Biomass and Bioenergy 215 (2026) 109634

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Unraveling the bioactive potential of grape pomace using deep eutectic solvents (DES)

Alba Pérez-Pérez [a,b,c], Adela Alvaredo-López-Vizcaíno [a,b], Tânia C.S. P. Pires [c], Tiane C. Finimundy [c], Filipa Mandim [c], Lillian Barros [c], Pablo G. Del-Río [a,b,*], Beatriz Gullón [a,b]

[a] Universidade de Vigo, Departamento de Enxeñaría Química, Facultade de Ciencias, Ourense, 32004, Spain
[b] Instituto de Agroecoloxía e Alimentación (IAA), Universidade de Vigo – Campus Auga, Ourense, 32004, Spain
[c] CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, 5300- 253, Portugal

ARTICLE INFO

Keywords:
Grape bagasse
Green solvents
Box-Behnken design
Polyphenols
Antioxidants
By-products

ABSTRACT

Grape pomace (GP) is the main by-product generated by the winemaking industry. Traditionally, the economic potential of this residue has been underestimated; however, it has recently attracted increasing interest as a promising source of high-value compounds. In this work, three deep eutectic solvents (DES) were assessed as green alternatives for the efficient extraction of phenolic compounds from GP. Amongst the tested solvents, the DES composed of choline chloride, acetic acid, and water (1:1:10 M ratio) showed the best performance. Under optimized extraction conditions (14.86 mL/g liquid-to-solid ratio (LSR), 60 °C, and 275.85 min), the total phenolic content (TPC) reached 113.71 mg GAE/g, while the condensed tannins content (CTC) was 125.37 mg CE/g. Antioxidant activity values were 165.25 mg TE/g for DPPH, 171.48 mg TE/g for ABTS, 102.88 mg TE/g for FRAP, and 367.79 mg TE/g for CUPRAC. Additionally, lipid peroxidation inhibition (TBARS) showed an IC_{50} of 337.02 μg/mL. HPLC-MS-ESI analysis of the optimal extract allowed the tentative identification of 18 phenolic compounds, mainly including phenolic acids, flavan-3-ols, anthocyanins, and tannins. Furthermore, the extract exhibited cytotoxic potential against gastrointestinal and liver cell lines with IC_{50} of 23 and 12 μg/mL, respectively; and antibacterial activity against *Escherichia coli*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimum inhibitory concentration (MIC) value of 33.35 mg/mL for all three bacterial strains. Overall, this study highlights red GP as a promising wine industry by-product for the recovery of bioactive compounds, although further studies are required to assess their safety and potential use as multifunctional ingredients.

1. Introduction

During the past few decades, the concept of sustainability has been gaining momentum as awareness of resource constraints and the overproduction of waste has grown. In this context, there is a serious challenge regarding the generation of millions of tons of waste and by-products by the agri-food sector, whose management involves considerable economic costs [1]. These by-products are often considered worthless and are generally burned or deposited in fields as fertilizers. However, these practices cause significant environmental, economic, and social impacts [2].

Among agri-food industries, the wine sector is of particular relevance worldwide, with a cultivated area of approximately 7 million hectares of grapes and an annual production of more than 200 million hectoliters [3]. This crop is especially important in Mediterranean countries, where the world's largest producers are located, namely Italy, France, and Spain, which together account for almost 50% of global wine production [3]. Nevertheless, the high productivity of this sector is accompanied by the generation of large amounts of waste, as more than 30 wt% of grapes are not used during winemaking. Consequently, wineries generate around 20 million tons of residues annually, mainly including grape pomace (GP), wine lees, wastewater, vine shoots, and grape stalks [4,5]. Among these, GP is one of the most important. Its production is estimated at 1 kg per 6 L of wine, and its traditional use results in significant air pollution and changes in soil properties that lead to pests and diseases [6,7].

* Corresponding author. Universidade de Vigo, Departamento de Enxeñaría Química, Facultade de Ciencias, 32004, Ourense, Spain.
E-mail address: pdelrio@uvigo.es (P.G. Del-Río).

https://doi.org/10.1016/j.biombioe.2026.109634
Received 19 April 2026; Received in revised form 26 May 2026; Accepted 28 May 2026
Available online 30 May 2026

Therefore, the valorization of wine by-products, particularly GP, represents a promising strategy to improve the sustainability and circularity of the wine sector [8,9]. The interest in GP valorization is closely related to its chemical composition and its potential as a source of valuable bioactive compounds. GP contains dietary fiber, sugars, pectin, lipids, proteins, minerals, and, particularly, phenolic compounds such as anthocyanins, flavanols, resveratrol, and phenolic acids [10,11]. Polyphenols have recently received extensive scientific awareness owing to their potential advantages on human health [12,13]. Numerous studies have demonstrated the distinct biological properties of phenolic compounds derived from GP. In this case in point, Ishimoto et al. [14] carried out an investigation which showed an anti-lipidemic and antioxidant effect attributed to the bioactive substances present in GP. Similarly, Pérez-Ortiz et al. [15] observed antiproliferative and antitumoral activities of white and red GP extracts through *in vitro* assays. Besides, Abreu et al. [16] reported that extracts obtained from GP of distinct cultivation varieties exhibit interesting antibacterial activity.

Conventional extraction methods, such as maceration or Soxhlet, remain widely applied for the recovery of phenolic compounds due to their simplicity and reproducibility, although they often involve long extraction times and the use of harmful organic solvents. These conditions may negatively influence extraction yield and even the stability or bioactivity of the target compounds. On the other hand, more advanced techniques, including ultrasound- or microwave-assisted extraction, can improve extraction efficiency, but they usually require specialized equipment and be restricted by limited scalability [17–19]. Within this framework, deep eutectic solvents (DES) offer a promising alternative that can be implemented within conventional extraction processes, providing enhanced solubility of phenolic compounds while contributing to a more sustainable and environmentally friendly approach. DES are mixtures of at least one hydrogen bond donor (HBD) and one acceptor (HBA) whose interactions lower the system's energy and melting point [20,21]. The industrial interest in DES stems from its favorable physicochemical properties, including low viscosity and density, low cost, safety, biodegradability, and sustainability [22]. Consequently, their applications have been explored in numerous fields, such as gasoline purification, the synthesis of sustainable biological membranes, and the recovery of phenolic compounds, among others [23–25].

To date, although DES have been increasingly explored for the recovery of bioactive compounds from agro-industrial residues, studies specifically addressing the optimization of phenolic compound extraction from GP using DES remain relatively limited. In particular, to the best of the authors' knowledge, the application of a choline chloride: acetic acid-based DES, in which acetic acid acts as the hydrogen bond donor, has not yet been reported for the extraction of bioactive compounds from red GP.

Therefore, the present study aims to develop and optimize a simple, low-cost, and environmentally friendly DES-based extraction strategy for the valorization of red GP, with particular emphasis on the recovery of phenolic-rich extracts exhibiting biologically relevant properties. First, DES were prepared from the following HBA/HBD pairs: sodium acetate/lactic acid, choline chloride/acetic acid, and choline chloride/citric acid. Then, the most suitable DES formulation was selected based on maximum recovery of antioxidant compounds and minimum solvent synthesis cost. Subsequently, the effect of the liquid-solid ratio, temperature, and extraction time was evaluated by applying a Box-Behnken experimental design. Furthermore, the gastrointestinal and hepatic toxicity, inhibitory potential against lipid oxidation (TBARS), and the antimicrobial activity of the optimized extracts were analyzed, as well as their phenolic profile determined by HPLC-MS-ESI.

2. Materials and methods

2.1. Raw materials

The red GP from the native Galician grape varieties Caíño Longo (15%), Brancellao (30%), Sousón (15%), and Mencía (40%) was kindly supplied by the Cuñas Davia winery (from the Ribeiro area, Spain) after the harvest in September 2022. It was collected after 10 days of maceration and fermentation, and the action of pectolytic and β-glucanase enzymes to achieve a higher recovery of phenols and sugars in the wine. It was mainly composed of seeds (54%), skin (43%) and stems (3%). After shaker drying (50 °C for 24 h), the material was milled to a particle size ≤1 mm, sealed in bags, and stored at −20 °C until the time of use.

2.2. Chemicals and reagents

All chemicals and reagents used were of analytical grade. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and m-phenylphenol were obtained from Alfa Aesar. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), iron (II) sulfate heptahydrate, and streptomycin sulfate were purchased from Acros Organics. Malt Extract Broth (MEB) was provided by Biolab®, and choline chloride by BLDpharm. Glacial acetic acid, ethanol, iron (III) chloride hexahydrate, L(+)-lactic acid, methanol, potassium persulfate, sodium hydroxide, and sulfuric acid were supplied by Carlo Erba. Dexamethasone, Tris Base, L-ascorbic acid, dimethyl sulfoxide (DMSO), and trichloroacetic acid were obtained from Enzo Life Science, Fisher BioReagents, Fisher Chemical, GRISP Research Solutions, and Labkem, respectively. Ketoconazole, malt agar, sterile saline solution, and Tween 80 were obtained from Frilabo. Cell culture reagents, including Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM:F12), heat-inactivated fetal bovine serum (FBS), insulin-transferrin-selenium solution (ITS solution II), non-essential amino acids solution (NEAA), penicillin-streptomycin solution, phosphate-buffered saline without calcium and magnesium (DPBS), and trypsin-EDTA, were supplied by PAN-Biotech GmbH. Ampicillin, methicillin, and p-iodonitrotetrazolium chloride (INT) were obtained from PanReac AppliChem. Citric acid anhydrous, sodium carbonate anhydrous, and hydrochloric acid were acquired from Scharlau. Neocuproine hydrochloride monohydrate was purchased from TCI Chemicals. (+)-Catechin and gallic acid were obtained from Sigma-Aldrich. 2,4,6-Tri-(2-pyridyl)-1,3,5-triazine (TPTZ), 4,6-dihydroxy-2-mercaptopyrimidine (thiobarbituric acid), resazurin, and vanillin were provided by Thermo Scientific. Ammonium acetate, copper (II) chloride dihydrate, Folin–Ciocalteu reagent, and sodium acetate were obtained from VWR Chemicals.

2.3. Experimental methods

2.3.1. Deep eutectic solvents (DES) synthesis

Drawing on earlier studies of phenolic recovery from plant materials [26–29], three candidate DES formulations were selected and formulated using the heating-stirring method. All the DES components were weighed in screw cap bottles according to the established proportions in Table 1. DES 1 was prepared both without water and with 30% water for the study of its extraction effect, while DES 3 required the addition of 30% water to facilitate mixability of the components. Once formulated, they were mixed on stirring and temperature-controlled plates (maximum 80 °C) until a homogeneous and transparent mixture was acquired. Finally, the DES were stored at room temperature until use.

2.3.2. Evaluation of DES extraction efficiency

For the identification of the optimal DES to recover phenolic compounds from red GP, a conventional solid-liquid extraction was utilized. First, the different DES were mixed with the GP to obtain a liquid-solid

Table 1
List of DES used in this work and their characteristics.

Abbreviations	Reagent 1 (HBA)	Reagent 2 (HBD)	Reagent 3	Molar ratio	Price (€/Kg)[a,b]
DES1	Sodium acetate	Lactic acid	-	(1:3)	14.10
DES1 +30%	Sodium acetate	Lactic acid	30% water	(1:3 + 30% water, w/w)	9.88
DES2	Choline chloride	Acetic acid	Water	(1:1:10)	25.10
DES3	Choline chloride	Citric acid	30% water	(2:1 + 30% water, w/w)	31.22

[a] For the calculation of the DES synthesis price, the data available on Sigma Aldrich's website (September 2023) were used.
[b] The price of water was not considered in the calculations.

ratio (LSR) of 15 mL/g (considering the moisture content of GP) and then placed in an orbital shaker with temperature control (Incu-Shake series from SciQuip Ltd, UK) at 50 °C for 2 h. The selected conditions were established based on previous literature [30] as well as the prior experience of research group (data not shown). After extraction, the solid residue was separated from the liquid extract by vacuum filtration, and the recovered liquid fraction was stored at −20 °C until further analysis.

Additionally, an extraction using an ethanol-aqueous solution (50% v/v) was performed under the same conditions as specified above, in order to evaluate the extraction efficiency of DES compared with a conventional solvent. The 50% ethanol concentration was selected based on previous literature [31,32], as hydroethanolic mixtures have been widely reported as suitable solvents for the recovery of phenolic and other bioactive compounds from plant matrices.

2.3.3. Optimization of extraction conditions by experimental design

Once the most efficient DES for the extraction of GP polyphenols was selected, the influence of different factors on the properties of the extracts and the determination of the optimal conditions were investigated. The independent variables analyzed included LSR (8, 14 and 20 mL/g), time (30, 165 and 300 min), and temperature (40, 50 and 60 °C), which were set grounded on prior experiments (data not shown). A Box-Behnken design of 15 experiments with three central points, in combination with response surface methodology (RSM), was used to assay the relationship amongst the aforementioned independent variables (coded as −1, 0, 1) and the response variables (TPC, CTC, DPPH, ABTS, FRAP and CUPRAC), which were fitted to the following second-order polynomial model equation (Eq. (1)):

$$y_j = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i<j} \sum_{j=1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad \text{Eq. 1}$$

where y_i reflects the dependent variables (j = 1-6), β_0, β_i, β_{ij}, and β_{ii} correspond to the regression coefficients obtained from the experimental data using the least-squares method, and x_i and x_j reflect the independent variables (dimensionless and normalized), that can oscillate between −1 and 1. A regression analysis in Microsoft Excel's Data Analysis Add-In (USA) was used to fit the experimental results. The adequacy of the model was determined by the lack of fit, the coefficient of determination (R^2), and the F-test value calculated by the analysis of variance.

With the aim of achieving the maximum values of the response variables simultaneously the multi-response surface optimization was used. The extracts were selected based on their phenolic and condensed tannin content, as well as their antioxidant performance evaluated through the DPPH, ABTS, FRAP and CUPRAC methods. The optimal extraction conditions were determined using the desirability function available in Statgraphics Centurion XVI software (version 16.1.11). For model validation, experiments under the optimum extraction conditions were performed in three replicated, and the experimental results were contrast to the predicted values.

2.4. Analytical methods

2.4.1. Raw material chemical characterization

The chemical characterization of the GP was performed complying with the procedures for the determination of extractives, moisture, ash and carbohydrate content proposed by NREL [33–36]. For the determination of extractives, two sequential extractions were assayed using water and ethanol (96%, v/v) to eliminate water and fat-soluble components, separately. Each resulting extract was assayed by quantitative post-hydrolysis [37] and subsequently, an aliquot of both untreated and post-hydrolysis extracts were analyzed via high performance liquid chromatography-HPLC (Agilent 1260 series, Palo Alto, CA, USA) for the quantification of solubilized compounds, which were added to the carbohydrate content of the extractives-free solid. The following columns and operating conditions were used for the HPLC analysis: (i) Rezex ROA-Organic acid H^+ column (Phenomenex) at 60 °C, mobile phase of 3 mM H_2SO_4 at 0.60 mL/min of flow, using a refractive index detector at 35 °C; and (ii) Aminex HPX-87P column (Bio-Rad) at 80 °C, mobile phase of ultrapure water at 0.40 mL/min of flow rate, refractive index detector at 35 °C. After quantitative acid hydrolysis (used for the determination of carbohydrate content of extractive-free solid), the spent solid was weighed and labeled as Klason lignin, whilst the liquid was injected in the HPLC as described before for the quantitation of monomeric sugars, galacturonic acid and acetic acid. Additionally, nitrogen was measured by elemental analysis, calculating the protein content by applying the factor 6.25 [38]. The assays were determined in three replicates.

2.4.2. Characterization of extraction liquors

The extracts resulting from all treatments of GP with DES were analyzed by spectrophotometric methods for determining the content in phenolics and condensed tannins and for the evaluation of antioxidant activity. In all cases, calibration curves were performed, and all samples were analyzed in three replicates.

2.4.2.1. Total phenolic content (TPC) and condensed tannin content (CTC) assays. For TPC analysis, the Folin-Ciocalteu method was used, as proposed by Singleton & Rossi (1965) [39]. Briefly, 150 μL of extract were blended with 1250 μL of Folin-Ciocalteu reagent diluted 1:10 (v/v) and, subsequently, 1000 μL of Na_2CO_3 7.50% (w/v) was added. After a 1 h incubation in the dark, the samples' absorbance was quantified at 760 nm. Gallic acid (20–200 μg/mL) was employed for the calibration curve, expressing the results as mg of gallic acid equivalents (GAE)/g of GP.

On the other hand, the tannin content was established complying with the method proposed by Broadhurst & Jones (1975) [40] with some modifications. For this, 450 μL of 4% (w/v) vanillin dissolved in methanol and 450 μL of 32% (v/v) H_2SO_4 were added to 75 μL of GP extract. Finally, the absorbance of the samples was quantified at 500 nm after a 15-min incubation. Tannin content was determined using catechin (100–375 μg/mL) for the calibration curve, expressing the content as mg of catechin equivalents (CE)/g of GP.

2.4.2.2. Antioxidant capacity assays. The antioxidant activity of the GP extracts was evaluated by DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays, following the methodologies described by Gullón et al. [41] and copper reducing antioxidant power capacity (CUPRAC) assay, according to the method proposed by Özyürek et al. [42]. These four colorimetric methods are based on the color change of the solution

under study, caused by the reduction of DPPH or ABTS radicals and ferric or cupric complexes by the antioxidants present in the extracts. Trolox was used as a standard for all antioxidant methods (5–50 μg/mL for DPPH assay, 50–450 μg/mL for ABTS assay, 25–225 μg/mL for FRAP assay and 5–80 μg/mL for CUPRAC assay) expressing the results as mg of Trolox equivalents (TE)/g of GP.

2.4.3. Bioactive properties assessment of optimized GP extract

The GP extract resulting from the optimum conditions predicted by the experimental design model was freeze-dried (LyoQuest-55 freeze-dryer, Telstar Technologies, Barcelona, Spain) and stored at −20 °C in a hermetically sealed vial for subsequent bioactivity analysis. Moreover, the DES (choline chloride: acetic acid: water, molar ratio 1:1:10) underwent the same preparation process as GP extract and was utilized as a negative control.

The lyophilized crude GP extract contained approximately 85% DES and 15% GP-derived bioactive compounds. Therefore, the results of the bioactivity assays were expressed considering only the GP-derived fraction (i.e., excluding the DES contribution) and reported as the mean of three replicates.

2.4.3.1. Thiobarbituric acid reactive substances (TBARS) formation inhibition. The capacity of freeze-dried extracts to inhibit lipid peroxidation was assayed via the TBARS formation inhibition assay employing the method priorly conveyed by Mandim et al. [43]. Briefly, 200 μL of extracts solutions (375.00–0.73 μg/mL) were blended with 100 μL of ascorbic acid (0.10 mM), 100 μL of iron sulfate (10 μM), and 100 μL of porcine brain cell homogenates (1:2, w/v), and the blend was incubated for 1 h at 37 °C. Subsequently, 500 μL of trichloroacetic acid (28%, w/v) and 380 μL of thiobarbituric acid (2%, w/v) were added and subjected to incubation at 80 °C for 20 min. Finally, a centrifugation was performed at 3000xg for 10 min, and the supernatant was quantified at 532 nm. DES was used as a negative control, and Trolox was tested as a positive control. The results obtained were reported in IC_{50} values (μg/mL of extract required to inhibit the lipid peroxidation by 50%) applying the following equation:

$$EC_{50} = \frac{C_{>50} - C_{<50}}{I_{>50} - I_{<50}} \cdot (50 - I_{<50}) + C_{<50} \quad \text{Eq. 2}$$

In the formula, $C_{>50}$ and $C_{<50}$ represent the concentration of the GP extract that produce lipid oxidation values above and below 50%, respectively. Similarly, $I_{>50}$ and $I_{<50}$ correspond to the specific inhibition percentages higher and lower than 50%, respectively. The inhibition percentages were calculated according to the following equation:

$$I(\%) = \frac{A_B - A_S}{A_B} \cdot 100 \quad \text{Eq. 3}$$

Where A_B denotes the absorbance of de blank at 532 nm (containing all reagents except GP extract), whereas A_S corresponds to the absorbance of the sample measured at 532 nm.

2.4.3.2. Cytocompatibility. The cytocompatibility of the lyophilized extracts was assessed using gastrointestinal and hepatic cells (Caco-2 and AML12, respectively). The tested cell lines were cultured in T75 culture flasks under controlled conditions, namely 37 °C, 5% CO_2, and a humidified atmosphere (SAFEGROW PRO CO_2 Incubator Bioair, Siziano, Italy). Caco-2 cells were habitually preserved in DMEM culture media supplemented with 10% heat-inactivated FBS, 1% penicillin and streptomycin solution, and 1% of non-essential amino acids (NEAA), AML-12 were maintained in DMEM:F12 medium supplemented with 10% FBS, 1% penicillin and streptomycin, insulin (10 μg/mL), transferrin (5.50 μg/mL), selenium (0.005 μg/mL), and dexamethasone (0.04 μg/mL). Caco-2 and AML12 cell lines tested were purchased from Cytion (catalog numbers 300137 and 300643, respectively) and used between passages 14-16 and 8–10, respectively.

Cell culture medium was substituted every 2 to 3 days, and when the cells fulfilled 80-90% confluence, they were separated using 0.25% Trypsin-EDTA. The cytocompatibility was assayed according to EN ISO 10993-5:2009 [44]. Briefly, the cells were seeded in 96-well plates at a density of 10,000 cells/well for Caco-2 and 15,000 cells/well for AML12, and after 24 h of incubation, the cells were exposed to various concentrations of the GP extract dissolved and successively diluted in DPBS (concentrations between 7.50 and 0.12 mg/mL, 1:2 successive dilutions) for another 24 h. The cytocompatibility of the samples was assayed using the resazurin reduction method. For this, the culture medium was carefully separated, and the adherent cells washed twice with 100 μL of DPBS before adding 100 μL/well of resazurin (44 μM, in the corresponding culture medium). After resazurin metabolization, the absorbance was quantified at 570 and 600 nm against a resazurin blank using a microplate reader (SpectraMax® iD3, Molecular Devices, LLC, San Jose, CA). DPBS and DES were used as negative controls, while the positive control was 10% DMSO. The metabolic activity of cells (expressed as percentage of viable cells relative to negative control) was determined according to the following equation:

$$\%\ Metabolic\ activity = \frac{AS_{570} - AS_{600}}{AC_{570} - AC_{600}} \cdot 100 \quad \text{Eq. 4}$$

Where AS_{570} represents the absorbance of the treated cell sample at 570 nm, corresponding to the maximum absorbance wavelength of the reduced form of resazurin (resorufin), while A_{600} corresponds to the absorbance of the same sample at 600 nm, associated with wavelength of maximum absorbance of non-reduced resazurin. Likewise, AC_{570} and AC_{600} denote the absorbance values of the negative control (untreated cells) recorded at 570 and 600 nm, respectively.

2.4.3.3. Antibacterial activity. For the assessment of the antibacterial activity, the optimal extracts were tested against bacterial strains responsible for food contamination and several clinical pathogens: *Enterobacter cloacae* (ATCC 49741), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* subsp (ATCC 13076), *Escherichia coli* (ATCC 25922), *Yersinia enterocolitica* (ATCC 8610), *Klebsiella pneumoniae* (VRI17214), *Morganella morganii* (VRU14272) and *Proteus mirabilis* (VRU17684) as Gram-negative bacteria; and *Listeria monocytogenes* (ATCC 19111), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA, VRI17654) and *Enterococcus faecalis* (VRU14041) as Gram-positive bacteria. All the above food contaminants were supplied by Frilabo (Porto, Portugal) and clinical bacteria were isolates from hospitalized patients at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). During the 24 h prior to the analysis, the bacteria were incubated in an appropriate fresh culture medium at 37 °C to maintain the exponential growth phase. Subsequently, the bacterial inoculum was prepared at $1.5 \cdot 10^6$ CFU/mL.

To determine the extracts antibacterial activity, the microdilution method combined to the INT (*p*-iodonitrotetrazolium chloride) colorimetric assay, as priorly stated by Pires et al. [45], was employed. In brief, 90 μL of each extract's concentration (between 66.70 and 0.52 mg/mL of dry GP extract) was blended with 10 μL of bacterial suspension (guaranteeing the presence of 1.50×10^6 CFU/mL) in a 96-well microplate and kept at 37 °C for 24 h. Thereafter, 40 μL of INT dye (0.2 mg/mL) was added, incubating again the microplates at 37 °C for 30 min. INT changes color from yellow to pink when microorganisms were viable; therefore, the minimum inhibitory concentration-MIC (expressed as mg/mL) was defined as the lowest extract concentration that did not exhibit a visible color change. Ampicillin (10 mg/mL), streptomycin (1 mg/mL), methicillin (1 mg/mL), imipenem (1 mg/mL), and vancomycin (1 mg/mL) were used as positive controls. The minimum bactericidal concentration (MB, expressed as mg/mL) was defined as the lowest extract concentration necessary to kill the microorganisms.

For this purpose, 10 μL from wells showing no color change were plated onto 7% sheep blood agar and incubated at 37 °C for 24 h.

2.4.4. Tentative identification of phenolic profile from grape pomace by HPLC-DAD-ESI/MS

The lyophilized liquid fraction recovered after DES extraction under the optimized conditions was mixed with distilled water to reach a final concentration of 10 g/L, filtrated through a 0.22 μm nylon filter, and injected into the HPLC system using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) equipped with a diode array detector (DAD) coupled to an electrospray ionization mass detector (ESI/MS), according to the method previously described by Mandim et al. [46]. A Spherisorb S3 ODS-2C18 column (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA), kept at 35 °C, was used. The mobile phases consisted of 0.1% formic acid in water (A) and 100% acetonitrile (B) under isocratic elution. Likewise, 280 and 370 nm were selected for DAD detection, corresponding to the identification of protocatechuic acid and flavonoids. For MS detection in negative ionization mode, a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source was used, with nitrogen as the sheath gas (50 psi), a spray voltage of 5 kV, a source temperature of 325 °C, a capillary voltage of −20 V, and a collision energy of 35 arbitrary units. Data acquisition was performed using the Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The tentative identification was carried out by comparing of the retention times (Rt), wavelength of maximum absorption (λ_{max}), pseudomolecular ion ($[M-H]^-$), and fragmentation patterns (MS^2) obtained in literature. Authentic standards of protocatechuic acid, (+)-catechin, (−)-epicatechin, gallic acid and malvidin-3-O-glucoside (Sigma-Aldrich/Thermo, ≥95% purity) were analyzed under the same chromatographic conditions and used to confirm peak identity and to support the tentative assignment of related compounds.

2.5. Statistical analysis

Data obtained from the different DES formulations were statistically analyzed using R software (version 4.2.1). Significant differences among DES formulations were determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with statistical significance set at $p < 0.05$.

3. Results and discussion

3.1. Grape pomace chemical composition

Table 2 presents the chemical characterization of the raw material, determined in g/100 g of GP on a dry basis ±standard deviation. As shown, the majority of GP constituents are ethanolic and aqueous extracts, which together account for more than 60% of the total composition. These fractions are particularly interesting owing to their high content of bioactive compounds, like polyphenols, oils, or soluble sugars. The aqueous extract content is analogous to that observed by Ioannidou et al. [47] for the pomace from the *Vitis vinifera* L. *cv. Grenache Rouge* grape variety, while the ethanolic extracts found in the current study (37.70%) were significantly higher than those determined by Ioannidou et al. [47] (2.90%) and other authors [48,49] using other organic solvents, such as heptane (7.50%) and dichloromethane (5.50%). The next most abundant component is Klason lignin (24.02%), whose levels are close to those reported in the literature for other grape varieties' pomace [50–52]. On the other hand, the total polysaccharide content was 11.74% (calculated as the sum of glucan, mannan, xylan, fructan and galactan), which is considerably lower than that published in other studies, like by Filippi et al. [53] (34.21%) for GP of the Agiorgitiko Nemeas variety or Coelho et al. [53] (26.50%) for Pinot Noir GP.This difference may be explained by the maceration process, since the bagasse used for this work was subjected to enzymatic treatment to enhance the solubilization of sugars and anthocyanins. Regarding protein content, Jin et al., 2019 [54] reported values of about 11.50–13.00% for four samples of red GP, which are comparable to the value reached in this study (11.66%). It is important to mention that the variation of this factor may be due, among other reasons, to the overestimation produced by the mixing of yeast biomass with pomace during the alcoholic fermentation stage. Finally, to a lesser extent, other compounds such as galacturonic acid, ash or acetyl groups were also found.

Table 2
Red GP composition. Content expressed in g per 100 g of raw material on a dry basis ±standard deviation.

Component	g/100 g GP
Glucan	4.05 ± 1.20
Mannan	2.41 ± 1.29
Xylan	2.02 ± 1.09
Fructan	1.30 ± 0.77
Galactan	1.96 ± 1.06
Acetyl groups	1.17 ± 0.17
Klason lignin	24.02 ± 0.41
Galacturonic acid	5.86 ± 2.72
Ashes	4.65 ± 0.01
Protein	11.66 ± 0.98
Aqueous extracts	22.58 ± 6.23
Ethanol extracts	37.70 ± 6.05

3.2. Screening of DES for the polyphenol extraction of GP

With the aim of identifying the most suitable solvent for the recovery of phenolic compounds from GP, three different DES were tested, as shown in Table 1. Since the DES differed in composition, they were compared as complete solvent systems rather than as individual HBA/HBD components. In addition, 50% ethanol (v/v) was employed as a reference solvent to evaluate the performance of the DES formulations in comparison with a conventional organic solvent. The extraction conditions were fixed at a LSR of 15 mL/g, a temperature of 50 °C and an extraction time of 2 h.

Fig. 1 shows the content of total phenols and condensed tannins, accompanied by the antioxidant activity (measured by DPPH, ABTS and FRAP assays) of the extracts obtained after GP extraction with different solvents. In terms of TPC, the results ranged between 47.00 and 110.62 mg GAE/g GP. These results are analogous with those stated by Marinaccio et al. [55] on Montepulciano GP. The solvent enabling the solubilization of more phenolics (measured by TPC) was the one made from chorine chloride and citric acid (DES 3), followed by the DES 2 (choline chloride: acetic acid: water) and 50% ethanol extracts.

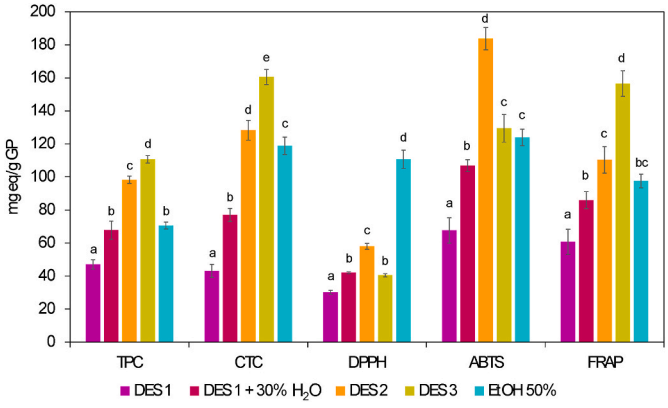

Fig. 1. Results regarding TPC (mg GAE/g GP), CTC (mg CE/g GP), DPPH (mg TE/g GP), ABTS (mg TE/g GP) and FRAP (mg TE/g GP) after GP extraction with the different DES and 50% EtOH at 50 °C for 2 h. Different letters indicate statistically significant differences at the 95% confidence level.

Conversely, DES 1 with 30% water and DES 1 were more inefficient for the extraction of phenols in GP. An analogous trend was noticed for condensed tannins, with the maximum value (160.53 mg CE/g GP) obtained using DES 3. Radulescu et al. [56], who studied the ethanolic extraction assisted by ultrasonication of a Romanian grape variety, and Deaconu et al. [57], who investigated the use of hydroethanolic solution in combination with a conventional treatment to extract Pinot Noir GP, reported condensed tannin recoveries of approximately 34 mg CE/g GP, substantially lower than those reached in the current study.

Concerning antioxidant activity, in general, the solvents followed a similar pattern to that observed for TPC and CTC, except for the DPPH assay, where the peak values of antioxidant activity were reached using EtOH 50%, followed by DES 2, while the lowest values were observed for DES 3 and DES 1. The highest antioxidant activity was assessed by the ABTS assay, reaching 183.73 mg TE/g GP for the extract obtained with DES 2. Comparable results were reported by Ben-Aziz et al. [58] for GP skin extracts of the Arinarnoa variety, while considerably lower ABTS values were obtained for Tempranillo GP extracted using ultrasound and microwave-assisted techniques [59].

Overall, these results indicate that DES can improve the obtaining of phenolic compounds with antioxidant capacity compared with conventional hydroalcoholic solutions (ethanol 50% v/v). A comparable observation was reported by Del-Castillo-Llamosas et al. [60] during the valorization of avocado seeds using choline chloride: glycerol DES. This phenomenon can be attributed to the ability of DES to establish extensive hydrogen-bonding networks with phenolic compounds, thereby enhancing their solubility and simultaneously protecting them from oxidative degradation [61,62]. Furthermore, it has also been shown that the presence of water in DES formulations not only facilitates their handling but also favors the extraction of target compounds. This effect may be related to changes in the physicochemical properties of DES upon water incorporation, particularly the increase in polarity and reduction viscosity, which collectively improve mass transfer kinetics and solute diffusion [63].

Based on these results, DES 3 and DES 2 were selected as the most suitable solvents to obtain the richest phenolic and tannins extracts, possessing the highest antioxidant activities. However, considering that the formulation of DES entails an economic cost derived from the nature of the compounds used, the price of the DES synthesis has been taken into account. According to the results shown in Table 1, the formulation of DES 3 entails a cost of 31.23 €/kg while that of DES 2 is 25.11 €/kg (about 20% less). Moreover, due to its higher water content, DES 2 exhibits a substantially lower viscosity than DES 3, as previously explained, which enhances mass transfer processes. On the other hand, the increased polarity of this system promotes the dissolution of moderate polar compounds such as polyphenols [61,63]. Accordingly, DES 2 was selected for the subsequent optimization of the extraction variables.

3.3. Optimization of GP extraction conditions

Three extraction conditions, namely LSR, extraction time and temperature, were explored and optimized to maximize the recovery of phenols (TPC) and tannins (CTC), as well as their antioxidant capacity (DPPH, ABTS, FRAP and CUPRAC), using a Box-Behnken design in combination with response surface methodology (RSM). The independent experimental parameters and the corresponding results for the dependent variables of 15 runs are posed in Table 3. On the other hand, Table 4 displays the regression coefficients belonging to each model, assuming a second-degree polynomial, as well as the statistical significance according to Student's t-test and Fisher's F test and correlation parameters (R^2). Since the significance level of the models was above 90%, the R^2 values were close to 0.9, and the F values were generally high, it can be presumed that the models showed appropriate adequacy and a satisfactory fit to the experimental data.

In addition, the significance of independent parameters interaction individually or in combination was evaluated. According to the significant regression coefficients (at a confidence level above 90%), the experimental results were adjusted to six quadratic regression equations for the TPC (y_1), CTC (y_2), DPPH (y_3), ABTS (y_4), FRAP (y_5) and CUPRAC (y_6):

$$\mathbf{TPC} = 93.72 - 10.67x_1 - 5.82x_3 + 7.76x_1x_3 + 7.92x_2x_2 \quad \text{Eq. 5}$$

$$\mathbf{CTC} = 109.22 - 18.33x_1 + 9.65x_3 - 9.16x_1x_2 + 7.05x_1x_3 + 9.87x_2x_3 - 8.53x_1x_1 \quad \text{Eq. 6}$$

$$\mathbf{DPPH} = 156.37 + 17.11x_1 + 26.06x_2 + 14.76x_3 + 30.96x_1x_2 + 29.07x_1x_3 - 30.06x_1x_1 \quad \text{Eq. 7}$$

$$\mathbf{ABTS} = 156.78 - 9.06x_1 + 21.98x_2 + 5.59x_3 - 8.01x_1x_2 - 14.15x_1x_3 - 12.75x_1x_1 \quad \text{Eq. 8}$$

$$\mathbf{FRAP} = 109.80 + 8.47x_1 + 4.43x_1x_2 + 3.54x_1x_3 - 7.68x_2x_2 - 6.20x_3x_3 \quad \text{Eq. 9}$$

Table 3
Experimental conditions of the Box-Behnken design (expressed as dimensional and dimensionless independent variables) and the corresponding results for the dependent variables y_1 to y_6.

Experiment	Independent Variables			Dependent Variables					
	LSR or x_1 (mL/g)	T or x_2 (°C)	t or x_3 (min)	TPC or y_1 (mg GAE/g GP)	CTC or y_2 (mg CE/g GP)	DPPH or y_3 (mg TE/g GP)	ABTS or y_4 (mg TE/g GP)	FRAP or y_5 (mg TE/g GP)	CUPRAC or y_6 (mg TE/g GP)
1	8 (−1)	40 (−1)	165 (0)	100.06	119.51	118.09	127.64	97.35	279.25
2	8 (−1)	50 (0)	30 (−1)	116.82	116.99	122.11	128.51	93.13	333.09
3	8 (−1)	50 (0)	300 (1)	90.32	116.52	109.23	163.83	93.33	306.62
4	8 (−1)	60 (1)	165 (0)	100.69	128.63	121.17	179.05	88.60	452.20
5	14 (0)	40 (−1)	30 (−1)	118.30	110.20	151.54	114.82	96.80	252.95
6	14 (0)	40 (−1)	300 (1)	94.33	115.43	155.76	132.91	96.80	259.18
7	14 (0)	50 (0)	165 (0)	96.08	108.96	154.63	161.56	107.68	266.84
8	14 (0)	50 (0)	165 (0)	92.64	108.83	161.71	153.27	113.37	249.59
9	14 (0)	50 (0)	165 (0)	92.43	109.86	152.78	155.53	108.35	326.23
10	14 (0)	60 (1)	30 (−1)	100.66	88.87	181.23	170.10	94.29	291.52
11	14 (0)	60 (1)	300 (1)	100.03	133.59	204.54	182.67	95.80	369.87
12	20 (1)	40 (−1)	165 (0)	82.60	98.65	97.05	120.95	104.22	326.80
13	20 (1)	50 (0)	30 (−1)	70.55	68.77	91.51	143.28	104.22	230.31
14	20 (1)	50 (0)	300 (1)	75.07	96.49	194.92	122.02	118.56	363.31
15	20 (1)	60 (1)	165 (0)	94.30	71.13	223.98	140.33	113.18	340.13

Table 4
Regression coefficients and statistical parameters reflecting the correlation and significance of the models.

Coefficient	TPC or y_1	CTC or y_2	DPPH or y_3	ABTS or y_4	FRAP or y_5	CUPRAC or y_6
b_0	93.72[a]	109.22[a]	156.37[a]	156.78[a]	109.80[a]	280.89[a]
b_1	−10.67[a]	−18.33[a]	17.11[b]	−9.06[b]	8.47[a]	−13.83
b_2	0.05	−2.70	26.06[a]	21.98[a]	−0.41	41.94[a]
b_3	−5.82[c]	9.65[a]	14.76[b]	5.59[c]	2.01	23.89[c]
b_1b_2	2.77	−9.16[b]	30.96[a]	−8.01[c]	4.43[b]	−39.90[b]
b_1b_3	7.76[c]	7.05[b]	29.07[a]	−14.15[b]	3.54[c]	39.87[b]
b_2b_3	5.83	9.87[a]	4.77	−1.38	0.38	18.03
b_1b_1	−7.22	−8.53[b]	−30.06[a]	−12.75[b]	−1.29	41.83[b]
b_2b_2	7.92[c]	3.80	13.76	−2.04	−7.68[a]	26.88
b_3b_3	1.69	−0.99	3.13	−4.62	−6.20[b]	−14.39
R-sq	0.887	0.978	0.957	0.962	0.960	0.926
R-sq (adj)	0.685	0.938	0.878	0.892	0.886	0.794
F	4.39	24.74	12.24	13.86	13.15	6.99
Standard error	7.21	4.62	13.87	7.21	3.01	26.48
Significance level (%)	94.111	99.874	99.344	99.507	99.444	97.730

[a] Highly significant coefficients (99% confidence level).
[b] Moderately significant coefficients (95% confidence level).
[c] Significant coefficients (90% confidence level).

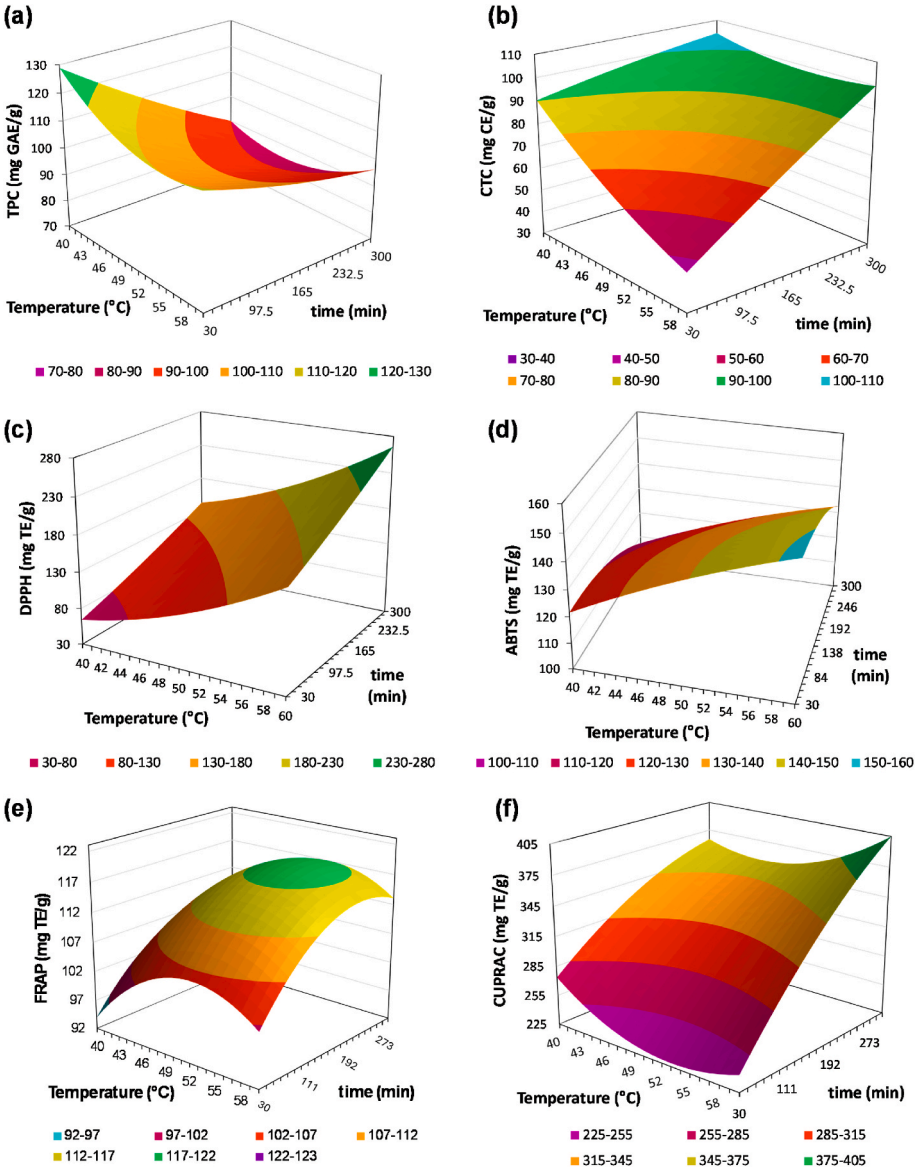

Fig. 2. Response surface of (a) TPC, (b) CTC, (c) DPPH, (d) ABTS, (e) FRAP and (f) CUPRAC as a function of extraction temperature and time. The independent variable set for all dependent parameters was LSR = 20 mL/g, except for TPC, which was LSR = 8 mL/g.

$$\mathbf{CUPRAC} = 280.89 + 41.94x_2 + 23.89x_3 - 39.90x_1x_2 + 39.87x_1x_3 + 41.83x_1x_1$$

Eq. 10

3.3.1. Total phenolic Content (TPC)

The experimental TPC values ranged from 70.55 GAE/g GP (experiment 13; LSR of 20 mL/g, 50 °C, 30 min) to 118.30 mg GAE/g GP (experiment 5; LSR of 14 mL/g, 40 °C, 30 min). These results imply that the extraction process is favored by moderate LSR values and lower treatment temperatures. Likewise, Rashid et al. [64], who applied ultrasound-assisted DES extraction for the valorization of apple bagasse, also reported that the extraction efficiency of TPC decreased at temperatures above 40 °C. In addition, the findings of this research are along the same lines as those obtained by Frontini et al. [65] for rosé GP extracted with choline chloride-based DES in an ultrasonic bath (28 kHz, 45 °C for 30 min). Nonetheless, a considerably lower TPC value (18.65 mg GAE/g) was reported by Marinaccio et al. [66] for Montepulciano d'Abruzzo GP extracted using menthol: thymol eutectic mixture (1:1 M ratio) in an ultrasound-assisted bath (36 °C for 20 min).

Attending to the regression coefficients (Table 4), TPC was extensively influenced by both LSR and extraction time, as well as by the quadratic effect of temperature and the interaction between LSR and extraction time. Similarly, the response surface plot for TPC (Fig. 2a) representing the effect of extraction temperature and time at a set LSR of 8 mL/g ($x_1 = -1$), indicates that temperature did not significantly affect the TPC values, whereas shorter extraction times favored higher TPC recovery. Prolonged extraction times may negatively affect total phenolic recovery due to the progressive degradation of polyphenols that may be caused by oxidation and hydrolysis reactions [67]. In literature, other studies have shown similar behavior. Del-Castillo-Llamosas et al. [60] reported a stronger influence of treatment time than extraction temperature on the recovery of phenolic compounds from avocado seed using a chlorine chloride:glycerol DES (1:1 M ratio). In addition, Zheng et al. [68] found that TPC values decreased after more than 30 min of ultrasound-assisted DES extraction of *Steraria italica*, likely on account of the degradation of phenolic compounds caused by the long extraction times combined with elevated temperatures.

3.3.2. Condensed Tannins Content (CTC)

As can be observed in the model coefficients presented in Table 4, CTC was negatively influenced by the independent variable LSR, its quadratic term, and the interaction between LSR and extraction temperature. In contrast, extraction time, as well as its interactions with LSR and temperature, presented a positive effect on the recovery of condensed tannins. An improvement in condensed tannin extraction with increasing reaction time was also observed in a study on the optimization of DES-based microwave extraction of *Rumex hastatus* [69]. Fig. 2b represents the combined effect of extraction temperature and time on CTC recovery at a fixed LSR of 20 mL/g ($x_1 = 1$). Its interpretation reveals, as above mentioned, that the recovery of condensed tannins increased with increasing residence time, however, temperature exhibited a negative influence on the extraction of these target compounds. Condensed tannins, also known proanthocyanidins, are structurally complex and highly reactive polyphenols whose extraction is strongly influenced by processing conditions, particularly temperature, which at high levels may promote their thermal degradation [70].

The maximum experimental value of CTC (Table 3) was 133.59 mg CE/g GP, obtained in experiment 11, under intermediate LSR condition (14 mL/g) and the highest tested temperature and extraction time (60 °C and 300 min, respectively). The results reported in this study are well above those obtained for pomace from two Romanian red grape varieties (12.25–33.57 mg CE/g GP) subjected to ultrasound-assisted ethanolic extraction [56]. Conversely, they are comparable to those obtained by Cardona-Jimenez et al. [71].

3.3.3. Antioxidant capacity

The impact of extraction parameters on the antioxidant capacity of GP extracts was studied by four complementary assays. The DPPH and ABTS assays measure the scavenging of radicals formed in each analysis system by antioxidant compounds found in the extracts. Meanwhile, the FRAP and CUPRAC assays are based on the ability of antioxidants to reduce ferric (Fe^{+3}) to ferrous ion (Fe^{+2}) and cupric (Cu^{+2}) to cuprous ion (Cu^{+}), respectively.

The experimental results of antioxidant activity obtained ranged from 91.51 to 223.98 mg TE/g GP for DPPH, 114.82–182.67 mg TE/g GP for ABTS, 88.60–118.56 mg TE/g GP for FRAP, and 230.31–452.20 mg TE/g GP for CUPRAC. For the DPPH and ABTS assays, the results found in this work were significantly higher than those observed for Tempranillo GP subjected to two extraction intensification technologies: ultrasound (44.08–51.04 mg TE/g GP and 55.44–73.97 mg TE/g GP, respectively) and microwaves (42.48–74.59 mg TE/g GP and 53.29–62.09 mg TE/g GP, respectively) [59]. In the same way, when antioxidant activity was assayed by the FRAP methodology, Moutinho et al. [72], who optimized the extraction of polyphenols from "Touriga Nacional" and "Sousão" GP, also reported poorer results than those observed in our work. Finally, the CUPRAC values acquired in this work were superior to those observed in for different Montepulciano grape skins extracts [73].

According to the regression coefficients depicted in Table 4, the linear terms of the extraction time and temperature displayed a significant positive impact on the results of DPPH, ABTS, and CUPRAC. On the other hand, DPPH and FRAP were positively influenced by LSR, whereas ABTS was negatively affected by it. Furthermore, the antioxidant activity was prompted by the interaction between LSR and treatment temperature and time. Regarding the quadratic effects, the quadratic term of LSR negatively affected DPPH and ABTS but positively influenced CUPRAC, while the quadratic terms of temperature and extraction time negatively affected FRAP values.

Fig. 2c-f shows the response surfaces for antioxidant activity quantified by DPPH, ABTS, FRAP, and CUPRAC, respectively, as a function of extraction temperature and time with LSR value set at the maximum assayed ($x_1 = 1$). In general, increasing the extraction temperature enhanced the antioxidant capacity of GP extracts. Similar trends were reported by Souadia et al., [74] during the optimization of ultrasound-assisted extraction with methanol from *Thymus algeriensis* Boiss, & Reut., where higher temperatures increased the DPPH values. This behavior can be attributed to compositional changes in the extract and the formation of thermally induced compounds with enhanced antioxidant properties [75]. However, in the case of the FRAP assay, antioxidant activity increased up to an optimal value, after which FRAP values decreased. This can be justified by the significant negative contribution of the quadratic term of temperature in the FRAP model equation. Benarfa et al. [76] reported similar findings when they extracted the flowers of *Deverra scoparia* Coss. & Durieu with methanol in an ultrasonic bath. This trend was also observed for the residence time in the results of the current work. On another side, treatment time led to a boost in antioxidant activity, except in the ABTS assay. An analogous pattern was confirmed by Gullón et al. [77] when investigating the antioxidant activity of purple corn cobs aqueous extracts using the ABTS method.

3.4. Optimization and validation of the experimental model

In order to assay the experimental conditions that jointly maximize the results of the independent variables (TPC, CTC, DPPH, ABTS, FRAP, and CUPRAC), a multi-response optimization of the dependent variables (LSR, temperature, and time of extraction) was conducted. To this end, the response values for each variable were transformed using a desirability function by means of Statgraphics Centurion XVI software. In turn, this function was used to decipher the combination of extraction variables assessed capable of providing simultaneous maximum

responses in TPC, CTC, and antioxidant activity estimated by DPPH, ABTS, FRAP, and CUPRAC.

Based on the model predictions, the optimal experimental conditions were as follows: LSR of 14.86 mL/g, temperature of 60 °C, and time of 275.85 min. To validate the suitability of the model, experiments were carried out (in triplicate) under the predicted optimal conditions, and the resulting extracts were analyzed for all dependent variables studied. The experimental results, along with the corresponding predicted values, are listed in Table 5. As can be seen, there is a strong agreement between the most predicted and experimental values, confirming the adequacy of the response surface methodology for quantitative prediction. These findings further demonstrate the suitability of the Box–Behnken design for accurate and reliable prediction of total phenolic content, condensed tannin content, and antioxidant activity in red GP extracts obtained using DES.

Nevertheless, the optimized extraction time of 275.85 min highlights the importance of balancing phenolic recovery with process efficiency, particularly when considering the scalability of DES-based extraction systems. In this regard, process intensification strategies, such as ultrasound- or microwave-assisted extraction, could contribute to reducing processing time while maintaining high extraction performance [78].

Under the optimal extraction conditions, the predicted values were 102.52 mg GAE/g GP for TPC and 122.39 mg CE/g GP for CTC, and antioxidant activities measured by the DPPH, ABTS, FRAP and CUPRAC assays were 223.98 mg TE/g GP, 172.72 mg TE/g GP, 101.72 mg TE/g GP and 372.20 mg TE/g GP, respectively. Overall, these results closely matched those obtained when the optimum conditions were determined for each response variable individually (128.92 mg GAE/g GP, 139.79 mg CE/g GP, 261.93 mg TE/g GP, 183.88 mg TE/g GP, 118.82 mg TE/g GP and 445.48 mg TE/g GP).

3.5. Evaluation of bioactivities of optimized GP extract

3.5.1. Antioxidant activity

The antioxidant capacity of the optimal GP extract was evaluated using the TBARS cell-based method, which determines the concentration needed to reach 50% inhibition of lipid peroxidation. GP extract displayed an IC_{50} of 50.55 μg/mL, whereas no activity was observed with DES. Given that higher antioxidant activity corresponds to lower IC_{50} values, the GP extract displayed approximately 5.50-fold lower bioactivity than Trolox ($IC_{50} = 9.10 \pm 0.30$ μg/mL), commonly used as the positive control [43]. The lower TBARS antioxidant activity of the GP extract compared to Trolox may be attributed to their complex composition. Plant extracts contain heterogeneous mixtures of compounds with varying reactivity, as well as potential interfering substances that may affect the assay response differently. In contrast, Trolox is a pure reference antioxidant with well-defined and highly efficient activity [79]. Nonetheless, Ueda et al. [80] reported TBARS assay values ($IC_{50} = 23$ μg/mL) comparable to those obtained in this work for GP extracts obtained by ultrasound-assisted extraction. Likewise, medicinal plants extracted with pulsed electric field, such as *Asteriscus graveolens* and *Ruta chalepensis*, showed antioxidant activity values close to those monitored for GP in this work [81].

Table 5
Predicted and experimental values under optimal conditions of simultaneous optimization of TPC, CTC, DPPH, ABTS, FRAP and CUPRAC (LSR = 14.86 mL/g, extraction temperature = 60 °C, and extraction time = 275.85 min).

Response	Predicted value	Experimental value[a]
TPC (mg GAE/g GP)	102.52	113.71 ± 1.16
CTC (mg CE/g GP)	122.39	125.37 ± 2.17
DPPH (mg TE/g GP)	223.98	165.25 ± 4.17
ABTS (mg TE/g GP)	172.72	171.48 ± 1.13
FRAP (mg TE/g GP)	101.72	102.88 ± 2.26
CUPRAC (mg TE/g GP)	372.20	367.79 ± 12.59

[a] Average value (n = 3) ± standard deviation (SD) from three extraction replicates.

3.5.2. Cytocompatibility

The results of the gastrointestinal and hepatic toxicity studies of the optimal GP extract and DES are presented in Fig. 3.

No cytotoxic effects were detected for any of the assayed concentrations in the DES, confirming its absence of toxicity in both cell lines tested. In contrast, the GP extract exhibited a concentration-dependent reduction in metabolic activity in both cell lines. Particularly, AML-12 hepatocytes showed a higher sensitivity compared to Caco-2 intestinal epithelial cells. Cytotoxic effects in AML12 cells were observed at concentrations above 12 μg/mL, whereas Caco-2 cells were viable up to 23 μg/mL. This result indicates a lower tolerance of hepatic cells to the bioactive compounds present in the extract.

The different sensitivities detected between both cell lines may be related to their distinct physiological characteristics. Caco-2 cells, due to their intestinal epithelial phenotype, behave as a selective barrier and therefore reveal low permeability to certain compounds [82]. In contrast, AML 12 cells, derived from mouse hepatocytes, exhibit higher metabolic activity due to their role in detoxification processes. These functions can increase cellular exposure and, consequently, susceptibility to potentially active or toxic molecules [83].

The absence of cytotoxicity in the DES across all tested concentrations suggests that the observed biological effects are exclusively associated with the compounds recovered from GP rather than the solvent system itself. These results may indicate potential safety concerns, particularly for hepatic cells. Nevertheless, further studies, including broader dose–response assays, additional cell models, and *in vivo* evaluations, are required to better establish the toxicological profile and safe use of the extract.

Similar findings have been reported in the literature. In the case in point, Valenzuela-Bustamente et al., [84] reported cytotoxic effects of Carménère GP extracts in Caco-2 cells at concentrations ≥100 μg/mL. Also, Ferreira-Santos et al. [10] reported reduced viability in Caco-2 cells exposed to GP extracts obtained from Croatina GP after ohmic heating treatment. Conversely, previous studies have reported no hepatotoxicity effects of GP extracts, as described by Peixoto et al. [85] or Gómez-Mejía et al. [86].

3.5.3. Antibacterial activity

In this work, the antibacterial potential of the GP extract obtained under optimal conditions and eutectic solvent was assessed against a panel of Gram-negative and Gram-positive foodborne and clinical bacterial strains. The outcomes, summarized in Table 6, demonstrate that the DES alone exhibits intrinsic antibacterial activity, particularly against *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*, with MIC values fluctuating between 16.7 and 33.35 mg/mL. However, no bactericidal activity was monitored for any of the tested conditions, as all MBC values were above the highest concentration evaluated (>66.7 mg/mL), indicating a predominantly bacteriostatic effect.

The GP extract into the DES matrix led to a selective enhancement of antibacterial activity depending on the bacterial strain. The most notable activity was detected against *Escherichia coli*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA), for which MIC values of 33.35 mg/mL were obtained. No bactericidal activity was witnessed at any of the assayed extract concentrations. Moreover, for all bacterial strains studied, the GP extract exhibited MIC values substantially higher than those of the standard antibiotics used as positive controls, indicating markedly lower antimicrobial potency. This lower activity may be attributed to the complex and heterogeneous composition of GP extracts, in which bioactive compounds are present at varying concentrations and may exert different mechanisms of action, in contrast to purified antibiotics with specific antimicrobial targets [87].

The antibacterial activity of GP extracts has been previously investigated by other authors, such as Sateriale et al. [88] and Grosu et al.

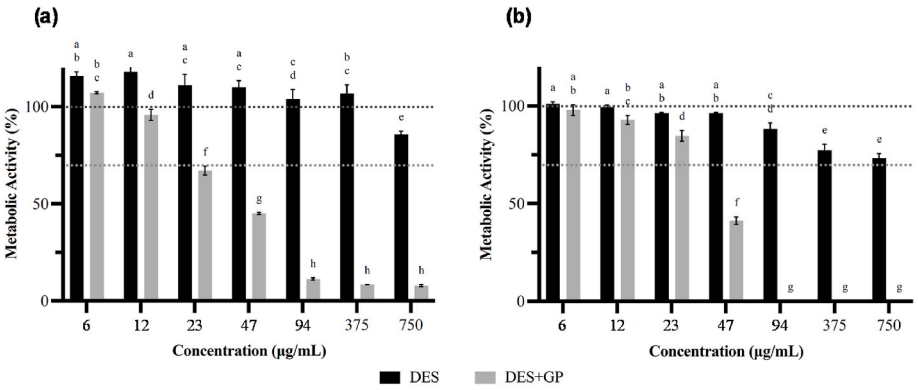

Fig. 3. Metabolic activity of AML12 (a) and Caco-2 (b) cells after 24 h of exposure to the eutectic solvent (DES) and the optimal GP extract (DES + GP). The horizontal lines indicate 70% and 100% cell viability. Different letters indicate significant differences ($p < 0.05$; Tukey's test).

Table 6
Antibacterial activity of the optimal GP extract and DES against food and clinical pathogens.

Antibacterial activity (mg/mL)														
	DES 2		DES 2 + GP		Streptomycin		Methicillin		Ampicillin		Imipenem		Vancomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria														
Enterobacter cloacae	16.7	>66.7	16.7	>66.7	0.007	0.007	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Escherichia coli	66.7	>66.7	33.35	>66.7	0.01	0.01	n.t.	n.t.	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	16.7	>66.7	16.7	>66.7	0.06	0.06	n.t.	n.t.	>10	>10	0.5	1	n.t.	n.t.
Salmonella enterica	66.7	>66.7	66.7	>66.7	0.007	0.007	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Yersinia enterocolitica	66.7	>66.7	66.7	>66.7	0.007	0.007	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Klebsiella pneumoniae	66.7	>66.7	66.7	>66.7	n.t.	n.t.	n.t.	n.t.	>10	>10	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	66.7	>66.7	66.7	>66.7	n.t.	n.t.	n.t.	n.t.	>10	>10	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	66.7	>66.7	66.7	>66.7	n.t.	n.t.	n.t.	n.t.	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Gram-positive bacteria														
Listeria monocytogenes	66.7	>66.7	33.35	>66.7	0.007	0.007	n.t.	n.t.	0.25	0.5	n.t.	n.t.	n.t.	n.t.
Staphylococcus aureus	33.35	>66.7	33.35	>66.7	0.007	0.007	0.007	0.007	0.25	0.5	n.t.	n.t.	n.t.	n.t.
MRSA	66.7	>66.7	33.35	>66.7	n.t.	n.t.	n.t.	n.t.	<0.15	<0.15	n.t.	n.t.	0.25	0.5
Enterococcus faecalis	33.35	>66.7	33.35	>66.7	n.t.	n.t.	n.t.	n.t.	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078

n.t. – not tested; MIC – minimum inhibitory concentration; MBC -minimum bactericidal concentration. Positive controls: streptomycin (1 mg/mL), methicillin (1 mg/mL), ampicillin (10 mg/mL).DES 2 consists of choline chloride, acetic acid, and water in a molar ratio of 1:1:10.

[89], who also observed significant inhibition of *Escherichia coli*, *Listeria monocytogenes* and MRSA growth (MIC = 40, 2.23 and 1.15 mg/mL, respectively) using hydroethanolic extracts of GP from Anglianico and Feteasсă Neagră varieties, respectively. Moreover, Fernández-Pérez et al. [90] demonstrated that the bioactive phenols present in the Graciano GP can be used to combat antibiotic resistance of bacteria such as *Escherichia coli*.

Current research has reported that the antimicrobial effects of GP extracts are primarily related to their rich phenolic composition (particularly flavonoids, flavanols and phenolic acids). These bioactive molecules can compromise the integrity of microbial cell membranes, disturb metabolic and enzymatic systems, and promote oxidative stress, ultimately leading to inhibition of microbial growth or cell death [91].

3.6. Phenolic compounds composition of GP extract

The phytochemical profile of the GP extract was determined by HPLC-DAD-ESI/MS. The results, shown in Table 7, exhibit the presence of 18 phenolic compounds in the extract, including 1 phenolic acid (protocatechuic acid), 2 flavan-3-ols (catechin and epicatechin), 4 condensed tannins (procyanidin dimer isomer I, procyanidin dimer isomer II, procyanidin dimer isomer III, B-type procyanidin pentamer), 8 hydrolysable tannins (trygalloyl-glucoside, digalloyl-HHDP-glucoside, punicalagin, tetragalloyl-glucose, tri-*O*-galloyl-β-D-glucose, pentagalloyl-glucose, *O*-galloyl-castalagin, vitilagin) and 3 anthocyanins (malvidin-3-*O*-glucoside, malvidin-*O*-diglucoside, malvidin-*O*-rutinoside).

These outcomes are consistent with prior statements, as GP is well known to contain anthocyanins such as malvidin-3-*O*-glucoside or diglucosides of malvidin, among others. In addition, flavan-3-ols such as catechin, epicatechin, and their oligomers (procyanidins), as well as galloylated derivatives and phenolic acids such as protocatechuic acid, are commonly detected in GP extracts [92].

The detection of phenolic compounds in GP extracts has also been extensively reported by other authors. For example, Vorobyova et al. [93] employed HPLC-MS and HPLC-DAD to detect epicatechin and catechin, among others, in extracts from GP obtained using a lactic acid and betaine-based DES. Likewise, these compounds, along with several procyanidin dimers, have also been reported in Tannat GP [94]. Anthocyanins, such as malvidin-3-*O*-glucoside have also been detected in samples of Cabernet Sauvignon GP after enzyme extraction [95] and in Sahibi GP extracts [96]. Similarly, Viola et al. [97] informed the presence of protocatechuic acid in GP extracts from Malbec and Bonarda varieties obtained using ethanol and ethyl acetate, respectively.

Evidence from previous studies indicates that some of the phenolic compounds identified in the GP extract are associated with potential biological activities. In particular, phenolic acids such as protocatechuic acid have been reported to exert several pharmacological effects, including antioxidant and anti-inflammatory activity, as well as antifungal, antimutagenic, antidiabetic, antibacterial, and analgesic properties [98,99]. Likewise, flavan-3-ols like catechin and epicatechin are well recognized for their antitumor, anti-inflammatory,

Table 7
Polyphenolic profile of the extract obtained from GP at optimum extraction conditions. Protocatechuic acid, (+)-catechin, (−)-epicatechin, gallic acid and malvidin-3-O-glucoside were classified as confirmed compounds, while the remaining compounds were categorized as tentatively identified.

Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative/Confirmed identification
Phenolic acids				
6.55	281	153	135(100)	Protocatechuic acid
Flavan-3-ols				
8.01	280	289	245(100)	Catechin
10.59	283	289	245(100)	Epicatechin
Condensed tannins (Proanthocyanidins)				
8.82	277	577	289(100)	Procyanidin dimer isomer I
9.16	280	578	289(100)	Procyanidin dimer isomer II
21.01	283	577	289(100)	Procyanidin dimer isomer III
22.76	287	1441	1315(25),1153(25),1151(55),1027(25),865(60),863(100),739(25),635.63(80),577(90),575(80),451(30),407(45),289(70),287(40)	B-type Procyanidin pentamer
Hydrolysable tannins				
9.85	273	635	483(100),271(23)	Trygalloyl-glucoside
14.11	283	785	633(100),615(16),483(37),313(8),301(7)	Digalloyl-HHDP-glucoside
14.89	289	1083	933(100),783(83),765(31),633(28),301(18)	Punicalagin
16.29	278	787	635(100),617(16),483(63),465(11),313(19)	Tetragalloyl-glucose
16.99	277	635	483(100),313(21),169(5)	tri-O-galloyl-β-D-glucose
25.29	281	939	635(65),301(100)	Pentagalloyl-glucose
29.48	283	1085	933(100),783(32),633(13),481(51),301(9)	O-galloyl-castalagin (carnusiin B)
33.31	283	801	765(100),301(10)	Vitilagin
Anthocyanins				
35.05	526	493	331(100)	Malvidin-3-O-glucoside
47.33	532	655	331(100)	Malvidin-O-diglucoside
51.42	532	639	331(100)	Malvidin-O-rutinoside

Rt - retention time; λ_{max} – wavelength of maximum absorption; $[M-H]^-$ – deprotonated ion; MS^2 – fragmentation pattern.

neuroprotective, and cardioprotective effects [100–104]. Anthocyanins, particularly malvidin-3-*O*-glucoside, have also been associated with metabolic benefits, including hypoglycemic and hypolipidemic activities [105,106], whereas punicalagin has shown important potential in the management of neurodegenerative disorders such as Alzheimer's disease [107]. According to previous studies, the anticancer potential of natural phenolic compounds may be mainly linked to their ability to modulate key cellular mechanisms, such as programmed cell death, cellular turnover, and telomerase regulation, ultimately limiting cancer cell growth [108]. In addition, the cardioprotective activity of polyphenols has been associated with their ability to reduce oxidative stress by decreasing reactive oxygen species and lipid peroxidation, while their capacity to inhibit LDL oxidation and its cytotoxic effects may contribute to the prevention of atherosclerosis [109].

4. Conclusions

In this study, the optimization of the GP phenolic compounds extraction using DES was investigated. The results revealed an appropriate correlation amongst the tested parameters and the measured responses (TPC, CTC, DPPH, ABTS, FRAP, and CUPRAC). LSR had the most significant impact (mainly negative), while time and temperature showed predominantly positive influences. Optimal conditions (LSR 14.86 mL/g, 60 °C, 275.85 min) yielded extracts containing 18 identified compounds, associated with antibacterial, antioxidant, and anticancer activities. The extract inhibited the growth of *Escherichia coli*, *Listeria monocytogenes*, and MRSA, without showing gastrointestinal or hepatic toxicity at 6 or 12 μg/mL of GP extract concentration. The model suitability was corroborated by the strong accordance between most of the predicted and experimental values. In summary, the results demonstrate that DES are a propitious alternative to organic solvents for the retrieval of highly valuable components from GP and to produce biocompatible and ready-to-use extracts with potential industrial applications as functional ingredients.

CRediT authorship contribution statement

Alba Pérez-Pérez: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Adela Alvaredo-López-Vizcaíno:** Formal analysis, Investigation, Writing – review & editing. **Tânia C.S. P. Pires:** Formal analysis, Investigation, Methodology. **Tiane C. Finimundy:** Formal analysis, Investigation, Methodology. **Filipa Mandim:** Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **Lillian Barros:** Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing. **Pablo G. Del-Río:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Beatriz Gullón:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

Acknowledgments

Authors acknowledge (i) University of Vigo and CISUG for financing Open Access publication, (ii) Ministry of Science and Innovation of Spain (VALWIDE project TED2021–132088B-I00 and AdChemFood project PID2022-138458OB-I00) and Interreg VI-B Sudoe 2021-2027 (NEWPOWER, S1/1.1/E0116) for funding, and (iii) Consellería de Cultura, Educación e Ordenación Universitaria (Xunta de Galicia) through the contract ED431B 2025/34 to group Bio2Val as Group with Growth Potential, programs partially funded by ERDF. Alba Pérez-Pérez (FPU21/02446) acknowledge funding from Ministry of Universities of Spain. Pablo G. Del-Río acknowledges funding from Consellería de Cultura, Educación, Formación Profesional e Universidades (Xunta de Galicia) (ED481D-2025/008). Authors acknowledge Servicio de Seguridad Alimentaria y Desarrollo Sostenible from Centro de Apoio

Científico e Tecnolóxico á Investigación (CACTI-Universidade de Vigo) for the use of scientific and technical services. This work was also supported by national funds through FCT/MCTES (PIDDAC): CIMO, UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); and national funding by FCT, P.I., through the institutional scientific employment program contract for L. Barros (CEEC-INST, DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and the research contract of F. Mandim through the project 20651_DiVina – NORTE2030-FEDER-02089700.

Data availability

Data will be made available on request.

References

[1] G. Domínguez-Rodríguez, M.C. García, M. Plaza, M.L. Marina, Revalorization of *Passiflora* species peels as a sustainable source of antioxidant phenolic compounds, Sci. Total Environ. 696 (2019) 1–13, https://doi.org/10.1016/j.scitotenv.2019.134030.

[2] P. Gullón, B. Gullón, A. Romaní, G. Rocchetti, J.M. Lorenzo, Smart advanced solvents for bioactive compounds recovery from agri-food by-products: a review, Trends Food Sci. Technol. 101 (2020) 182–197, https://doi.org/10.1016/j.tifs.2020.05.007.

[3] OIV, Statistical Report on Global Vitiviniculture, International Organisation of Vine and Wine, 2024. https://www.oiv.int. (Accessed 7 November 2025).

[4] I. Dávila, P. Gullón, J. Labidi, Influence of the heating mechanism during the aqueous processing of Vine shoots for the obtaining of hemicellulosic oligosaccharides, Waste Manag. 120 (2021) 146–155, https://doi.org/10.1016/j.wasman.2020.11.014.

[5] R. Ferrer-Gallego, P. Silva, The wine industry By-Products: applications for food industry and health benefits, Antioxidants 11 (2022) 1–21, https://doi.org/10.3390/antiox11102025.

[6] H. Zemni, R. Khiari, M. Lamine, Y. Houimli, S. Chenenaoui, A. Ben Salem, Grape marc skin valorization: from waste to valuable polyphenol source, Chem. Afr. 7 (2024) 765–776, https://doi.org/10.1007/s42250-023-00800-6.

[7] T. Ilyas, P. Chowdhary, D. Chaurasia, E. Gnansounou, A. Pandey, P. Chaturvedi, Sustainable green processing of grape pomace for the production of value-added products: an overview, Environ. Technol. Innov. 23 (2021) 1–25, https://doi.org/10.1016/j.eti.2021.101592.

[8] A. Mir-Cerdà, O. Nuñez, M. Granados, S. Sentellas, J. Saurina, An overview of the extraction and characterization of bioactive phenolic compounds from agri-food waste within the framework of circular bioeconomy, TrAC, Trends Anal. Chem. 161 (2023) 1–12, https://doi.org/10.1016/j.trac.2023.116994.

[9] A. Blasi, A. Verardi, P. Sangiorgio, The zero-waste economy: from food waste to industry, in: Membrane Engineering in the Circular Economy: Renewable Sources Valorization in Energy and Downstream Processing in Agro-Food Industry, 2022, pp. 63–100, https://doi.org/10.1016/B978-0-323-85253-1.00006-X.

[10] P. Ferreira-Santos, C. Nobre, R.M. Rodrigues, Z. Genisheva, C. Botelho, J. A. Teixeira, Extraction of phenolic compounds from grape pomace using ohmic heating: chemical composition, bioactivity and bioaccessibility, Food Chem. 436 (2024) 137780, https://doi.org/10.1016/j.foodchem.2023.137780.

[11] R. Sirohi, A. Tarafdar, S. Singh, T. Negi, V.K. Gaur, E. Gnansounou, B. Bharathiraja, Green processing and biotechnological potential of grape pomace: current trends and opportunities for sustainable biorefinery, Bioresour. Technol. 314 (2020) 123771, https://doi.org/10.1016/j.biortech.2020.123771.

[12] A.Z. Barakat, R.I. Bassuiny, A.M. Abdel-Aty, S.A. Mohamed, Diabetic complications and oxidative stress: the role of phenolic-rich extracts of saw palmetto and date palm seeds, J. Food Biochem. 44 (2020) 1–13, https://doi.org/10.1111/jfbc.13416.

[13] A.M. Abdel-Aty, A.Z. Barakat, R.I. Bassuiny, S.A. Mohamed, Statistical optimization, characterization, antioxidant and antibacterial properties of silver nanoparticle biosynthesized by saw palmetto seed phenolic extract, Sci. Rep. 13 (2023) 15605, https://doi.org/10.1038/s41598-023-42675-0.

[14] E.Y. Ishimoto, S.J.V. Vicente, R.J. Cruz, E. Torres, Hypolipidemic and antioxidant effects of grape processing by-products ihigh-fat/cholesterol diet-induced hyperlipidemic hamsters, Food Sci. Technol.(Brazil) 40 (2020) 558–567, https://doi.org/10.1590/fst.32619.

[15] J.M. Pérez-Ortiz, L.F. Alguacil, E. Salas, I. Hermosín-Gutiérrez, S. Gómez-Alonso, C. González-Martín, Antiproliferative and cytotoxic effects of grape pomace and grape seed extracts on colorectal cancer cell lines, Food Sci. Nutr. 7 (2019) 2948–2957, https://doi.org/10.1002/fsn3.1150.

[16] T. Abreu, C. Luís, J.S. Câmara, J. Teixeira, R. Perestrelo, Unveiling potential functional applications of grape pomace extracts based on their phenolic profiling, bioactivities, and circular bioeconomy, Biomass Convers. Biorefinery 15 (2025) 31491–31506, https://doi.org/10.1007/s13399-025-06578-6.

[17] J.F. Osorio-Tobón, Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds, J. Food Sci. Technol. 57 (2020) 4299–4315, https://doi.org/10.1007/s13197-020-04433-2.

[18] S. Cao, J. Liang, M. Chen, C. Xu, X. Wang, L. Qiu, X. Zhao, W. Hu, Comparative analysis of extraction technologies for plant extracts and absolutes, Front. Chem. 13 (2025), https://doi.org/10.3389/fchem.2025.1536590.

[19] P. Tapia-Quirós, M. Granados, S. Sentellas, J. Saurina, Microwave-assisted extraction with natural deep eutectic solvents for polyphenol recovery from agrifood waste: mature for scaling-up? Sci. Total Environ. 912 (2024) 168716 https://doi.org/10.1016/j.scitotenv.2023.168716.

[20] F.M. Perna, P. Vitale, V. Capriati, Deep eutectic solvents and their applications as green solvents, Curr. Opin. Green Sustainable Chem. 21 (2020) 27–33, https://doi.org/10.1016/j.cogsc.2019.09.004.

[21] A. Shishov, A. Pochivalov, L. Nugbienyo, V. Andruch, A. Bulatov, Deep eutectic solvents are not only effective extractants, TrAC, Trends Anal. Chem. 129 (2020) 1–13, https://doi.org/10.1016/j.trac.2020.115956.

[22] K.A. Omar, R. Sadeghi, Physicochemical properties of deep eutectic solvents: a review, J. Mol. Liq. 360 (2022) 119524, https://doi.org/10.1016/j.molliq.2022.119524.

[23] J.O. Airouyuwa, H. Mostafa, A. Riaz, C. Stathopoulos, S. Maqsood, Natural deep eutectic solvents and microwave-assisted green extraction for efficient recovery of bioactive compounds from By-Products of date fruit (*Phoenix dactylifera* L.) processing: modeling, optimization, and phenolic characterization, Food Bioprocess Technol. 16 (2023) 824–843, https://doi.org/10.1007/s11947-022-02960-8.

[24] R. Manurung, A. Syahputra, M.A. Alhamdi, Purification of palm biodiesel using deep eutectic Solvent (DES) based Choline Chloride (ChCl) and 1,2-Propanediol ($C_3H_8O_2$), J. Phys., Conf. Ser. 1028 (2018), https://doi.org/10.1088/1742-6596/1028/1/012032.

[25] R. Castro-Muñoz, A. Msahel, F. Galiano, M. Serocki, J. Ryl, S. Ben Hamouda, A. Hafiane, G. Boczkaj, A. Figoli, Towards azeotropic MeOH-MTBE separation using pervaporation chitosan-based deep eutectic solvent membranes, Sep. Purif. Technol. 281 (2022), https://doi.org/10.1016/j.seppur.2021.119979.

[26] M. Panić, V. Gunjević, G. Cravotto, I. Radojčić Redovniković, Enabling technologies for the extraction of grape-pomace anthocyanins using natural deep eutectic solvents in up-to-half-litre batches extraction of grape-pomace anthocyanins using NADES, Food Chem. 300 (2019) 125185, https://doi.org/10.1016/j.foodchem.2019.125185.

[27] E. Hernández-Corroto, M. Plaza, M.L. Marina, M.C. García, Sustainable extraction of proteins and bioactive substances from pomegranate peel (*Punica granatum* L.) using pressurized liquids and deep eutectic solvents, Innov. Food Sci. Emerg. Technol. 60 (2020), https://doi.org/10.1016/j.ifset.2020.102314.

[28] M. Patsea, I. Stefou, S. Grigorakis, D.P. Makris, Screening of natural sodium acetate-based low-transition temperature mixtures (LTTMs) for enhanced extraction of antioxidants and pigments from red vinification solid wastes, Environ. Process. 4 (2017) 123–135, https://doi.org/10.1007/s40710-016-0205-8.

[29] S. El Kantar, H.N. Rajha, N. Boussetta, E. Vorobiev, R.G. Maroun, N. Louka, Green extraction of polyphenols from grapefruit peels using high voltage electrical discharges, deep eutectic solvents and aqueous glycerol, Food Chem. 295 (2019) 165–171, https://doi.org/10.1016/j.foodchem.2019.05.111.

[30] S.A. Siddiqui, A. Ali Redha, M. Salauddin, I.A. Harahap, H.P.V. Rupasinghe, Factors affecting the extraction of (Poly)Phenols from natural resources using deep eutectic solvents combined with ultrasound-assisted extraction, Crit. Rev. Anal. Chem. 55 (2025) 139–160, https://doi.org/10.1080/10408347.2023.2266846.

[31] L. Sillero, M. Marcial, I. Olaizola, J.M. Banales, F. Hernández-Ramos, X. Erdocia, A. Morales, Sustainable recovery of bioactive compounds from forest residues using microwave-assisted extraction, Sep. Purif. Technol. 379 (2025) 134993, https://doi.org/10.1016/j.seppur.2025.134993.

[32] K.J.A. Lim, A.A. Cabajar, C.F.Y. Lobarbio, E.B. Taboada, D.J. Lacks, Extraction of bioactive compounds from mango (*Mangifera indica* L. *var. Carabao*) seed kernel with ethanol-water binary solvent systems, J. Food Sci. Technol. 56 (2019) 2536–2544, https://doi.org/10.1007/s13197-019-03732-7.

[33] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J. Wolfe, Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory (NREL), 2008, pp. 3–5.

[34] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Ash in Biomass. Laboratory Analytical Procedure (LAP), 1, National Renewable Energy Laboratory (NREL), 2008, pp. 1–5, https://doi.org/NREL/TP-510-42619.

[35] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP), 1, National Renewable Energy Laboratory (NREL), 2008, pp. 1–15, https://doi.org/NREL/TP-510-42618.

[36] A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Extractives in Biomass. Laboratory Analytical Procedure (LAP), 1, National Renewable Energy Laboratory (NREL), 2008, pp. 1–9, https://doi.org/10.1016/j.rmr.2016.02.006.

[37] P.G. del Río, A. Pérez-Pérez, G. Garrote, B. Gullón, Manufacturing of hemicellulosic oligosaccharides from fast-growing Paulownia wood via autohydrolysis: microwave versus conventional heating, Ind. Crops Prod. 187 (2022) 115313, https://doi.org/10.1016/j.indcrop.2022.115313.

[38] J.B. Sluiter, R.O. Ruiz, C.J. Scarlata, A.D. Sluiter, D.W. Templeton, Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods, J. Agric. Food Chem. 58 (2010) 9043–9053, https://doi.org/10.1021/jf1008023.

[39] V.L. Singleton, J.A. Rossi, Colorimetric of total phenolics with phosphomolybdic—phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[40] R.B. Broadhurst, W.T. Jones, Analysis of condensed tannins using acidified vanillin, J. Sci. Food Agric. 29 (1978) 788–794, https://doi.org/10.1002/jsfa.2740290908.
[41] B. Gullón, G. Eibes, M.T. Moreira, I. Dávila, J. Labidi, P. Gullón, Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of Vine shoots, Ind. Crops Prod. 107 (2017) 105–113, https://doi.org/10.1016/j.indcrop.2017.05.034.
[42] M. Özyürek, K. Güçlü, R. Apak, The main and modified CUPRAC methods of antioxidant measurement, TrAC, Trends Anal. Chem. 30 (2011) 652–664, https://doi.org/10.1016/j.trac.2010.11.016.
[43] F. Mandim, M.I. Dias, J. Pinela, P. Barracosa, M. Ivanov, D. Stojković, M. Soković, C. Santos-Buelga, L. Barros, I.C.F.R. Ferreira, Chemical composition and in vitro biological activities of cardoon (*Cynara cardunculus* L. var. *altilis* DC.) seeds as influenced by viability: chemical prospection and bioactivity of cardoon seeds, Food Chem. 323 (2020), https://doi.org/10.1016/j.foodchem.2020.126838.
[44] ISO, 10993-5:2009 - Biological Evaluation of Medical Devices – Part 5: Tests for in Vitro Cytotoxicity, International Organization for Standardization, Geneva (Switzerland), 2009.
[45] T.C.S.P. Pires, M.I. Dias, L. Barros, M.J. Alves, M.B.P.P. Oliveira, C. Santos-Buelga, I.C.F.R. Ferreira, Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica* Borkh. cv Bravo de Esmolfe), Food Chem. 240 (2018) 701–706, https://doi.org/10.1016/j.foodchem.2017.08.010.
[46] F. Mandim, S.A. Petropoulos, M.I. Dias, J. Pinela, M. Kostić, M. Soković, C. Santos-Buelga, I.C.F.R. Ferreira, L. Barros, Phenolic composition and biological properties of *Cynara cardunculus* l. Var. *altilis* petioles: influence of the maturity stage, Antioxidants 10 (2021), https://doi.org/10.3390/antiox10121907.
[47] S.P. Ioannidou, A.G. Margellou, M.D. Petala, K.S. Triantafyllidis, Pretreatment/fractionation and characterization of winery waste streams within an integrated biorefinery concept, Sustain. Chem. Pharm. 27 (2022) 100670, https://doi.org/10.1016/j.scp.2022.100670.
[48] H. Wikberg, S. Grönqvist, P. Niemi, A. Mikkelson, M. Siika-aho, H. Kanerva, A. Käsper, T. Tamminen, Hydrothermal treatment followed by enzymatic hydrolysis and hydrothermal carbonization as means to valorise agro- and forest-based biomass residues, Bioresour. Technol. 235 (2017) 70–78, https://doi.org/10.1016/j.biortech.2017.03.095.
[49] J.A.S. Mendes, S.O. Prozil, D.V. Evtuguin, L.P.C. Lopes, Towards comprehensive utilization of winemaking residues: characterization of grape skins from red grape pomaces of variety *Touriga Nacional*, Ind. Crops Prod. 43 (2013) 25–32, https://doi.org/10.1016/j.indcrop.2012.06.047.
[50] K. Filippi, H. Papapostolou, M. Alexandri, A. Vlysidis, E.D. Myrtsi, D. Ladakis, C. Pateraki, S.A. Haroutounian, A. Koutinas, Integrated biorefinery development using winery waste streams for the production of bacterial cellulose, succinic acid and value-added fractions, Bioresour. Technol. 343 (2022), https://doi.org/10.1016/j.biortech.2021.125989.
[51] L. Guardia, L. Suárez, N. Querejeta, C. Pevida, T.A. Centeno, Winery wastes as precursors of sustainable porous carbons for environmental applications, J. Clean. Prod. 193 (2018) 614–624, https://doi.org/10.1016/j.jclepro.2018.05.085.
[52] B.M. Pedras, G. Regalin, I. Sá-Nogueira, P. Simões, A. Paiva, S. Barreiros, Fractionation of red wine grape pomace by subcritical water extraction/hydrolysis, J. Supercrit. Fluids 160 (2020), https://doi.org/10.1016/j.supflu.2020.104793.
[53] K. Filippi, N. Georgaka, M. Alexandri, H. Papapostolou, A. Koutinas, Valorisation of grape stalks and pomace for the production of bio-based succinic acid by *Actinobacillus succinogenes*, Ind. Crops Prod. 168 (2021), https://doi.org/10.1016/j.indcrop.2021.113578.
[54] Q. Jin, J.O. Hair, A.C. Stewart, S.F.O. Keefe, A.P. Neilson, Y. Kim, M. Mcguire, A. Lee, H. Huang, Compositional characterization of different industrial white and red grape pomaces in Virginia and the potential valorization of the major components, Foods 8 (2019) 1–15, https://doi.org/10.3390/foods8120667.
[55] L. Marinaccio, G. Gentile, E.J. Llorent-Martínez, G. Zengin, D. Masci, F. Flamminii, A. Stefanucci, A. Mollica, Valorization of grape pomace extracts against cranberry, elderberry, rose hip berry, goji berry and raisin extracts: phytochemical profile and *in vitro* biological activity, Food Chem. 463 (2025), https://doi.org/10.1016/j.foodchem.2024.141323.
[56] C. Radulescu, R.L. Olteanu, C.L. Buruleanu, M. Nechifor, I.D. Dulama, R. M. Stirbescu, I.A. Bucurica, S.G. Stanescu, A.L. Banica, Polyphenolic screening and the antioxidant activity of grape pomace extracts of Romanian white and red grape varieties, Antioxidants 13 (2024) 1–20, https://doi.org/10.3390/antiox13091133.
[57] M. Deaconu, A. Abduraman, A.-M. Brezoiu, N.K. Sedky, S. Ioniță, C. Matei, L. Ziko, D. Berger, Anti-Inflammatory, antidiabetic, and antioxidant properties of extracts prepared from pinot noir grape marc, free and incorporated in porous silica-based supports, Molecules 29 (2024), https://doi.org/10.3390/molecules29133122.
[58] M. Ben Aziz, F. Garcia, L. Mouls, H. Fulcrand, H. Hajjaj, Proanthocyanidins and anthocyanins contents, chromatic and antioxidant properties of red grape pomaces from Morocco, J. Food Meas. Char. 13 (2019) 2051–2061, https://doi.org/10.1007/s11694-019-00126-3.
[59] L.C. Marianne, A.G. Lucía, M.S.M. de Jesús, H.M. Eric Leonardo, M. Mendoza-Sánchez, Optimization of the green extraction process of antioxidants derived from grape pomace, Sustain. Chem. Pharm. 37 (2024), https://doi.org/10.1016/j.scp.2023.101396.
[60] A. Del-Castillo-Llamosas, F. Rodríguez-Rebelo, B. Rodríguez-Martínez, A. Mallo-Fraga, P.G. Del-Río, B. Gullón, Valorization of avocado seed wastes for antioxidant phenolics and carbohydrates recovery using Deep Eutectic Solvents (DES), Antioxidants 12 (2023), https://doi.org/10.3390/antiox12061156.
[61] J. Serna-Vázquez, M.Z. Ahmad, G. Boczkaj, R. Castro-Muñoz, Latest insights on novel deep eutectic solvents (DES) for sustainable extraction of phenolic compounds from natural sources, Molecules 26 (2021) 5037, https://doi.org/10.3390/molecules26165037.
[62] D. Rente, A. Paiva, A.R. Duarte, The role of hydrogen bond donor on the extraction of phenolic compounds from natural matrices using deep eutectic systems, Molecules 26 (2021) 2336, https://doi.org/10.3390/molecules26082336.
[63] M. Vilková, J. Płotka-Wasylka, V. Andruch, The role of water in deep eutectic solvent-base extraction, J. Mol. Liq. 304 (2020), https://doi.org/10.1016/j.molliq.2020.112747.
[64] R. Rashid, S. Mohd Wani, S. Manzoor, F.A. Masoodi, M. Masarat Dar, Green extraction of bioactive compounds from apple pomace by ultrasound assisted natural deep eutectic solvent extraction: optimisation, comparison and bioactivity, Food Chem. 398 (2023) 133871, https://doi.org/10.1016/j.foodchem.2022.133871.
[65] A. Frontini, A. Luvisi, C. Negro, M. Apollonio, R. Accogli, M. De Pascali, L. De Bellis, Polyphenols extraction from different grape pomaces using natural deep eutectic solvents, Separations 11 (2024) 1–16, https://doi.org/10.3390/separations11080241.
[66] L. Marinaccio, G. Gentile, G. Zengin, S. Pieretti, A. Stefanucci, A. Cichelli, A. Mollica, Ultrasound assisted deep eutectic solvent-based extraction of Montepulciano d' Abruzzo grape seeds for the recovery of the grape seed oil and its biological evaluation, Food Chem. X (2025) 26, https://doi.org/10.1016/j.fochx.2025.102273.
[67] Z. Diaconeasa, Time-dependent degradation of polyphenols from thermally-processed berries and their in vitro antiproliferative effects against melanoma, Molecules 23 (2018) 2534, https://doi.org/10.3390/molecules23102534.
[68] B. Zheng, Y. Yuan, J. Xiang, W. Jin, J.B. Johnson, Z. Li, C. Wang, D. Luo, Green extraction of phenolic compounds from foxtail millet bran by ultrasonic-assisted deep eutectic solvent extraction: optimization, comparison and bioactivities, Lwt 154 (2022), https://doi.org/10.1016/j.lwt.2021.112740.
[69] N. Anis, D. Ahmed, DES-based microwave- and ultrasound-assisted extraction of natural products from *Rumex hastatus* as per response surface methodology, Sustain. Chem. Pharm. 36 (2023), https://doi.org/10.1016/j.scp.2023.101289.
[70] M. Molnar, M. Jakovljević Kovač, V. Pavić, A comprehensive analysis of diversity, structure, biosynthesis and extraction of biologically active tannins from various plant-based materials using deep eutectic solvents, Molecules 29 (2024) 2615, https://doi.org/10.3390/molecules29112615.
[71] M.E. Cardona Jimenez, J. Gabilondo, R.M. Bodoira, L.M. Agudelo Laverde, P. R. Santagapita, Extraction of bioactive compounds from pecan nutshell: an added-value and low-cost alternative for an industrial waste, Food Chem. 453 (2024), https://doi.org/10.1016/j.foodchem.2024.139596.
[72] J. Moutinho, I. Gouvinhas, R. Domínguez-Perles, A. Barros, Optimization of the extraction methodology of grape pomace polyphenols for food applications, Molecules 28 (2023), https://doi.org/10.3390/molecules28093885.
[73] A. Mollica, G. Scioli, A. Della Valle, A. Cichelli, E. Novellino, M. Bauer, W. Kamysz, E.J. Llorent-Martínez, M.L.F. De Córdova, R. Castillo-López, G. Ak, G. Zengin, S. Pieretti, A. Stefanucci, Phenolic analysis and in vitro biological activity of red wine, pomace and grape seeds oil derived from *Vitis vinifera* l. Cv. montepulciano d'abruzzo, Antioxidants 10 (2021) 1–17, https://doi.org/10.3390/antiox10111704.
[74] A. Souadia, A. Djemoui, L. Souli, K. Haiouani, A.V. Atoki, D. Djemoui, M. Messaoudi, S. Hegazy, H. Alsaeedi, A. Barhoum, Ultrasound-assisted extraction for maximizing total phenolics, flavonoid content, and antioxidant activity in *Thymus algeriensis*: Box-Behnken experimental design, Biomass Convers. Biorefinery 15 (2025) 12979–12994, https://doi.org/10.1007/s13399-025-06494-9.
[75] A. Antony, M. Farid, Effect of temperatures on polyphenols during extraction, Appl. Sci. (Switzerland) 12 (2022), https://doi.org/10.3390/app12042107.
[76] A. Benarfa, N. Gourine, S. Hachani, M. Harrat, M. Yousfi, Optimization of ultrasound-assisted extraction of antioxidative phenolic compounds from *Deverra scoparia* coss. & durieu (flowers) using response surface methodology, J. Food Process. Preserv. 44 (2020) 1–10, https://doi.org/10.1111/jfpp.14514.
[77] P. Gullón, G. Eibes, J.M. Lorenzo, N. Pérez-Rodríguez, T.A. Lú-Chau, B. Gullón, Green sustainable process to revalorize purple corn cobs within a biorefinery frame: Co-production of bioactive extracts, Sci. Total Environ. 709 (2020), https://doi.org/10.1016/j.scitotenv.2019.136236.
[78] L.M. Anaya-Esparza, E.F. Aurora-Vigo, Z. Villagrán, E. Rodríguez-Lafitte, J. M. Ruvalcaba-Gómez, M.Á. Solano-Cornejo, V.M. Zamora-Gasga, E. Montalvo-González, H. Gómez-Rodríguez, C.E. Aceves-Aldrete, N. González-Silva, Design of experiments for optimizing ultrasound-assisted extraction of bioactive compounds from plant-based sources, Molecules 28 (2023) 7752, https://doi.org/10.3390/molecules28237752.
[79] N. Chaves, A. Santiago, J.C. Alías, Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used, Antioxidants 9 (2020) 76, https://doi.org/10.3390/antiox9010076.
[80] J.M. Ueda, K.R. Griebler, T.C. Finimundy, D.B. Rodrigues, T.C.S.P. Pires, J. Gonçalves, I.P. Fernandes, E. Pereira, L. Barros, S.A. Heleno, R.C. Calhelha, Polyphenol composition by HPLC-DAD-(ESI-)MS/MS and bioactivities of extracts from grape agri-food wastes, Molecules 28 (2023), https://doi.org/10.3390/molecules28217368.
[81] N. Pallarés, H. Berrada, E. Ferrer, W. Rached, J. Pinela, F. Mandim, T.C.S.P. Pires, T.C. Finimundy, F.J. Barba, L. Barros, Green and innovative extraction: phenolic profiles and biological activities of underutilized plant extracts using pulsed

electric fields and maceration, Foods 14 (2025) 1–19, https://doi.org/10.3390/foods14020222.
[82] N.J. Darling, C.L. Mobbs, A.L. González-Hau, M. Freer, S. Przyborski, Bioengineering novel in vitro Co-culture models that represent the human intestinal mucosa with improved Caco-2 structure and barrier function, Front. Bioeng. Biotechnol. 8 (2020) 992, https://doi.org/10.3389/fbioe.2020.00992.
[83] Z. Zhou, M.-J. Xu, B. Gao, Hepatocytes: a key cell type for innate immunity, Cell. Mol. Immunol. 13 (2016) 301–315, https://doi.org/10.1038/cmi.2015.97.
[84] P. Valenzuela-Bustamante, P. Cornejo, N. Nolan, A. Concepción-Alvarez, R. Bridi, M.Á. Rincón-Cervera, O. Porras, A.C. de Camargo, M.F. Arias-Santé, Valorization of Carménère grape pomace: extraction, microencapsulation, and evaluation of the bioactivity of polyphenols in Caco-2 cells, Int. J. Mol. Sci. 26 (2025) 1–21, https://doi.org/10.3390/ijms26167994.
[85] C.M. Peixoto, M.I. Dias, M.J. Alves, R.C. Calhelha, L. Barros, S.P. Pinho, I.C.F. R. Ferreira, Grape pomace as a source of phenolic compounds and diverse bioactive properties, Food Chem. 253 (2018), https://doi.org/10.1016/j.foodchem.2018.01.163.
[86] E. Gómez-Mejía, C.L. Roriz, S.A. Heleno, R. Calhelha, M.I. Dias, J. Pinela, N. Rosales-Conrado, M.E. León-González, I.C.F.R. Ferreira, L. Barros, Valorisation of black mulberry and grape seeds: chemical characterization and bioactive potential, Food Chem. 337 (2021), https://doi.org/10.1016/j.foodchem.2020.127998.
[87] N. Oulahal, P. Degraeve, Phenolic-Rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides? Front. Microbiol. 12 (2022) 753518 https://doi.org/10.3389/fmicb.2021.753518.
[88] D. Sateriale, G. Forgione, M. Di Rosario, C. Pagliuca, R. Colicchio, P. Salvatore, M. Paolucci, C. Pagliarulo, Vine-winery byproducts as precious resource of natural antimicrobials: in vitro antibacterial and antibiofilm activity of grape pomace extracts against foodborne pathogens, Microorganisms 12 (2024), https://doi.org/10.3390/microorganisms12030437.
[89] A.C. Grosu, F.C. Diguță, M.-C. Pristavu, A. Popa, F. Badea, M. Dragoi Cudalbeanu, A. Orțan, I. Dopcea, N. Băbeanu, Exploring the phytochemical profiles, and antioxidant and antimicrobial activities of the hydroethanolic grape pomace extracts from two Romanian Indigenous varieties, Fermentation 10 (2024), https://doi.org/10.3390/fermentation10090470.
[90] R. Fernández-Pérez, S. Ayuso, C. Moreta, M.J. Saiz-Abajo, M. Gastón-Lorente, F. Ruiz-Larrea, C. Tenorio, Chemical profile and antibacterial activity of *Vitis vinifera* L. cv graciano pomace extracts obtained by green supercritical CO2 extraction method against multidrug-resistant *Escherichia coli* strains, Foods 14 (2025) 1–21, https://doi.org/10.3390/foods14010017.
[91] M. Galante, M.E. Brassesco, C. Maragoni Santos, C. Beres, A.E.C. Fai, I. Cabezudo, Grape pomace as a natural source of antimicrobial agents for food preservation, Front. Nutr. 12 (2025), https://doi.org/10.3389/fnut.2025.1650450.
[92] M. Campo, M. Khatib, C. Cassiani, N. Mulinacci, A. Durazzo, M. Lucarini, P. Vignolini, Chemical characterization of Grechetto grape pomace for potential valorization in a biorefinery perspective, Eur. Food Res. Technol. 251 (2025) 2239–2252, https://doi.org/10.1007/s00217-025-04760-8.
[93] V. Vorobyova, M. Skiba, O. Horodniuk, L. Khrokalo, G. Vasyliev, Betaine-based deep eutectic grape Pomace extract mediated synthesis of silver nanoparticles with antibacterial activities, BioNanoScience 13 (2023) 1726–1740, https://doi.org/10.1007/s12668-023-01211-0.
[94] V. Olt, J. Báez, R. Curbelo, E. Boido, E. Dellacassa, A. Medrano, A.M. Fernández-Fernández, Gastrointestinal digestion impact on phenolics and bioactivity of tannat grape pomace biscuits, Molecules 30 (2025) 1–17, https://doi.org/10.3390/molecules30153247.
[95] D.M. Piazza, D. Romanini, M.R. Meini, High-efficiency novel extraction process of target polyphenols using enzymes in hydroalcoholic media, Appl. Microbiol. Biotechnol. 107 (2023) 1205–1216, https://doi.org/10.1007/s00253-023-12386-7.
[96] T. Ahad, D. Majid, S. Naqash, H.A. Makroo, S.J. Rashid, B.N. Dar, Exploring bioactive compounds in lyophilized grape pomace extracts: comparative analysis of traditional and innovative extraction methods, J. Food Meas. Char. 19 (2025) 1325–1336, https://doi.org/10.1007/s11694-024-03044-1.
[97] C.M. Viola, M.E. Danilovich, R. Torres-Carro, M.M. Moreira, F. Rodrigues, E. Cartagena, M.R. Alberto, M.A. Blázquez, M.E. Arena, Phenolic-Rich wine pomace extracts as antioxidant and antipathogenic agents against *Pseudomonas aeruginosa*, Antibiotics 14 (2025) 1–20, https://doi.org/10.3390/antibiotics14040384.
[98] J. Cadena-Iñiguez, E. Santiago-Osorio, N. Sánchez-Flores, S. Salazar-Aguilar, R. M. Soto-Hernández, M. de la L. Riviello-Flores, V.M. Macías-Zaragoza, I. Aguiñiga-Sánchez, The cancer-protective potential of protocatechuic acid: a narrative review, Molecules 29 (2024), https://doi.org/10.3390/molecules29071439.
[99] C. Yuan, Y. Wang, L. Zhang, D. Wang, Procatechuic acid and protocatechuic aldehyde increase survival of Caenorhabditis elegans after fungal infection and inhibit fungal virulence, Front. Pharmacol. 15 (2024) 1–12, https://doi.org/10.3389/fphar.2024.1396733.
[100] M.A. Sunil, V.S. Sunitha, P. Santhakumaran, M.C. Mohan, M.S. Jose, E. K. Radhakrishnan, J. Mathew, Protective effect of (+)–catechin against lipopolysaccharide-induced inflammatory response in RAW 264.7 cells through downregulation of NF-κB and p38 MAPK, Inflammopharmacology 29 (2021) 1139–1155, https://doi.org/10.1007/s10787-021-00827-6.
[101] P. Thomas, J. Dong, (-)-Epicatechin acts as a potent agonist of the membrane androgen receptor, ZIP9 (SLC39A9), to promote apoptosis of breast and prostate cancer cells, J. Steroid Biochem. Mol. Biol. 211 (2021), https://doi.org/10.1016/j.jsbmb.2021.105906.
[102] E.J. Hid, J.I. Mosele, P.D. Prince, C.G. Fraga, M. Galleano, (−)-Epicatechin and cardiometabolic risk factors: a focus on potential mechanisms of action, Pflugers Arch 474 (2022) 99–115, https://doi.org/10.1007/s00424-021-02640-0.
[103] J. Ling, Y. Wu, X. Zou, Y. Chang, G. Li, M. Fang, (-)-Epicatechin reduces neuroinflammation, protects mitochondria function, and prevents cognitive impairment in sepsis-associated encephalopathy, Oxid. Med. Cell. Longev. 2022 (2022), https://doi.org/10.1155/2022/2657713.
[104] Y.-H. Chan, J.W.E. Moss, J.O. Williams, N. Ferekidis, N. Alshehri, T.R. Hughes, J. Menendez-Gonzalez, S.F. Plummer, D.R. Michael, N.P. Rodrigues, D.P. Ramji, (+)-Catechin attenuates multiple atherosclerosis-associated processes in vitro, modulates disease-associated risk factors in C57BL/6J mice and reduces atherogenesis in LDL receptor deficient mice by inhibiting inflammation and increasing markers of plaque, Mol. Nutr. Food Res. 67 (2023), https://doi.org/10.1002/mnfr.202200716.
[105] X. Zhao, Z. Chai, J. Wang, D. Hou, B. Li, L. Zhang, W. Huang, Assessment on malvidin-3-glucoside interaction with TLR4 via multi-spectroscopic analysis and molecular docking, Spectrochim. Acta Mol. Biomol. Spectrosc. 318 (2024), https://doi.org/10.1016/j.saa.2024.124460.
[106] D. Li, Y. Lv, G. Du, Y. Zhu, B. Shi, X. Ye, M. Zhang, Malvidin-3-O-glucoside ameliorates the development of vascular endothelial dysfunction in type 2 diabetes mellitus by inhibiting arginase 1 expression, Food Biosci. 68 (2025) 1–10, https://doi.org/10.1016/j.fbio.2025.106592.
[107] M. Nasehi, M.-H. Mohammadi-Mahdiabadi-Hasani, M.-R. Zarrindast, S. Zarrabian, Punicalagin effect on total sleep deprivation memory deficit in male wistar rats, J. Integr. Neurosci. 20 (2021) 87–93, https://doi.org/10.31083/J.JIN.2021.01.378.
[108] S. Bakrim, N. El Omari, N. El Hachlafi, Y. Bakri, L.H. Lee, A. Bouyahya, Dietary phenolic compounds as anticancer natural drugs: recent update on molecular mechanisms and clinical trials, Foods 11 (2022) 3323, https://doi.org/10.3390/foods11213323.
[109] N. Hedayati, A. Yaghoobi, M. Salami, Y. Gholinezhad, F. Aghadavood, R. Eshraghi, M.H. Aarabi, M. Homayoonfal, Z. Asemi, H. Mirzaei, M. Hajijafari, A. Mafi, M. Rezaee, Impact of polyphenols on heart failure and cardiac hypertrophy: clinical effects and molecular mechanisms, Front. Cardiovasc. Med. 10 (2023), https://doi.org/10.3389/fcvm.2023.1174816.