.* (2022)[25] found similar values to those achieved by Sánchez-Martínez *et al.* (2022)[7] using an ethanol : water mixture with tangerines (*Citrus reticulata* cv. (Kinnow), 130.6 ± 2.04 μg mL^{-1}), instead of oranges. Nevertheless, the amount of orange by-products in the world is comparatively much higher than that of tangerines, and so it is the interest in valorizing them. Other research on citrus varieties, such as by Peron *et al.* (2024),[26] demonstrated notable cholinesterase inhibition activity using different extraction methods, but these studies primarily used traditional solvent systems that do not align with the green chemistry principles (Ballesteros-Vivas *et al.* 2021).[27] These comparisons emphasize that while some methods show promise, there remains a need for further optimization to enhance both bioactivity and environmental performance simultaneously.

3.1.2. GXL to reduce ethyl acetate consumption. Once we confirmed that it was not possible to improve the yield and bioactivity results simultaneously using PLE with greener solvents such as water and ethanol, the next step consisted of developing gas expanded liquid (GXL) extraction to minimize the use of ethyl acetate. The use of water or ethanol as solvents in the GXL system was not further investigated, as both the current results (3.1.1) and previous findings[4,7] consistently showed markedly lower extraction yields and reduced bioactivity under such conditions. These conventional solvents failed to solubilize key neuroprotective compounds effectively under GXL conditions. Ethyl acetate, in contrast, consistently enabled the recovery of highly bioactive fractions, particularly those with acetylcholinesterase inhibitory activity. Therefore, our strategy focused on minimizing the use of ethyl acetate within the GXL system rather than replacing it with less efficient solvents. This approach ensures scientific robustness and aligns with green chemistry principles by reducing solvent consumption while maintaining extract quality. As such, a comparative analysis with water and ethanol was deemed unnecessary.

GXLs are formed when a gas, typically carbon dioxide (CO_2), is dissolved in a liquid solvent (*e.g.*, ethyl acetate) under moderate pressure, leading to an expanded phase that improves solvent penetration and mass transfer.[28] This method not only enhances the extraction efficiency but also significantly reduces the amount of liquid solvent required, as CO_2 effectively lowers the viscosity and increases the diffusivity of the liquid phase, thus optimizing the solubilization of target compounds. Previous studies have demonstrated the effectiveness of GXLs in extracting bioactive compounds such as polyphenols, flavonoids, and terpenoids from natural sources while adhering to green chemistry principles by reducing solvent volumes and organic waste.[29] Given these benefits, we optimized the GXL extraction process (using CO_2 and ethyl acetate) to minimize the use of ethyl acetate while maintaining the bioactivity levels of compounds extracted from orange juice by-products. Besides, the second objective was to obtain extracts using lower temperature in view of future scale-up of the process.

The GXL optimization was performed using a full factorial experimental design (3^2), with the factors being the percentage of CO_2 in ethyl acetate (ETAC) ranging from 10 to 50% and the temperature ranging from 50 to 100 °C, while the yield and acetylcholinesterase (AChE) inhibition served as the response variables. All other extraction parameters were kept constant: sample mass at 50 g, sand mass at 100 g, pressure at 100 bar, cell volume at 300 mL, static extraction type, and an extraction time of 30 minutes. The results of this optimization can be seen in Table 3.

The results of the gas expanded liquid (GXL) extraction experiments demonstrate that the yield and acetylcholinesterase (AChE) inhibition vary significantly with the CO_2 concentration and temperature. The highest yield was observed in

Table 3 Gas expanded liquid (GXL) extraction of orange by-product optimization and the results obtained

#	CO_2 (%)	Temperature (°C)	Yield (%)	AChE (IC_{50} μg mL^{-1})
GXL1	10	50	1.65 ± 0.11	104.95 ± 10.89
GXL2	30	50	0.49 ± 0.03	102.62 ± 13.21
GXL3	50	50	1.48 ± 0.13	82.39 ± 8.78
GXL4	10	75	2.69 ± 0.02	110.64 ± 1.03
GXL5	30	75	4.15 ± 0.33	173.67 ± 20.11
GXL6	50	75	2.56 ± 0.05	146.26 ± 11.90
GXL7	10	100	3.71 ± 0.17	137.96 ± 8.71
GXL8	30	100	5.06 ± 0.22	147.46 ± 15.55
GXL9	50	100	3.41 ± 0.18	125.63 ± 11.12
GXL10	30	75	4.22 ± 0.13	264.75 ± 22.51
GXL11	30	75	4.40 ± 0.24	192.85 ± 18.08

GXL8 (5.06%) with 30% CO_2 at 100 °C, indicating that moderate CO_2 levels combined with higher temperatures optimize the extraction efficiency. In contrast, lower CO_2 levels, such as 10% in GXL2, resulted in significantly lower yields (0.49% at 50 °C). Increasing CO_2 to 50% (*e.g.*, GXL9) also achieved a high yield (3.40%), though still less effective than with 30% CO_2 under the same temperature conditions.

Regarding AChE inhibition, the lowest IC_{50} value (highest inhibition) was achieved in GXL3 (82.39 μg mL^{-1}) with 50% CO_2 at 50 °C, suggesting that higher CO_2 concentrations combined with lower temperatures enhance bioactivity. However, increasing the temperature to 100 °C (*e.g.*, GXL8 and GXL9) generally led to higher IC_{50} values (lower inhibition), indicating a potential decline in bioactivity at elevated temperatures. On using 30% CO_2 at 75 °C (*e.g.*, GXL10 and GXL11), yields were relatively high (4.20–4.40%), but IC_{50} values increased substantially, reaching up to 264.75 μg mL^{-1}, showing reduced AChE inhibition.

The inclusion of three replicated runs at the center point in the experimental design allowed for a robust estimation of pure error and an assessment of process stability. Quantitative analysis revealed an average yield of 4.26% with a low standard deviation of 0.13 (RSD = 3.03%), indicating high precision and minimal inherent variability for this response. In contrast, the AChE (IC_{50}) response, with a mean of 210.42 μg mL^{-1} and a higher standard deviation of 48.02 (RSD = 22.82%), demonstrated a variability of extracted compounds in the raw material used for extractions. These quantitative measures of pure error are critical for statistically distinguishing significant factor effects from background noise and for assessing the reproducibility of the system under the center point conditions.

Comparison with pressurized liquid extraction (PLE) results from the work of Sánchez-Martínez *et al.* (2022),[7] which achieved an extraction yield of 2.1% and AChE IC_{50} of 137.1 μg mL^{-1}, highlights that GXL extraction can enhance the yield twofold without significantly compromising neuroprotective activity. Notably, the conditions in GXL3 (50% CO_2 and 50 °C) produced the highest neuroprotective activity, making these the optimal parameters for maximizing bioactivity.

3.2. Environmental impact comparison of extraction processes

The environmental impact of an extraction process is a critical consideration, particularly when optimizing methods for bioactive compound recovery from agricultural by-products. Life cycle assessment (LCA) is a widely used tool to quantify and compare the environmental burdens associated with different extraction techniques, providing insights into their sustainability profiles. In this study, we performed an LCA to evaluate and compare the environmental impacts of three extraction methods for acetylcholinesterase-inhibitory compounds from orange by-products: the PLE method developed by Sánchez-Martínez *et al.* (2022),[7] the present optimized GXL extraction, and a classical maceration method developed by Sanchez-Martinez *et al.* (2021).[4] The LCA was conducted using SimaPro V9.3.03 software, with data sourced from the Ecoinvent V3.8 database and assessed using the CML-IA non-baseline method. Several impact categories, such as global warming potential, acidification, and ecotoxicity, were analyzed to provide a comprehensive environmental footprint of each method.

Studies by Midolo *et al.* (2024)[30] on citrus waste valorization using LCA demonstrated the importance of evaluating diverse environmental impact categories, particularly in processes involving chemical solvents. Additionally, Manakas *et al.* (2025)[31] highlighted the environmental advantages of novel technologies, which have proved to reduce solvent use and emissions in citrus processing. By comparing the three different processes in terms of their environmental burdens, our study aims to identify the most sustainable and efficient approach for bioactive compound extraction from citrus by-products, thus contributing to the development of eco-friendly technologies in the food processing industry.

3.2.1. System boundaries and LCA results. Defining the system boundaries and LCA inventory are the first steps to perform the environmental analysis. The system boundaries (Fig. 3), including the selected conditions for each extraction process, were defined from a gate-to-gate perspective, focusing specifically on the extraction processes, beginning after the orange peels have been dried and ground. This approach isolates the environmental impacts directly associated with the extraction techniques, allowing for a more precise comparison of the PLE, GXL, and classical maceration methods. The LCA inventory (Table 1) includes detailed data on energy consumption, solvent use, and emissions associated with each extraction technique. Inputs such as the quantities of CO_2, ethyl acetate, and other solvents, as well as electricity and water usage, were recorded, ensuring a comprehensive representation of each process. The outputs, including waste products, emissions, and the yields of bioactive compounds, were also captured to accurately quantify their environmental impacts. By clearly establishing the system boundaries and compiling a detailed LCA inventory, this section provides the foundational data required for calculating and interpreting the environmental burdens associated with each extraction method.

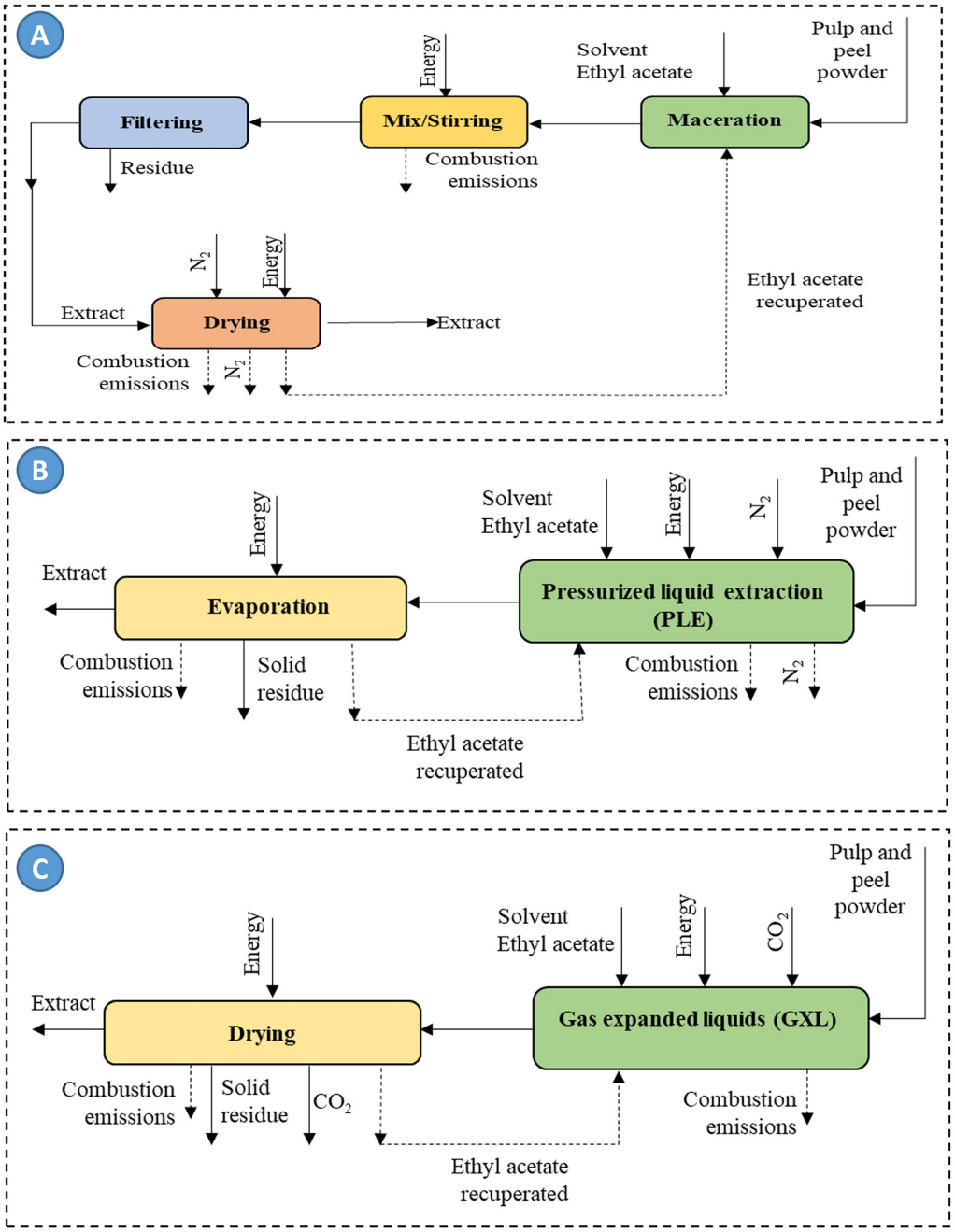

Fig. 3 System boundaries employed in the life cycle assessment (LCA) of orange by-product valorization through three different extraction processes: (A) maceration with ethyl acetate, room temperature, and 24 h; (B) PLE extraction with ethyl acetate, 100 °C, 30 min, and 10 MPa; (C) GXL extraction with CO_2 : ethyl acetate 1 : 1, 50 °C, and 10 MPa.

The bar graph shown in Fig. 4A illustrates the relative environmental impacts of the three extraction methods (maceration, PLE and GXL) normalized to the impact of the maceration method (100%) as a reference point. The results clearly show that the maceration process has the highest environmental burden across all evaluated categories, serving as the baseline for comparison. This fact is evident in categories like global warming potential (18.8 kg CO_2 eq.) and malodorous air emissions (401 000 m^3 air), highlighting its inefficiency and high resource demand. In contrast, the GXL method consistently demonstrates the lowest environmental impact; for instance, its global warming potential is only 1.52 kg CO_2 eq., and malodorous air emissions are substantially reduced to 6350 m^3 air, emphasizing its environmentally-friendly nature and outperforming both PLE and maceration in every category analyzed. The PLE method, while generally more sustainable than maceration, shows intermediate values, such as a global warming potential of 3.42 kg CO_2 eq.

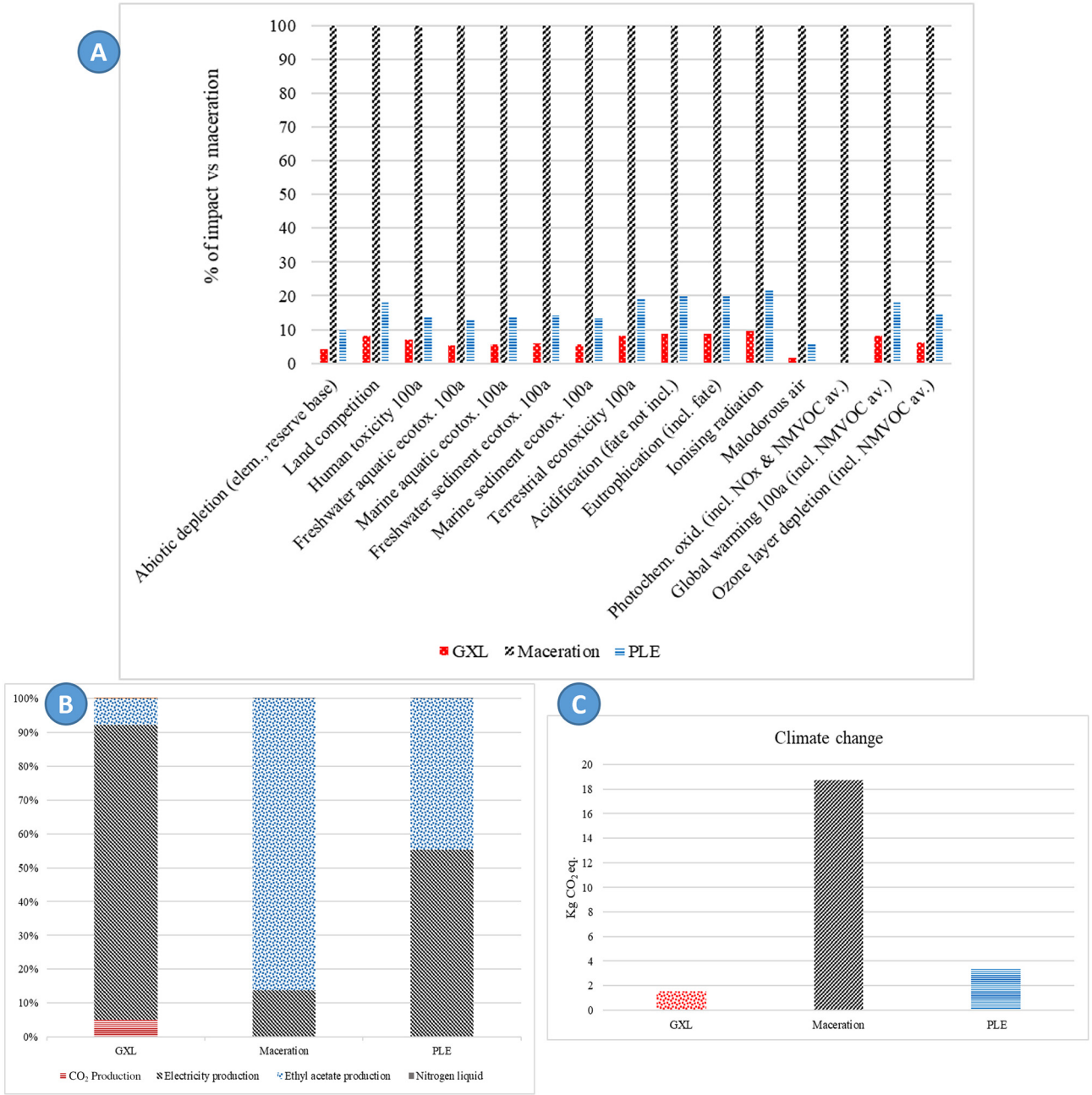

Fig. 4 Results of the LCA of maceration, PLE and GXL applied to orange by-product extraction. (A) Life cycle impacts; (B) precursor contributions to impact categories; and (C) climate change category comparison in kg CO_2 equivalents.

and malodorous air emission of 22 600 m^3 air, indicating improvements but still higher environmental impact than that of GXL. Notably, GXL shows the lowest impacts in critical areas like acidification (0.0106 kg SO_2 eq.) and eutrophication (0.006 kg PO_4 eq.), further confirming its environmental superiority. Overall, these results emphasize the superior environmental performance of GXL extraction, suggesting that it is the most sustainable option among the three methods evaluated.

To determine the contribution of various precursors to the environmental impacts, we first identified the contributions within each impact category. Then, the contributions specific to each of the three extraction methods were assessed. The primary precursors identified were CO_2 production, electricity, and ethyl acetate production. Fig. 4B highlights these precursors as the most significant contributors to the environmental impacts of the extraction processes analyzed. The results indicate that electricity production accounts for 87% of the impacts in the GXL process, 14% in maceration, and 55% in PLE. Meanwhile, ethyl acetate production is responsible for 8% of impacts in GXL, 86% in maceration, and 45% in PLE.

These findings highlight that electricity and ethyl acetate production are the dominant environmental impact contributors across all extraction processes. By combining emissions from electricity and ethyl acetate production, we found around 95% of the total environmental impacts in the GXL process and 100% in both maceration and PLE methods. Based on these observations, key points for reducing environmental burdens are discussed in the subsequent sections.

SDG 13 [32] states that climate change is a critical global issue that needs urgent attention. Because of that, this section focuses on the climate change category. Fig. 4C shows the results for the climate change (global warming potential) category. As can be noticed, the maceration process releases 18.80 kg CO_2 eq. per hour. This is the extraction method that is more affected in this category. It is followed by PLE, which emits 3.40 kg CO_2 eq. per hour and GXL that emits 1.50 kg CO_2 eq. per hour. The climate change category is affected mainly by electricity generation and ethyl acetate production. For instance, electricity production causes 96% of the impacts in GXL, 78% in maceration, and 97% in PLE. On the other hand, ethyl acetate production causes 1% of the impacts in GXL, 22% in maceration, and 4% in PLE. Our results align with the findings of Joglekar *et al.* (2019)[33] since both emphasize the importance of identifying environmental hotspots and optimizing processes to reduce the environmental burden of citrus waste processing. In their work, the authors study the LCA of a citrus waste biorefinery, finding significant contributions coming from specific processing steps like "hydrolysis and flashing", which accounted for around 60% of the several environmental impact indicators. In comparison, our study demonstrates that optimizing extraction methods through gas expanded liquid (GXL) technology substantially reduces the GWP, with GXL emitting only 1.50 kg CO_2 equivalent per hour, significantly lower than conventional methods such as maceration (18.80 kg CO_2 equivalent per hour). Joglekar *et al.* recommend advanced technologies like microwave- and ultrasound-assisted steps to reduce impacts, while our study further supports this approach by showing that GXL technology—a modern and green extraction method—can achieve a significant reduction in environmental indicators such as GWP and acidification potential. These results suggest that advanced technologies are not only effective for biorefineries but also for optimizing smaller-scale extraction processes, ultimately contributing to the development of more sustainable and eco-friendly systems.

The LCA results shown in the present study agree with those presented by Teigiserova *et al.* (2022)[34] and Midolo *et al.* (2024)[30] in the importance of optimizing both electricity consumption and solvent use in citrus by-product processing to minimize environmental impacts. Teigiserova *et al.* (2022)[34] assessed the LCA of a biorefinery using orange peel waste (OPW) for producing limonene, citric acid, and animal feed. They found that the environmental performance, particularly in the climate change category, was heavily influenced by electricity inputs, with CO_2 emissions ranging from 4388 kg CO_2 eq. per tonne of OPW using the current electricity mixture, down to 594 kg CO_2 eq. when using wind energy. In our study, we also observed that electricity is a major contributor, accounting for up to 96% of the GWP in the GXL process, which is consistent with Teigiserova *et al.*'s findings. This emphasizes that using renewable energy sources can significantly reduce the environmental footprint, as shown in their study where renewable energy reduced emissions substantially. In this sense, Midolo *et al.* (2024)[30] focused on the extraction of pectin and limonene from OPW and noted that the use of ethanol as a solvent, coupled with high electricity consumption, led to a considerable environmental impact, especially for pectin extraction. The authors conclude that by switching to more sustainable solvents, the environmental footprint of the process could be reduced by 73.4%. Our LCA results align with these insights, as the ethyl acetate used in our PLE and GXL processes also emerged as a significant impact contributor. In our study, ethyl acetate accounted for 8% of the impacts in the GXL method and up to 86% in maceration. Together, these comparisons reinforce the conclusion that optimizing solvent use and electricity sources is critical in reducing the environmental impact of citrus by-product extraction. These findings emphasize the importance of integrating green technologies and renewable energy sources in developing sustainable extraction processes for citrus by-product valorization.

3.3. Greenness assessment

In addition to the comprehensive LCA, the AGREEprep greenness assessment method was applied to evaluate and compare the environmental performance of the three extraction methods; maceration, PLE and GXL. While LCA provides a detailed and rigorous quantification of environmental impacts across multiple categories and life cycle stages, it can be data-intensive and complex to implement, especially in early research or development stages. Therefore, incorporating a simpler, rapid, and user-friendly tool like AGREEprep allows for an additional layer of evaluation focused specifically on the greenness of extraction processes considering them as sample preparation steps. This method enables researchers to visualize and score the environmental friendliness of laboratory-scale procedures using 10 criteria derived from the principles of green analytical chemistry.[15]

The extraction conditions outlined in Table 1 were inputted into AGREEprep software, and the resulting greenness pictograms are shown in Fig. 5. The maceration method received a score of 0.53 (Fig. 5A), PLE scored 0.64 (Fig. 5B), and GXL scored 0.69 (Fig. 5C). The most notable differences among the methods were observed in criterion 5 (waste generation) and criterion 8 (energy consumption). The GXL method significantly outperformed the others by reducing solvent usage by over 95% and energy consumption by 90% compared to maceration and 44% compared to PLE. These improvements directly contributed to its higher AGREEprep score.

To the best of our knowledge, this study is the first to combine AGREEprep and LCA to evaluate the environmental sustainability of extraction processes applied to food by-products. While both methods are independently valuable, their

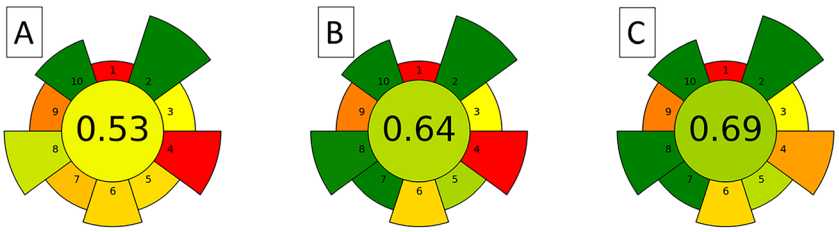

Fig. 5 AGREEprep greenness assessment pictograms of compared methods: (A) maceration, (B) PLE and (C) GXL.

combined use offers a powerful, complementary approach. LCA provides a broad, system-level evaluation of environmental impacts, while AGREEprep delivers a focused, intuitive, and criteria-based analysis of the sample preparation stage (extraction process in our case). The integration of AGREEprep enhances LCA by highlighting specific environmental hotspots, such as solvent use and energy consumption, within the extraction process. In the present work, AGREEprep helped quantify and visualize the greenness of maceration, PLE, and GXL methods, reinforcing LCA findings with more accessible, interpretable metrics. For instance, the significantly higher AGREEprep score of the GXL method aligned with its lower environmental impacts found in the LCA, particularly in waste generation and energy use. This synergy allows for a more nuanced and actionable sustainability assessment. AGREEprep serves not only as a supporting tool for LCA but also as a rapid screening method for early-stage process development, guiding researchers toward greener practices. Together, these tools enable more informed, balanced decisions in designing eco-friendly extraction strategies aligned with green chemistry and circular economy goals.

3.4. Economic assessment

Following the environmental assessment, the economic feasibility of the three extraction processes was evaluated to determine the most cost-effective approach for producing neuroprotective fractions from orange by-products. This economic analysis compares operational costs, including raw materials, energy consumption, and equipment maintenance, to provide a comprehensive understanding of the trade-offs between environmental sustainability and economic viability. The goal is to identify an extraction method that balances low environmental impact with economic efficiency, facilitating the industrial-scale adoption of sustainable citrus waste valorization.

Fig. 6 provides a cost comparison of the three extraction methods evaluated: maceration, PLE and GXL. For the maceration process, operational costs constitute 60% of the total, raw materials account for 32%, investment costs represent 7%, and utilities make up 1%. In contrast, the GXL process shows that operational costs are 75%, raw materials are 4%, investment costs are 20%, and utilities are 1%. The PLE process reveals that operational costs are 84%, raw materials are 4%, investment costs are 11%, and utilities are 1%. Overall, the maceration process has the highest costs due to two main factors: (1) maceration has a low extraction yield (0.51%, according to Sanchez-Martinez *et al.* 2021[4]), requiring larger quantities of feedstock, raw materials, and equipment to produce the same amount of the product as GXL and PLE and (2) maceration involves three processing steps (maceration and stirring, filtering, and drying), compared to only two steps in GXL and PLE, resulting in higher costs for equipment, waste management, labor, and maintenance.

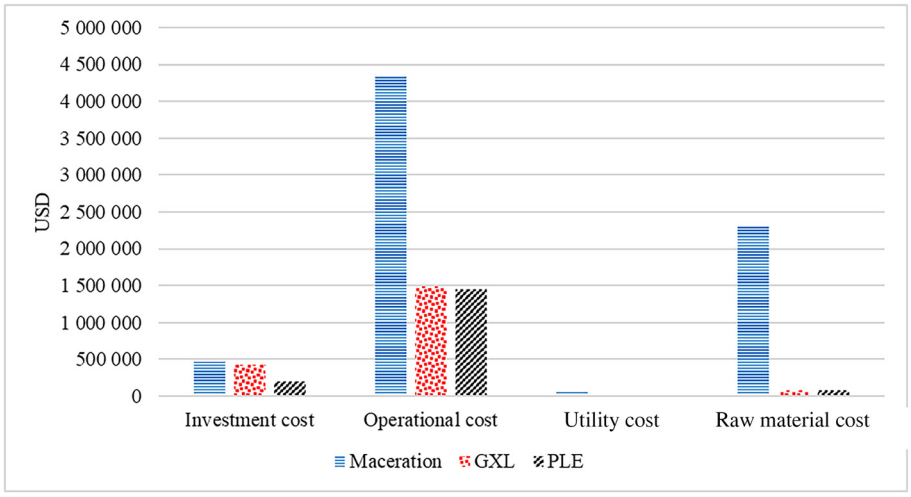

Fig. 6 Comparison of costs for the maceration, GXL, and PLE extraction processes.

Comparatively, the GXL process incurs the second-highest costs, while PLE demonstrates the lowest overall costs, except for utilities where GXL is marginally lower. The PLE process benefits from a higher extraction yield (73%), which reduces the amount of feedstock and equipment size needed, thus lowering costs. Profitability was assessed using return on investment (ROI) and payback time indicators, with a selling price range of $100–$1000 USD per kg due to the lack of an established market price for neuroprotective orange extracts. Tables 4 and 5 present the ROI and payback time for each process. PLE exhibited the best economic performance, achieving the highest ROI and shortest payback period, due to its lower costs. Conversely, maceration had the lowest ROI and the longest payback time, reflecting its higher overall costs. All methods showed a payback time shorter than the expected plant lifetime, indicating profitability. To achieve profitability, the minimum selling prices required were $180 USD per kg for PLE, $190 USD per kg for GXL, and $530 USD per kg for maceration, further demonstrating the economic efficiency of the PLE process.

Table 4 Results of the ROI indicator for different orange extract selling prices for the maceration, PLE and GXL extraction processes

	ROI (%)		
Selling price (USD per kg)	Maceration	PLE	GXL
100	—	—	—
180	—	18	—
190	—	49	14
200	—	76	30
300	—	347	162
400	—	618	291
500	—	888	419
530	5	969	458
600	93	1158	548
700	206	1429	677
800	318	1699	806
900	431	1970	935
1000	544	2240	1063

Table 5 Results of the payback time indicator for different orange extract selling prices for the maceration, PLE and GXL extraction processes

	Payback time (years)		
Selling price (USD per kg)	Maceration	PLE	GXL
100	—	—	—
180	—	4.42	—
190	—	2.01	4.94
200	—	1.3	3.02
300	—	0.29	0.62
400	—	0.16	0.34
500	—	0.11	0.24
530	7.08	0.1	0.22
600	1.08	0.09	0.18
700	0.49	0.07	0.15
800	0.31	0.06	0.12
900	0.23	0.05	0.11
1000	0.18	0.04	0.09

Finally, this economic analysis allowed us to determine the economic performance and viability of extraction systems. This methodology can be implemented to other extraction methods for the production of high value compounds derived from different biomass residues in a context of circular bioeconomy and sustainability.

3.5. Multi-criteria decision analysis

Multi-criteria decision analysis (MCDA) is a structured decision-making tool that integrates multiple performance indicators into a single comparative framework. To support decision-making regarding the industrial implementation of the evaluated extraction methods, a MCDA was conducted by integrating environmental and economic indicators. The analysis considered carbon footprint (global warming potential) as an environmental indicator and return on investment and payback time as economic indicators, with a higher weight assigned to environmental impact to reflect the study's sustainability-driven objectives. Using normalized values and a simple additive weighting method, GXL emerged as the top-ranked method due to its significantly lower environmental footprint, despite the slightly lower economic performance compared to PLE. While PLE demonstrated the highest ROI and shortest payback time, its environmental impact was notably higher than that of GXL. Maceration ranked the lowest in all categories. These findings reinforce the conclusion that GXL offers the most balanced and sustainable solution for the valorization of citrus by-products, particularly when environmental criteria are prioritized. This supports its selection as the most suitable method for future industrial applications aligned with green chemistry and circular economy principles.

4. Conclusions

This study demonstrates the potential of orange by-products as a profitable source for producing neuroprotective extracts using environmentally sustainable extraction techniques. Through a comprehensive evaluation of maceration, GXL, and PLE processes, the results showed that citrus by-products, typically regarded as waste or low-cost animal feed, can be transformed into high-value bioactive extracts. The optimization of solvents played a critical role in this study; although greener solvents like water and ethanol were initially tested, they did not effectively extract the neuroprotective fractions. Consequently, GXL (using CO_2 and ethyl acetate) was employed as an innovative approach, reducing the use of the most effective solvent, ethyl acetate, while maintaining high bioactivity in the extracts. This strategic reduction aligns with green chemistry principles and significantly lowers the environmental impact of the extraction process.

Among the methods analyzed, PLE and GXL emerged as the most economically and environmentally viable. PLE showed the highest profitability with the lowest costs, highest ROI, and shortest payback time, while GXL demonstrated the lowest environmental footprint due to minimized solvent use

and reduced emissions. These findings highlight that by integrating advanced and optimized technologies such as PLE and GXL, it is possible to balance profitability and sustainability in the valorization of citrus by-products. Such methods offer a scalable and eco-friendly pathway for the production of neuroprotective products, supporting circular bioeconomy principles and sustainable biorefinery development.

Author contributions

Brenda L. S. Porto: formal analysis, investigation, and writing – original draft; Berenice Acevedo-García: formal analysis, investigation, methodology, writing – original draft, and writing – review & editing; Ayla Elmi Kashtiban: formal analysis, investigation, and writing – original draft; Tulio Miranda Sepulveda: investigation; Miguel Herrero: conceptualization, funding acquisition, and project administration; Alejandro Cifuentes: conceptualization, funding acquisition, and project administration; Jose A. Mendiola: conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, writing – original draft, and writing – review & editing; Elena Ibáñez: conceptualization, data curation, methodology, supervision, validation, and writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the tables supplied in the text and SI.

Supplementary information is available. See DOI: **https://doi.org/10.1039/D5GC02153G**

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References

1 M. González-Miquel and I. Díaz, Chapter 6 – Valorization of Citrus Waste through Sustainable Extraction Processes, in *Food Industry Wastes (Second Edition)*, Academic Press, 2020, pp. 113–133.
2 Y. Shan, Preface, in *Comprehensive Utilization of Citrus By-Products*, Academic Press, 2016, pp. vii–viii.
3 European Commission-Directorate-General for Agriculture and Rural, European Commission|Oranges short-term outlook, **https://agridata.ec.Europa.eu/extensions/dashboardsto/sto_oranges.html**, (accessed 14 July 2025).
4 J. D. Sánchez-Martínez, M. Bueno, G. Alvarez-Rivera, J. Tudela, E. Ibañez and A. Cifuentes, *Food Funct.*, 2021, **12**, 302–314.
5 A. E. Kashtiban, C. O. R. Okpala, A. Karimidastjerd and S. Zahedinia, *Explor. Foods Foodomics*, 2024, **2**, 125–154.
6 A. d. P. Sánchez-Camargo, J. A. Mendiola and E. Ibáñez, Gas Expanded-Liquids, in *Supercritical and Other High-pressure Solvent Systems: For Extraction, Reaction and Material Processing*, The Royal Society of Chemistry, 2018, pp. 512–531.
7 J. D. Sánchez-Martínez, G. Alvarez-Rivera, R. Gallego, M. B. Fagundes, A. Valdés, J. A. Mendiola, E. Ibañez and A. Cifuentes, *Food Chem.:X*, 2022, **13**, 100242.
8 B. Gilbert-López, J. A. Mendiola, J. Fontecha, L. A. M. Van Den Broek, L. Sijtsma, A. Cifuentes, M. Herrero and E. Ibáñez, *Green Chem.*, 2015, **17**, 4599–4609.
9 V. L. Singleton and J. A. Rossi, *Am. J. Enol. Vitic.*, 1965, **16**, 144–158.
10 L. Montero, M. Herrero, E. Ibáñez and A. Cifuentes, *J. Chromatogr. A*, 2013, **1313**, 275–283.
11 R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radical Biol. Med.*, 1999, **26**, 1231–1237.
12 M. Koel, *Green Chem.*, 2016, **18**, 923–931.
13 S. Armenta, S. Garrigues and M. De La Guardia, *TrAC, Trends Anal. Chem.*, 2008, **27**, 497–511.
14 Y. Zou, W. Tang and B. Li, *Green Chem.*, 2024, **26**, 4975–4986.
15 W. Wojnowski, M. Tobiszewski, F. Pena-Pereira and E. Psillakis, *TrAC, Trends Anal. Chem.*, 2022, **149**, 116553.
16 F. Pena-Pereira, W. Wojnowski and M. Tobiszewski, *Anal. Chem.*, 2020, **92**, 10076–10082.
17 R. Turton, *Analysis, Synthesis, and Design of Chemical Processes*, Prentice Hall, 2012.
18 Richard Turton|Analysis Synthesis and Design of Chemical Processes 5th Edition, **https://richardturton.faculty.wvu.edu/publications/analysis-synthesis-and-design-of-chemical-processes-5th-edition**, (accessed 14 July 2025).
19 J. Qiu, Y. Wang, F. Lü, N. Liao, J. Li, X. Hua, H. Zhang, B. Xu and P.-J. He, *Green Chem.*, 2025, **27**, 4611–4620.
20 J. Cristóbal, C. Caldeira, S. Corrado and S. Sala, *Bioresour. Technol.*, 2018, **259**, 244–252.
21 A. C. Dimian, P. Iancu, V. Plesu, A.-E. Bonet-Ruiz and J. Bonet-Ruiz, *Chem. Eng. Res. Des.*, 2019, **141**, 198–219.

22 E. Cruz Sánchez, J. M. García-Vargas, I. Gracia, J. F. Rodríguez and M. T. García, *Processes*, 2022, **10**, 2708.
23 T. Brazdauskas, L. Montero, P. R. Venskutonis, E. Ibañez and M. Herrero, *J. Chromatogr. A*, 2016, **1468**, 126–135.
24 N. M. A. Abd El-Aziz, M. G. Shehata, T. Alsulami, A. N. Badr, M. R. Elbakatoshy, H. S. Ali and S. A. El-Sohaimy, *Pharmaceuticals*, 2022, **16**, 12.
25 P. Sharma, V. Dadwal, S. N. Rahmatkar, M. Gupta and D. Singh, *J. Sci. Ind. Res.*, 2022, **81**, 445–454.
26 G. Peron, G. Bernabé, S. Marcheluzzo, G. Zengin, K. Ibrahime Sinan, J. Hošek, J. Treml, I. Kaja, M. Paccagnella, P. Brun, I. Castagliuolo, M. Zancato and S. Dall'Acqua, *J. Funct. Foods*, 2024, **116**, 106147.
27 D. Ballesteros-Vivas, B. Socas-Rodríguez, J. A. Mendiola, E. Ibáñez and A. Cifuentes, *Curr. Opin. Green Sustainable*, 2021, **31**, 100522.
28 J. A. Mendiola, Green Processes in Foodomics. Gas-Expanded Liquids Extraction of Bioactives, in *Comprehensive Foodomics*, Elsevier, Oxford, 2021, pp. 744–753.
29 A. D. P. Sánchez-Camargo, N. Pleite, J. A. Mendiola, A. Cifuentes, M. Herrero, B. Gilbert-López and E. Ibáñez, *Electrophoresis*, 2018, **39**, 1875–1883.
30 G. Midolo, G. Cutuli, S. M. C. Porto, G. Ottolina, J. Paini and F. Valenti, *Sci. Rep.*, 2024, **14**, 21418.
31 P. Manakas, A. T. Balafoutis, C. Kottaridi and A. Vlysidis, *Biomass Convers. Biorefin.*, 2025, **15**, 6525–6544.
32 Goal 13|Department of Economic and Social Affairs, **https://sdgs.un.org/goals/goal13**, (accessed 14 July 2025).
33 S. N. Joglekar, P. D. Pathak, S. A. Mandavgane and B. D. Kulkarni, *Environ. Sci. Pollut. Res.*, 2019, **26**, 34713–34722.
34 D. A. Teigiserova, L. Hamelin, L. Tiruta-Barna, A. Ahmadi and M. Thomsen, *Sci. Total Environ.*, 2022, **812**, 152574.