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Polyphenol extraction from Lacrima di Morro d'Alba grape pomace and Ascolana Tenera olive leaves as an effective recovery of agricultural side-streams

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ABSTRACT

This study investigates green extraction methods for recovering bioactive compounds from grape pomace and olive leaves, aiming to enhance the valorization of agricultural side-streams within a sustainable framework. Conventional and innovative extraction techniques including liquid-solid extraction (SLE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) were evaluated to determine their efficacy in yielding polyphenols and antioxidants, with particular attention to flavonoids, anthocyanins, and phenolic acids. Findings demonstrate that UAE optimally recovers flavan-3-ols, phenolic acids, and as anthocyanins the malvidin-3-galactoside from Lacrima di Morro d'Alba grape pomace. Otherwise for Ascolana Tenera olive leaves there is not a clear selectivity of both pre-treatment and extraction procedures applied for the recovery of oleuropein, hydroxytyrosol and tyrosol. This work underscores the potential of agri-food wastes to serve as renewable resources, fostering circular bioeconomy principles and contributing to environmental sustainability.

1. Introduction

It is well known that to face the escalating environmental challenges and a growing global population, the concepts of green economy and sustainability have emerged as crucial frameworks for encouraging a harmonious balance between economic growth and ecological integrity (Staniškis et al., 2022). Food waste is an important topic that raises ethical and environmental concerns, which is critical to this point. Nowadays, the problem of food waste and by-products is a key challenge for the implementation of sustainable resource management practices. Agri-food waste both of raw materials and food production has a negative impact on the environment, the nutritional status of the population, and food security (Hoehn et al., 2023). In the EU more than 60 million tonnes are generated annually as food waste. Concerning the wine industry, among 13 % of the grape processed become grape pomace as one of the main by-products, which consists of seeds, stems and skin (Drevelegka & Goula, 2020); while for the olive leaves, during the entire olive oil production process, about 25 kg/year of twigs and leaves are generated per tree (Markhali & Teixeira, 2024). Generally, such residues have been used mainly as animal feed, organic fertilizers, waste-to-energy incineration or discarded in the land (Drevelegka & Goula, 2020; Garcia-Garcia et al., 2019). However, these agri-food by-products and waste are considered precious resources due to the abundance of valuable compounds, such as phenolic compounds, flavonoids, anthocyanin pigments, tannins, stilbens, esters, and other active molecules with substantial health benefits (Chowdhary et al., 2021; Khwaldia et al., 2022; Sarker et al., 2023). For this purpose, in the past decade, many studies have been focused on the formulation of value-added products starting from food waste and by-products, including edible ingredients, functional foods, nutraceuticals, and cosmetics (Pattnaik et al., 2021). Despite numerous possibilities for food waste management, the transformation of waste into valuable resources through sustainable manners is limited. Consequently, the potential of food waste remains unexplored mainly due to the lack of sufficient research and policymaking (Sarker et al., 2023). Overall, the integration of green economy principles, sustainable practices, and innovative technologies is essential for addressing the complex challenges of food waste and resource management. Indeed, technologies and techniques

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aimed at extract bioactive compounds guaranteeing the sustainability issue as well, have been studied and developed to enhance the extraction yield of target molecules, considering energy consumption and cost benefits (Barba et al., 2016; Jha & Sit, 2022; Osorio-Tobón, 2020).

Addressing this issue not only requires innovative strategies to minimize waste but also involves the valorisation of local production, ensuring that food processing and agricultural practices are both economically viable and environmentally sustainable. The valorisation of the local production, contributing the use of the locally sourced materials, promotes and supports the regional economies as well (Salvador et al., 2022).

In this regard, grape pomace and olive leaves produced in the Marche region have been considered, in order to valorise local production as well as contributing to fully address the sustainability issues. Thus, in this work the recovery of polyphenolic compounds from grape pomace and olive leaves (pruning) was assessed by performing different extraction methods: conventional liquid extraction (LSE) (ethanol 70 %), ultrasound (UAE) and microwave (MAE) assisted extraction and supercritical CO_2 (SFE) extraction. The investigation of the different extraction technologies has never been studied in these local plant varieties. Then, the characterization of the differently obtained extracts was carried out by using spectrophotometric assays (1,1-diphenyl-2-picrylhydrazyl reducing activity (DPPH), total polyphenol content (TPC), total flavonoid content (TFC), total tannins content (TTC), and total anthocyanins content (TAC)) and HPLC-MS-MS and HPLC-DAD chromatography techniques were performed respectively in grape pomace and olive leaves extracts.

2. Materials and methods

2.1. Standards and reagents

Analytical standards of oleuropein, tyrosol and hydroxytyrosol were supplied by Sigma Aldrich (Milan, Italy). Stock solutions were prepared at 1000 μg/mL by dissolving 10 mg of each standard in 10 mL of HPLC grade methanol (EMSURE® Reag. pH Eur; Merck, Darmstadt, Germany). LC-MS-grade solvents, being methanol and 2-propanol, were supplied by Merck (Darmstadt, Germany) while ultrapure water was obtained from a Milli-Q SP water system (Millipore, Bedford, MA). Captiva PTFE 13 mm, 0.45 μm syringeless filter was bought from Agilent Technology (Santa Clara, CA, USA). Cyanidin-3-glucoside chloride, delphinidin-3,5-diglucoside chloride, delphinidin-3-galactoside chloride, petunidin-3-glucoside chloride, malvidin-3-galactoside chloride, quercetin-3-glucoside and kaempferol-3-glucoside were purchased from PhytoLab (Vestenbergsgreuth, Germany). The remaining 31 analytical standards of the 38 phenolic compounds were supplied by Sigma-Aldrich (Milan, Italy). Standard working solutions at various concentrations were prepared daily by appropriate dilution of the stock solutions with HPLC-grade methanol. Formic acid (99 %) was obtained from Merck (Darmstadt, Germany). Analytical-grade hydrochloric acid (37 %) was obtained from Carlo Erba Reagents (Milan, Italy). All solvents and solutions were filtered through a 0.2 μm polyamide filter from Sartorius Stedim (Goettingen, Germany). Before HPLC analysis, all samples were filtered with Phenex™ RC 4 mm 0.2 μm syringeless filter, Phenomenex (Castel Maggiore, BO, Italy). The Folin–Ciocolteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), Trolox equivalent ($C_{14}H_{18}O_4$), rutin equivalent ($C_{27}H_{30}O_{16}$), tannic acid equivalent ($C_{76}H_{52}O_{46}$), sodium carbonate (Na_2CO_3), sodium acetate($C_2H_3O_2Na$), ethanol (C_2H_5OH) and methanol (CH_3OH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH), potassium chloride (KCl), sodium nitrate ($NaNO_2$) and aluminium chloride ($AlCl_3$) were acquired from Carlo Erba reagents (Milan, Italy). The DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Glentham Life Sciences (Corsham, UK). All chemicals and reagents were analytical grade.

2.2. Sample preparation

Grape pomace, an ancient Lacrima di Morro d'Alba red grape variety, was provided by a local winery, Romagnoli (Marche, Italy), while the olive leaves (*Oleaeuropaea L.*) cultivar *Ascolana Tenera* were collected from a private farm located in Castignano, Ascoli Piceno, Marche, Italy (42°56′17.3″N 13°37′19″E). All samples were grown in the region of Marche (Italy) and harvested in 2023. In order to observe differences, in terms of the bioactive compounds' yield, raw materials have been pre-treated considering the logistic and scale-up process. Indeed, grape pomace were freeze-dried (technique most commonly used in the literature Wojdyło et al., 2009) and then the possibility of eliminating this process was evaluated by replacing it with air-drier, drying this at 40 °C for 14 h. While for olive leaves, drying at 40 °C for 24 h was evaluated and compared with fresh matrix as it is. In both cases, the most applicable options from a scale-up point of view were evaluated.

2.3. Extraction procedures

Before each extraction procedures, all samples were milled, using a kitchen grinder, until a fine powder was obtained and passed through a sieve (0.6 mm) to unify the particle size around 300 μm,.

All the extraction methodologies and pretreatment applied on grape pomace and olive leaves and relative samples are represented in the Table 1.

2.3.1. Conventional solid-liquid extraction

Solid-liquid extraction (SLE) was employed as a traditional technique for extracting bioactive compounds. Briefly, 1 g of the sample was mixed with 10 mL of a 70 % ethanol/water solution (maintaining a 1:10 drug-to-solvent ratio). This mixture was stirred magnetically at 250 rpm at room temperature for 4 hours, protected from light. After extraction, the mixture was centrifuged (IEC CL10 Centrifuge, Thermo Fisher Scientific, Waltham, USA) at 5000 rpm, at 4 °C, for 10 min, filtered through paper filter, and the resulting extracts were stored at −20 °C until they were analyzed.

2.3.2. Ultrasound-assisted extraction

The ultrasound-assisted extraction (UAE) technique was used to extract bioactive compounds from grape pomace and olive leaves according to the method of Alessandroni et al., 2024 with some modifications. For all samples, 1 g of material was added to 10 mL of ethanol 70 % in screw-capped test tubes. Extraction was performed in the dark at room temperature (25 °C) in a temperature-controlled sonication bath (FALC, Treviglio, Italy) at a frequency of 59 kHz for 30 min. Then, the extraction mixture was centrifuged at 5000 rpm, at 4 °C, for 10 min (IEC CL10 Centrifuge, Thermo Fisher Scientific, Waltham, USA). Finally, supernatant was kept at −20 °C until use.

2.3.3. Microwave-assisted extraction

The microwave-assisted extraction (MAE) of bioactive compounds from grape pomace and olive leaves were conducted using a commercial oven (Samsung, Suwon, South Korea). The microwave frequency was set at 2.45 GHz with a power of 500 W. The samples were processed individually in a treatment chamber equipped with a rotating rack. The temperature was monitored at both the beginning and end of the process, reaching approximately 60 °C. For the extraction, 10 g of powdered sample was placed in a closed vessel with 100 mL of a 70 % ethanol/water solution (maintaining a 1:10 drug-to-solvent ratio) and irradiation time was set to 3 min. Ethanol was chosen because it is a GRAS (Generally Recognized As Safe) solvent by the U.S. Food and Drug Administration (FDA) and authorized for food extraction by the European Commission under Directive 2009/32/EC. Furthermore, ethanol is known for its low toxicity, environmental friendliness, high effectiveness in polyphenol extraction, and suitability for large-scale processes

Table 1
Overview on matrix treatment applied for grape pomace and olive leaves.

Samples characteristics					
Grape pomace			Olive leaves		
Pre-treatment	*Extraction procedure*	*Sample name*	*Pre-treatment*	*Extraction procedure*	*Sample name*
Freeze-dried	Solid-liquid extraction	GFD-SLE	Fresh	Solid-liquid extraction	OLF-SLE
	Ultrasound-assisted extraction	GFD-UAE		Ultrasound-assisted extraction	OLF-UAE
	Microwave-assisted extraction	GFD-MAE		Microwave-assisted extraction	OLF-MAE
	Supercritical fluid extraction with carbon dioxide	GFD-SFE		Supercritical fluid extraction with carbon dioxide	OLF- SFE
Air-dried	Liquid extraction	GD-SLE	Air-dried	Liquid extraction	OLD-SLE
	Ultrasound-assisted extraction	GD-UAE		Ultrasound-assisted extraction	OLD-UAE
	Microwave-assisted extraction	GD-MAE		Microwave-assisted extraction	OLD-MAE
	Supercritical fluid extraction with carbon dioxide	GD-SFE		Supercritical fluid extraction with carbon dioxide	OLD- SFE

(Gil-Martín et al., 2022). Following extraction, the mixture was cooled using an ice bath. The cooled extract was transferred to centrifuge tubes, centrifuged at 5000 rpm, at 4 °C, for 10 min (IEC CL10 Centrifuge, Thermo Fisher Scientific, Waltham, USA), and filtered using filter paper. The resulting extract was stored at −20 °C until further use.

2.3.4. Supercritical fluid extraction with carbon dioxide

The operating condition of SFE ranges were determined by preliminary trials in which experimental and instrumental parameters, such as temperature, pressure, time, and co-solvent, were varied. The preliminary trials were guided by baseline conditions found in the literature (Da Porto & Natolino, 2024; Fiori et al., 2009; Manna et al., 2015; Pazir et al., 2021; Vatai et al., 2009). The best conditions were selected according to the concentration and profile of polyphenols present in extracts. Experimental activities were conducted using an SFT-120XW (Supercritical Fluid Technologies, Inc., US) bench-scale reactor. 5 g of materials ($\phi \leq 10$ mm) were placed in a bag and then loaded into the 100 mL volume extractor vessel. The setup featured two pressure control systems on the inlet and outlet valves, while the CO_2 flow rate was managed with a SFT Nex10 Liquid Carbon Dioxide Pump (Supercritical Fluid Technologies, Inc., US). The CO_2 flow rate was controlled by a restrictor valve and kept constant at 10 mL/min. The temperature of the treatment chamber was set to 50 °C for olive leaves and 45 °C for grape pomace, with pressure at 31 MPa. The SFE system included a co-solvent supply line, which utilized LL-Class Module Pump (Supercritical Fluid Technologies, Inc., US). The ethanol flow rate was set to 2 mL/min for 17.5 min, achieving a co-solvent percentage of 35 % of the treatment chamber volume. The total extraction time was 1 h and 15 min, given by alternating phases: 10 min of static phase followed by 15 min of dynamic phase. The extracts obtained at the end of the extraction were recovered in glass vials and stored at −20 °C until analysis.

2.4. Spectrophotometric assays (UV–Vis)

2.4.1. DPPH radical scavenging activity

The antioxidant activity was assessed via the DPPH method, which involves spectrophotometric measurement of the reduction of the 2,2-DiPhenyl-1-Picrylhydrazyl radical by antioxidant compounds. This evaluation was performed both on grape pomace extracts and olive leaves extracts, adapting the methodology set by Abouelenein et al., 2023 with some modifications. For each sample, 0.5 mL of extract, diluted 1:10, was combined with 4.5 mL of a 0.1 mM methanolic DPPH solution. The mixture was incubated for 30 min in darkness at room temperature. The decrease in DPPH radical concentration was then measured at 517 nm using an Agilent Cary 8454 UV–Vis spectrophotometer. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as the reference antioxidant, and results were expressed in terms of mg Trolox equivalents (TE) per mL extracts.

2.4.2. Determination of total phenolic contents (TPC)

The Total Phenolic Content (TPC) was determined spectrophotometrically, following the procedure described by Abouelenein et al., 2023 with some modifications. Briefly, to each tube, 0.5 mL of sample, diluted 1:10, was added, followed by 2.5 mL of Folin–Ciocalteau reagent and 7 mL of a 7.5 % w/w Na_2CO_3 solution in water. The reaction mixture was allowed to stand for 2 hours in darkness at room temperature, and absorbance was measured at 765 nm. TPC was quantified using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per mL of extracts.

2.4.3. Determination of total flavonoid contents (TFC)

The Total Flavonoid Content (TFC) was assessed using a slightly modified method described by Abouelenein et al., 2023. In a 15 mL tube, 0.5 mL of the sample solution, diluted 1:10, 0.15 mL of NaNO2 (0.5 M), 3.2 mL of methanol (30 % v/v), and 0.15 mL of AlCl3·6H2O (0.3 M) were combined. After 5 min, 1 mL of NaOH (1 M) was added to the mixture, which was then well mixed, and the absorbance was measured at 506 nm against a blank. The calibration curve for TFC was established using a rutin standard solution, adhering to the procedure. TFC was reported as mg rutin equivalents (RE) per ml of extracts.

2.4.4. Determination of total tannins contents (TTC)

The Total Tannins Content (TTC) was determined by the Folin-Ciocalteu method according to Alessandroni et al., 2024. The tannins content was expressed in terms of mg of tannic acid equivalents (TAE) per mL of grape pomace extracts.

2.4.5. Determination of total anthocyanins contents (TAC)

The TAC was determined by using the pH differential method, reported by Abouelenein et al., 2023. The TAC was estimated using the following formula:

$$TAC = [(A510nm - A700nm)pH1.0 - (A510nm - A700nm)pH4.5]MW \times TV \times DF \times 1000 / (\varepsilon \times L \times SW)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside, which equals to 449.2 g/mol, TV represents the extract total volume, DF indicates the dilution factor, ε is the extinction coefficient, which equals to 22,400 L/(mol × cm), L is the cuvette length of 1 cm, and SW represents the weight of sample or starting material.

The results were expressed as mg of cyanidin-3-glucoside equivalent (CGE) per mL of grape pomace extracts

2.5. Olive leaves extracts polyphenols HPLC-DAD quantification

The quantification of main olive oil polyphenols was performed on the obtained extracts using a 1260 Infinity II high-performance liquid chromatography instrument coupled with a diode array detector (HPLC-DAD) (Agilent Technologies, Santa Clara, CA, USA) according to a previously validated method (Ricciutelli et al., 2017). Results of oleuropein, hydroxytyrosol and tyrosol were quantified by using the calibration curves of the analysed compounds.

2.6. Grape pomace extracts: HPLC-ESI-MS/ MS polyphenols quantification

Thirty-six distinct phenolic compounds pertaining to various chemical classes, including phenolic acids, flavonols, flavan-3-ols, flavones, proanthocyanidins, anthocyanins and non-phenolic acids, were analyzed in the extracts. The analysis was conducted following a method previously described by Abouelenein et al., 2023 with some modifications. Briefly, 2 μL of filtered sample was injected in an Agilent 1290 Infinity series HPLC system, linked to an Agilent Triple Quadrupole 6420 from Agilent Technology (Santa Clara, California, USA), equipped with an electrospray ionization (ESI) source, able to operate in both negative and positive ionization modes. Data collection was performed using the MassHunter MS Optimizer software (Agilent). Chromatographic separation was achieved with a Synergi Polar–RP C18 column (250 mm × 4.6 mm, 4 μm) provided by Phenomenex (Chesire, UK), employing a mobile phase composed of (A) water and (B) methanol, each containing 0.1 % formic acid, at a flow rate of 0.8 mL/min. The gradient elution schedule was developed as follow: 0–1 min, isocratic phase at 20 % B; 1–25 min, linear increase from 20 % to 85 % B; 25–26 min, isocratic phase at 85 % B; 26–32 min, return to 20 % B, remaining for 8 min. The ESI source's drying gas was heated to 350 °C, with a nebulizer pressure at 55 psi, a capillary voltage of 4000 V and a gas flow rate maintained at 12,000 mL/min. Quantification was based on the integrated peak areas of the predominant product ions after detection through dynamic-multiple reaction monitoring mode.

2.7. Statistical analysis

The analysis of variance (ANOVA) was performed on the analytical replicates of the different extraction methods, while the *t*-test was performed on the analytical replicas of the same extraction method among the different pretreatments. The significance level used was $p \leq 0.05$. In addition, a multivariate statistical analysis was performed using the principal component analysis (PCA) with the objective of first comparing the raw data between samples with the analysed variables including specific phenolic acids and spectrophotometric assays. The statistic software used was XLSTAT version 2021.4.

3. Results and discussion

3.1. Grape pomace spectrophotometric results

3.1.1. DPPH results for grape pomace

The DPPH assays based on the ability of stable free radical to react with H-donors including phenolics (Roginsky & Lissi, 2005).. This assay represents a useful strategy to evaluate extraction methods and provide a preliminary characterization of samples before chromatographic analysis (Abouelenein et al., 2023). The value resulting from this test will be lower the more hydroxyl groups are present in the extracted compounds and results are expressed in mg of Trolox Equivalent/mL (mgTE/mL). Notably, compounds containing the structures of catechol and hydroquinone exhibited as well high antioxidant capacity, due to the reaction of phenoxy radicals which induced the formation of the hydroxy groups (Yamauchi et al., 2024). The results of the different extraction methodologies are present in Fig. 1A.

IC50 value represents the sample concentration necessary to scavenge the 50 % of DPPH free-radical. The IC50 values for grape pomace was 0.2293 mg/mL.

The DPPH values are very close to each other across all methods, ranging from about 0.227 to 0.235 mg TE/mL. The observed differences were related to the extraction conditions, methods and the pretreatment of samples. In terms of antioxidant activity, SFE is the most effective, when applied to both dried and lyophilized samples, whereas MAE, independently of the pre-treatment applied, and SLE for dried sample show the highest DPPH values. In fact, grape pomace processed with SFE and UAE, both dried or freeze-dried, showed values lower than the IC50.

Regarding the grape pomace pretreatment, there is no substantial difference between dried and freeze-dried samples, except for the liquid extraction. These results could suggest that the drying methodology does not significantly affect the antioxidant capacity of the extracts.

Comparing the results of this study, with those reported in the literature, they are higher than those of Ruberto et al., 2007; in fact, their results range between 0.014–0.039 mg TE/mL, whereas the range of this study is between 0.22–0.23 mg TE/mL. This may be due to the cultivar variability of the pomace, which have different profiles of bioactive compounds.

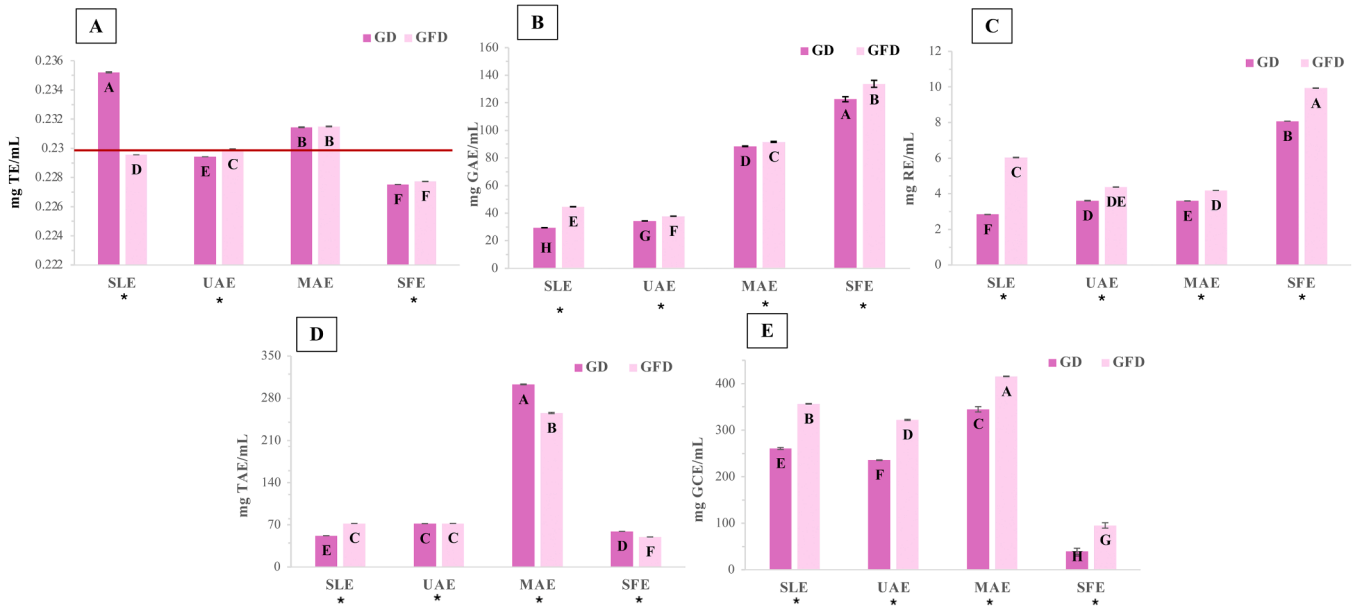

Fig. 1. a) Antiradical activity by DPPH method b) Total polyphenol content (TPC) c) Total flavonoid content d) Total tannins content e) total anthocyanins content of the pomace extracts obtained by the different methodologies applied.
Different letters indicate statistically significant differences between the different samples.
(*) indicates statistically significant differences at $p < 0.05$ (Student's *t*-test) between the two pretreatments applied on the grape pomace matrix.

3.1.2. TPC results for grape pomace

The total polyphenol content (TPC) of grape pomace extracts was determined using the Folin–Ciocalteu assay and the results are shown in Fig. 1B The total phenolic content ranged from the lowest value 29.44 mg GAE/mL (GD-SLE) to the highest 133.78 mg GAE/mL (GFD-SFE). Results were in good accordance with those obtained by HPLC-MS/MS analysis (Table 1S).

The data clearly shows that different methods have a great variation of effectiveness in extracting phenolic compounds from grape pomace. The most effective method for polyphenolic extraction is the SFE, even if it has the greater variability, followed by MAE. SLE and UAE are less effective; they showed consistent results but are significantly less effective in polyphenolic extraction compared to the first two methods. Focusing on the pretreatment, lyophilization seems to slightly improve the extraction efficiency across all methods, possibly due to better preservation of the compounds during the drying process. In fact, according to Casagrande et al., 2019, the TPC of grape pomace varied from 17.91 to 35.10 mg GAE/mL results, very similar to this study.

The obtained results are in accordance with Iora et al., 2015, according to which the total phenolic contents of grape pomace varied between 3014.55 and 5101.82 mg GAE/100 g d.m., and to Tournour et al., 2015 where TPC values range from 69.3 to 131.7 mg GAE/mL.

3.1.3. TFC and TTC results for grape pomace

The Total Flavonoid Content (TFC) and the Total Tannins Content (TTC) was determined by the Folin-Ciocalteau assay, and the results are shown respectively in Fig. 1C and 1D The TFC ranged from the lowest value of 2.83 mg RE/mL, which corresponds to the LSE for GD, to the highest value 9.93 mg RE/mL, which corresponds to the SFE method for GFD.

While the TTC ranged from 49.77 mg TE/mL, relative to the SFE for GFD sample, to 302.92 mg TE/mL, MAE of then GD.

Related to total flavonoid assays, the most effective extraction methodology is SFE, yielding the highest TFC values for both GD (8.07 mg RE/mL) and GFD (9.93 mg RE/mL) samples. For dried ones the best method is the SFE followed by UAE and MAE, which are quite similar, and the least efficient method is SLE. Also, for GFD the best methodology is the SFE, followed by the SLE, UAE and the least effective MAE. Related to the impact of the drying method, lyophilization produces generally higher values in TFC, compared to conventional drying, across all extraction methodologies. The main difference for SLE between the two pre-treatment applied was around 36 % and for SFE about 10 %. In both cases, the best performing results was obtained in freeze-dried grape pomace. These results are in accordance with the literature, in fact according to Xu et al., 2016 the flavonoids content ranged from 1.01 mg CE/mL to 91.75 mg CE/mL with differences among the different types of wine cultivar, the obtained results fall within the range of reported values. Instead, they are lower compared to the outcomes of Tseng & Zhao, 2012, where the flavonoids content ranged from 30.16 to 106.61 mg CE/mL; this could depend on the different type of cultivar and the geographical location. Related to tannins content, MAE is the most effective method for tannins, yielding much higher TTC values (302.92 mg TE/mL for GD and 255.53 mg TE/mL for GFD) compared to other methods. The UAE has reported great results for tannins extraction, as well as the LSE, while the least effective method is the SFE.

Regarding the impact of drying method, we cannot assess that a treatment is largely better than the other; we can say that some extraction methodology has better results with the lyophilized samples (LSE or UAE technique with the lyophilized samples), while some with the dried ones (MAE or SFE from GD yield better results).

The results obtained are in line with those reported in literature; in fact, TTC for fresh pomace sample ranged from 11.02 to 16.02 mg of catechin/mL, depending on the different variety, while it was higher for dried grape pomace sample (ranging from 20.91 to 24.53 mg of catechin/mL)(Carmona-Jiménez et al., 2018).

3.1.4. TAC results for grape pomace

The Total Anthocyanins Content (TAC) was determined by using the pH differential method and the results are shown in Fig. 1E.

For both dried and freeze-dried samples, the MAE technique yields the highest mean values (344.71 mg CGE/mL and 415.55 respectively mg CGE/mL). The least effective methodology for anthocyanins extraction is the SFE, which yields the lowest value for both sample types (39.65 mg CGE/mL for GD and 95.27 mg CGE/mL for GFD). This data suggests that the choice of the pretreatment and the extraction method has a significant impact on the anthocyanins content. According to Rockenbach et al., 2011, the highest TAC among several species of red grape (Isabel, Pinot Noir, Cabernet, and Primitivo) was reported to be around 911 mg/100 g d.m.. This result is higher of that of our study, owing to the difference in the processing of the raw material and the cultivar. Moreover, the obtained results are higher compared to Carmona-Jiménez et al., 2018; in fact, in their study the dried pomace sample ranged from 1.72 to 6.57 mg of malvidin equivalent/mL. This could depend on the differences in the extraction methodologies. Regarding how the thermal process affect the grape material, the drying process does not affect directly the amount of anthocyanins; while as stated by Wojdyło et al., 2009 the most suitable treatment was the freeze drying, by reporting similar values to dried sample.

3.2. Olive leaves spectrophotometric results

3.2.1. DPPH results for olive leaves

To measure the antioxidant activity of the extract, the DPPH radical scavenging capacity was measured in olive leaf extracts, and results are reported in Fig. 2A. The IC50 values for olive leaves extracts was 0.2312 mg/mL.

The DPPH values are very close to each other across all methods, ranging from 0.227 to 0.232 mg TE/mL. Analyses of the results related to dried olive leaves extracted with UAE and MAE, plus those related to fresh leaves extracted with SFE, reveals no statistically significant differences. Additionally, these values represent the lowest antioxidant activity compared to other extraction methods. Comparing these findings with those reported in the literature, very few studies reported the value for the cultivar Ascolana tenera; indeed Zhang et al., 2022, that investigated the potential antioxidant activity of 32 cultivar of olive leaves, showed higher value for DPPH with respect to the cultivar of this study. This is probably due to different microclimate; in fact, it is planted in a research garden in China. Moreover, extraction methods and pretreatment procedure can influence results. In the present study also the fresh leaves extract with microwave demonstrates a good antioxidant activity of 0.23 mg TE/mL. Regarding pretreatment, there is a notable difference between fresh and dried samples. Across all extraction methods, OLD yielded higher values than fresh leaves, except in the case of SFE, where the highest value was obtained from fresh leaves. This outcome suggests a higher extraction efficiency in fresh olive leaves, potentially due to the high-water content of the matrix, except for SFE. As demonstrated by Rosa et al., 2019, using ethanol-water solution as a co-solvent in SLE enhances extraction efficiency. This trend for fresh leaves in SFE is also supported by the results of TPC and TFC. Furthermore, MAE method employed in the current study exhibited good extraction for OLF, showing value of antiradical activity close to IC50. In contrast, extracts obtained by solid-liquid extraction and UAE showed low values, particularly for OLF. This supports the notion that novel extraction technologies can maximize yield in less time, improve quality, and are considered more environmentally friendly.

3.2.2. TPC results for olive leaves

The total phenolic content (TPC) of Olea europaea leaf extracts, measured by the Folin-Ciocalteu method, varied with the extraction technique employed. The data clearly shows that different methods have a great variation of effectiveness in extracting phenolic compounds from olive leaves (Fig. 2B). The highest yield of total phenolics was extracted

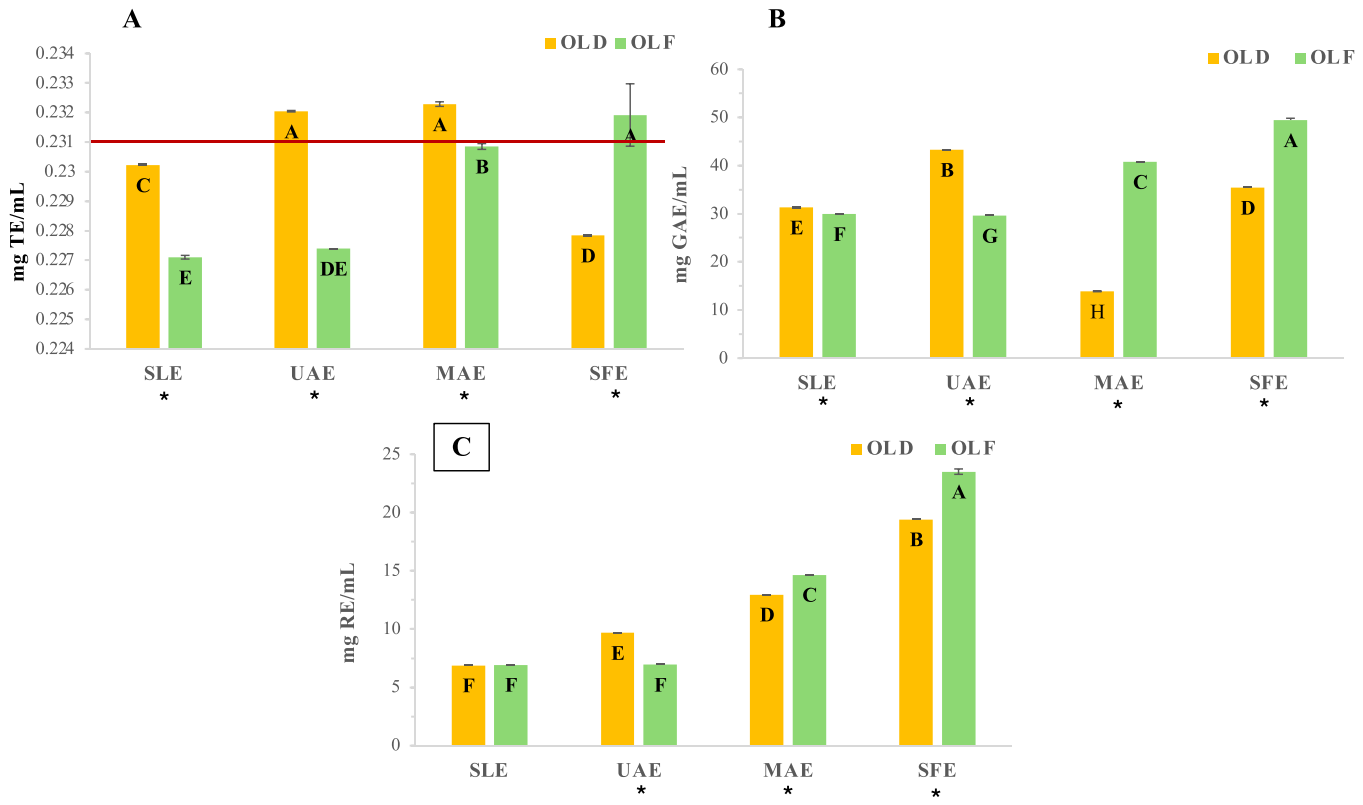

Fig. 2. a) Antiradical activity by DPPH method b) Total polyphenol content (TPC) c) Total flavonoid content of the olive leaves' extracts by the different methodologies applied.
Different letters indicate statistically significant differences between the different samples.
(*) indicates statistically significant differences at $p < 0.05$ (Student's *t*-test) between the two pretreatments applied on the olive leaves matrix.

from OLF by SFE: 49.43 ± 0.35 mg GAE/mL. When ethanol is used as a co-solvent, this enhances the polarity of CO_2 and increases the recovery of polyphenols. Also, the increase in extraction separation of the phenolic compounds from the leaves is attributed to ethanol affecting the cell membrane permeability through the action on the phospholipid bilayer structure (Kyriakoudi et al., 2024). Whereby the same method of extraction for OLD gave the lower value of TPC, which is 35.48 ± 0.03 mg GAE/mL, with significant differences among them to show the importance of pretreatment of a matrix. Comparing these findings to the literature, Kyriakoudi et al., 2024 found that dried and homogenized leaves of the Koroneiki variety (Olea europaea var. microcarpa alba) treated with SFE at different temperatures (35–90 °C) and with various co-solvents had significantly higher TPC values, ranging from 59.19 ± 4.73 to 299.97 ± 5.43 mg GAE/mL. Furthermore, the TPC obtained using SFE, as reported by Caballero et al., 2020 were lower for olive pruning residues, with values between 7.94 and 11.54 mg GAE/mL., compared to our findings. A. D. Rosa et al., 2019 also observed lower TPC values, ranging from 2.21 to 11.49 mg GAE/mL, across all tested temperatures (20, 45, and 60 °C) and pressures (8, 16.5, and 25 MPa) during supercritical fluid extraction. Moreover, in the same study, Rosa et al., 2019 found higher TPC values when using pressurized liquid extraction, particularly when ethanol was employed as solvent in combination with a temperature of 60 °C. In the present study, the ultrasound-assisted extraction (UAE) method proved to be the second most effective for extracting phenolic compounds, achieving a high yield from dried olive leaves with a concentration of 43.28 ± 0.04 mg GAE/mL. In contrast, using the same extraction conditions for fresh olive leaves resulted in a lower yield of 29.64 ± 0.03 mg GAE/mL. These are in line with the literature, particularly with the results reported by Irakli et al., 2018 who found similar extraction efficiency for olive leaves dried at 40 °C. On the contrary, Zhang, et al., 2022 documented a phenolic content range of 7.76–8.79 mg GAE/mL. for different olive leaf varieties from Spain, Italy, and China. Focusing on the pretreatment of the olive leaves, our results are aligned with previous studies, especially those by Zhang, et al., 2022 who demonstrated that drying processes enhance the release of extractable phenolic compounds from leaves. Compared to fresh leaves, hot air drying effectively increased the content of iridoids, such as oleuropein and secoxyloganin. Focusing attention on the other two extraction methods, MAE provided high extraction efficiency on fresh olive leaves, with a value of 40.80 ± 0.01 mg GAE/mL. The value shows a significant difference in TPC, compared to the obtained value from dried olive leaves, that was 13.90 ± 0.09 mg GAE/mL. These values are higher compared to those found by Di Meo et al., 2022 for other varieties of Italian olive cultivars, extracted using 70 % ethanol under different microwave power levels and at different extraction times. These include the varieties Caiazzana, Coratina, Ortice, Frantoio, and Leccino. LSE method also provided good results, with only slight differences between fresh and dried olive leaves, which respective TPC values were 29.92 ± 0.02 mg GAE/mL and 31.36 ± 0.05 mg GAE/mL. It is crucial to note that the synthesis and the concentration of phenolic compounds in olive leaves may be strongly influenced by the position of leaves on the olive tree, mineral content of the soil, exposure to sunlight, geographical location, cultivar, season, and drying techniques applied to the leaves (Ghomari et al., 2019).

3.2.3. *TFC results for olive leaves*

The TFC was between the lowest value obtained for liquid extraction of OLD, at 6.88 ± 0.01 mg RE/mL, and the highest value obtained for SFE on OLF at 23.53 ± 0.01 mg RE/mL. As shown in Fig. 2C, the yield of flavonoids increases with the use of novel extraction technologies. The most effective method for flavonoid extraction was the SFE, which achieved a content of 23.53 ± 0.01 mg RE/mL for OLF and 19.41 ± 0.01

mg RE/mL for OLD. This result may be attributed to the types of flavonoids present on the leaf surface (e.g., in leaf waxes), which are typically highly methylated and lack sugar substitution, making them easier to extract using SFE (Kyriakoudi et al., 2024). Comparing our data with the literature, Putnik et al., 2017 showed lower flavonoid levels when using a temperature (60 °C) similar to ours. They also tested the efficiency of the extraction by increasing the temperature to 100 °C, which allowed them to extract more flavonoids, but the levels were still below our results. In the contrary, Kyriakoudi et al., 2024 reported that a temperature of 90 °C increased flavonoid content in the range of 28.39–98.72 mg RE/mL. On the other hand, Rosa et al., 2019 obtained similar results (24.12 ± 1.05 mg CAT/mL) using pressurized liquid extraction at a pressure of 10.3 MPa, a temperature of 40 °C, and ethanol as a co-solvent. Our results demonstrate a high extraction yield in fresh olive leaves, which may be due to the high-water content during extraction with SFE. In fact, as demonstrated by Rosa et al., 2019 using an ethanol-water solution as a co-solvent in pressurized liquid extraction enhances the efficiency of flavonoid extraction. According to our results, the second most effective extraction method for flavonoids was MAE, which yielded values of 14.65 ± 0.02 mg RE/mL for fresh and 12.95 ± 0.01 mg RE/mL for dried leaves. When comparing those results with the literature, they are higher than those reported by Di Meo et al., 2022, who studied MAE of leaves from various Italian cultivars (*Caiazzana, Coratina, Ortice, Frantoio*, and *Leccino*) using 70 % ethanol under different microwave power levels and extraction times. This difference may be related to the fact that the initial matrices were of different cultivars that presented different profiles of bioactive compounds, besides the distinct methods of pretreatment and time-temperature exposition of leaves. When considering UAE and SLE, the efficiency of extraction decreases. These two extraction methods yielded quite similar results, with values of 6.96 ± 0.01 mg RE/mL for fresh leaves extracted using UAE, 6.92 ± 0.01 and 6.88 ± 0.01 mg RE/mL for fresh and dried leaves extracted with classic solvent extraction. Data indicate a higher flavonoid concentration in the extract using UAE from dried leaves at 9.69 ± 0.01 mg RE/mL. This runs in opposition to the trend observed in other methods of extraction, which gave higher concentrations of flavonoids from OLF. This difference may be attributed to the ultrasound's capability to enhance solvent penetration into dry plant material, which can increase pore size in cellular tissues and boost diffusion rates across cell walls, resulting in more efficient extraction (Alessandroni et al., 2024). Comparing the results obtained by UAE, we notice that our extraction method is more efficient that the method reported by Hannachi et al., 2019, which obtain as best results of extraction condition for flavonoid content 2.41 mg RE/mL. Indeed, for UAE at similar temperature but with longer time of treatment, Şahin et al., 2015 obtained similar results (9.98±0.02 mg CE/mL). It is important to note that both biotic and abiotic factors can qualitatively and quantitatively affect the flavonoid composition of olive leaves. These factors include sunlight exposure, geographic location, cultivar type, seasonal variations, and the pretreatment methods applied.

3.3. Grape pomace extracts polyphenols HPLC-ESI-MS/MS results

Phenolic compounds in grapes and wine play a significant role in determining the quality and authenticity of wine and are considered specific markers during the winemaking process. These compounds are secondary metabolites, meaning they are not directly involved in the plant's growth or reproduction, but they play the role of defence against pathogen and UV radiation. In the context of winemaking, phenolic compounds undergo various transformations during fermentation and aging, contributing to the wine's flavor, color, mouthfeel, and antioxidant properties (Pascual et al., 2016).

Phenolics can be classified as flavonoids (e.g. anthocyanins, flavan-3-ols, flavonols) and non-flavonoids (e.g. phenolic acids, stilbenes). In the current study, 29 phenolic compounds were quantified in the different extracts obtained from grape pomace with different extraction methodologies and pretreatment: 3 flavan-3-ols, 5 anthocyanins, 9 flavonols, 1 dihydrochalcones, 1 flavanone and 10 phenolic acids. (Fig. 3, Table 1S and Fig. 1Sa).

Considering the sum of HPLC-MS/MS analyzed compounds, TPC were 311.90 mg/kg for GD-UAE extract, that is the highest values among all treatments; on the other side, the lowest values was 44.30 mg/kg for GFD-MAE. In general, the more suitable pretreatment for phenolic compounds is the drying process, which does not affect the bioactive compounds, whereas it seems to enhance extraction. Regarding extraction methodologies, the best one is the UAE, followed by SFE, liquid extraction and the least effective microwave assisted extraction.

3.3.1. Flavan-3-ols

Flavan-3-ols are a group of flavonoids that include important compounds for human diet, such as catechins and proanthocyanidins. These molecules are easily found in fruits and vegetables; for example, catechins influence the bitterness and astringency of wine; they also play a role in antioxidant activity, while proanthocyanidins contribute to the astringency, structure, and aging of wine, especially in red wines (Pascual et al., 2016).

The three major compounds related to this phenolic group are catechin, epicatechin and procyanidin B2.

Regarding the extraction methodology, for total catechins the more suitable technology is the UAE, while the SLE and the MAE are the least effective for this group of compounds. Interestingly, SFE, which was ineffective for anthocyanins, showed good results for catechin and epicatechin. Procyanidin B2 shows the highest variability, with zero values in some methods and very high values in others, while epicatechin is consistently the least abundant of the three compounds.

Procyanidin B2 is the compound with the greater variability; in fact, in the samples where it is present, it shows often higher values compared to catechin and epicatechin, suggesting it might be the most abundant flavan-3-ol in these samples.

The last detected compound is epicatechin; it is present in all the samples in lower concentration than the other two molecules; for catechin, the best methodology for its extraction is UAE. The amount of catechin and epicatechin is lower, as compared to different study in literature (Rockenbach et al., 2011), but it is quite interesting that different studies did not identify the procyanidin B2 in their samples.

Focusing on the pretreatment, it is possible to assert that the freeze-dried treatment affects directly the final concentration of procyanidin B2; in fact, it is not detected in all the lyophilized sample, except for the ultrasound one.

3.3.2. Anthocyanins

Anthocyanins are highly concentrated pigments in the grape skin, responsible for red colour of grape but also of red wine.

Moreover, this group of compounds could have a therapeutic effect, such as the prevention of microbial infection, on hypertension and liver dysfunction (Pazir et al., 2021).

These compounds show particularly high concentrations in some extracts, especially with the GFD-UAE and GD-UAE methods. The main anthocyanins detected in grape skin are 3-O-glycosides of malvidin, petunidin, cyanidin, peonidin and delphinidin Abouelenein et al., 2023. In this study five different anthocyanins were detected in the different extracts; malvidin-3-galactoside was the predominant one, followed by petunidin-3-glucoside and delphidin 3,5 diglucoside. The predominant anthocyanin is malvidin-3-galactoside, that represents consistently the most abundant anthocyanin across all extraction methods, while cyanidin-3-glucoside is generally the least abundant. Petunidin-3-glucoside was detected previously in the Lacrima di Morro d'Alba wine at 23.9 ± 7.4 mg/kg (Boselli et al., 2008); in the current study it is detected in the same concentration only for the GFD-UAE method. In addition, all samples were characterized by the complete absence of the anthocyanin pelargonidin-3-rutinoside, as also in Boselli et al., 2008, while Abouelenein et al., 2023 detected low contents of this

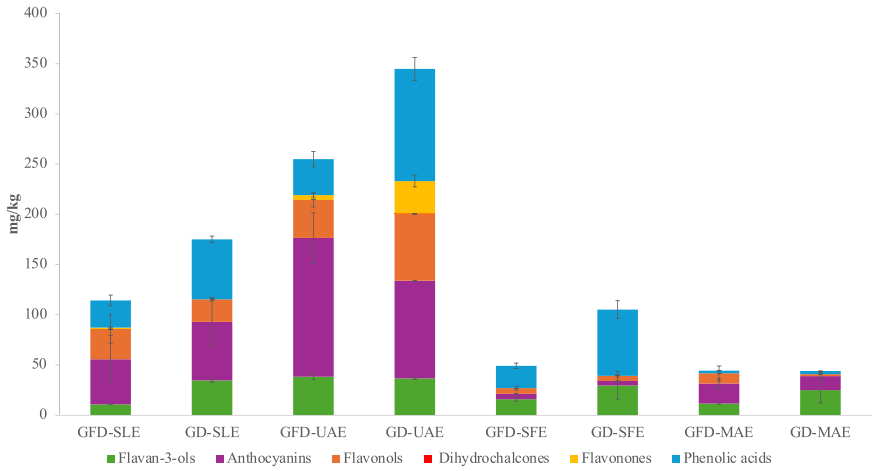

Fig. 3. Phenolic compounds determined by HPLC-MS/MS of the different grape pomace extracts divided according to the phenolic classes.

anthocyanins in grape pomace.

Regarding the extraction methodology, the ultrasound assisted methods is consistently the most effective technology, yielding the highest concentrations for all anthocyanins, particularly for malvidin-3-galactoside. Whereas SFE method is the least effective, the obtained result suggest that different anthocyanins respond differently to various extraction methods, some compounds being more suitable for specific extraction techniques than others.

Regarding the pretreatment of the grape pomace, the dried samples often show higher concentrations than their freeze-dried counterparts; differences are particularly significant for LSE and MAE methods. For UAE extraction, GFD samples show higher concentrations than GD samples.

3.3.3. *Flavonols*

Flavonols are one of the most important and abundant species of phenols; these compounds are mainly known for their colouring effect (interacting with anthocyanins and able to enhance colour intensity) and for their high antioxidant activity.

In flavonols, the three predominant structures are myricetin, quercetin, and kaempferol, compounds that are reported to be mostly present also in grape pomace, along with isorhamnetin (Liang et al., 2012). In the present study 9 flavonols have been identified, but not all compounds are present in each sample. Quantified flavonols are: 2 quercitrin derivatives (isoquercitrin and quercitrin), 2 kaempferols (kaempferol-3-glucoside and kaempferol), myricetin, isorhamnetin, quercetin, rutin and hyperoside. In the analysed samples, flavonols represented the least abundant group of compounds. Among samples, myrecetin is the most abundant flavonoids in all sample, except in GFD-SFE and GD- SFE where it is not present, while kaempferol-3-glucoside is the least abundant, being present only in GFD-UAE and GD-UAE samples. Regarding the extraction methodology, the best one for this group of compounds are the SLE and UAE methods, that generally yielded the highest content across all samples. MAE seems to be less effective overall, particularly for dried samples; interestingly enough, SFE yielded the lowest total flavonoid content, but showed selectivity for specific compounds (e.g., relatively high quercetin extraction). Focusing on the pretreatment, freeze-drying process generally retains more total flavonoids compared to the drying one; in fact, the GFD samples have the highest content across most extraction methods: the difference is particularly significant for the MAE and SLE. Furthermore, drying treatment seems to particularly reduce kaempferol content, while increasing quercetin content. Related to literature, in the present study quercetin 3-o-glucoside is not detected, whereas Negro et al. (2021) suggests this compound as the most present. The highest quercetin content (GFD-UAE, 7.86 mg/kg) is higher compared to Rockenbach et al., 2011, (5.7 mg/kg), but lower compared to Onache et al., 2022 (36.4, 262.0 and 89.5 mg/kg).

One interesting aspect is the highest amount of kaempferol and isorhamnetin in all the extraction methods, compared to different study; this suggests that the concentration of these two compounds could depend on different matrix, applied pretreatment and co-solvent used for extraction. (Abouelenein et al., 2023; Ruberto et al., 2007).

3.3.4. *Phenolic acid*

The main phenolic acid components in pomace are gallic acid, vanillic acid, tartaric acid, and coumaric acid (Casagrande et al., 2019). The two main groups of phenolic acids are hydroxybenzoic (HBA) and hydroxycinnamic (HCA). Protocatechuich, vanillin, gallic and syringic are the main HBA acids present in wine. Regarding the HCA acids, the most present in wine are q-coumaric, caffeic, ferulic, and cis and trans cinnamic: caftaric, cutaric and fertaric. In the present study 10 different phenolic acids were identified and quantified; among hydroxybenzoates the main compounds identified were: gallic acid, 4-hydroxy benzoic acid, 3-hydroxybenzoic acid, vanillic acid, syringic acid and ellagic acid. Among the hydroxycinnamic acids chlorogenic acid, caffeic acid, p-coumaric and ferulic acid were identified (Casagrande et al., 2019). The most effective extraction methodology for this group of compounds is UAE (111.5 mg/kg GD-UAE and 35.8 mg/kg GFD-UAE); this method is particularly effective for gallic, syringic and ellagic acid. Furthermore, this methodology coupled with drying as pretreatment is the best methodology among all; this is the only method able to extract p-coumaric and ferulic acids. SFE and LSE are both effective; in particular, the SFE is effective for chlorogenic acid, 4-hydroxy benzoic acid, and 3-hydroxy benzoic acid, while LSE shows good results for several samples. The least effective extraction is the microwave assisted: it gave acceptable results only for gallic acid in both GFD and GD samples.

Focusing on the pretreatment, dried samples generally yield higher concentrations of phenolic acids to the freeze dried one, suggesting that the drying method could affect the extractability of some acids. In fact, several compounds, such as 4-hydroxy benzoic acid, 3-hydroxy benzoic acid, vanillic acid, p-coumaric acid, and ferulic acid are only detected in dried samples.

The obtained results are in accordance with previous studies that quantified the total phenolic acid contents from 60.5 to 973.5 mg/kg for red grape peels. In general, a wide variabilities of the phenolic acids content occur between grape pomace and wine; this depends on the cultivar, climatic condition, harvesting time and methodology and ripening stages (Boselli et al., 2008).

Previously, wines of Lacrima di Morro D'Alba were reported to contain gallic acid as the most abundant phenolic acid in all wine samples (from 261 to 473 mg/kg) (Boselli et al., 2008); in this study on grape pomace of the same cultivar, the gallic acid is one of the most

present phenolic acids, but in lower amount. However, one of the most abundant compounds in grape pomace, according to literature, is the vanillic acid; this compound in our study was detected in only two samples in good amount (13.22 mg/kg GD-LSE and 21.65 mg/kg GD-UAE). The obtained result is higher compared to Peixoto et al., 2018 (4.0 mg/kg), but lower compared to other studies (Milinčić et al., 2021).

Fig. 4 shows the PCA score and loading plots, as well as correlations between grape pomace extracts' samples and all the performed analysis, both spectrophotometric assays and phenolic compounds concentrations. A total 75.72 % of data variability was explained by the sum of the two principal components (PCs). The extraction procedures, independently of applied pre-treatment, showed substantial effect on the specific phenolic compounds and spectrophotometric assays of grape pomace extracts, resulting in four distinct groups, as shown in the graphs. By analysing the distribution of the various extracts in relation to the measured variables, it is observed that the samples extracted with MAE are clearly distinguished from the others, being positioned at the top left. This indicates a significantly different chemical composition compared to other extraction methods, suggesting a potential and lower efficiency in the recovery of certain phenolic compounds. Samples extracted with SFE are grouped in the lower left of the graph. This suggests that this extraction method produces a unique phenolic profile, probably less correlated to the presence of key compounds such as anthocyanins.

Samples extracted with LSE are arranged close together, suggesting a similar phenolic composition between these samples. Their position in the graph indicates the extraction of specific phenolic compounds, which distinguishes them from other extraction methods.

Samples extracted using UAE, independently of the pre-treatment applied, were well clustered in the right part of the graph, far from the other extraction methods, where mostly of the identified phenolic compounds were located. This distribution indicates that UAE is particularly effective in recovering anthocyanins, flavonols, procyanidin-B2 and phenolic acids.

Superimposing the two images, GFD-LSE and GD-LSE samples are positively correlated to the content of TAC, DPPH and flavan-3-oils. In contrast, GFD-UAE and GD-UAE samples showed a positive correlation with the presence of anthocyanins, flavonoids and phenolic acids. The GFD- SFE and GD- SFE samples are positively correlated to flavonoid content, while the GFD-MAE and GD-MAE samples are associated with the TTC test.

3.4. Olive leaves HPLC-DAD

The predominant phenolic compounds of the obtained olive leaves' extracts from *Olea Europaea* L., cultivar Ascolana Tenera, are shown in Table 2 and Fig. 1Sb Among the most representative as oleuropein, hydroxytyrosol and tyrosol on olive leaves, in the current study other phenolic components have been investigated (vanillic acid, ferulic acid and p-cumaric acid); however, the latter have not been detected in all olive leaves samples' extracts. The large variability of phenolic compounds observed in olive leaves' extracts may derive from various factors, including plant age, environmental growth conditions (sunlight, temperature and availability of water), physiological stage and other variables that have not fully explored (Markhali & Teixeira, 2024)

Several studies described oleuropein to be the most representative phenolic in olive leaves (Di Meo et al., 2022; D'Avanzo et al., 2024). In particular, oleuropein plays an important role in the prevention of diseases and showed beneficial effects such as antioxidant, anti-inflammatory, antiatherosclerotic, and anticancerogenic properties.

Oleuropein is a phenolic secoiridoid glycoside which consists of hydroxytyrosol, elenolic acid, and a glucose molecule, due to the free hydroxytyrosol being formed by β-glycosidase hydrolysis. As well as oleuropein for hydroxytyrosol has been demonstrated the same biological properties, while for tyrosol lower bioactivity compared to hydroxytyrosol has been observed (D'Avanzo et al., 2024).

As shown by spectrophometric assays, concerning the specific compounds' quantification of olive leaves' extracts different behaviors have

Table 2
Phenolic compounds (mg/Kg) determined by HPLC-DAD of the different olive leaves extracts.

Sample	Tyrosol	Oleuropein	Hydroxytyrosol
OLF-SLE	$22.94\pm0.30_{B}$	$629.82\pm8.12_{D}$	$439.46\pm36.32_{D}$
OLD-SLE	$24.90\pm0.39_{A}$	$669.75\pm10.13_{C}$	$450.32\pm20.97_{D}$
OLF-UAE	$25.15\pm0.95_{A}$	$730.47\pm9.51_{B}$	$1323.2 \pm 47.93_{C}$
OLD-UAE	$25.09\pm0.02_{A}$	$757.57\pm2.27_{A}$	$3309.96\pm15.73_{B}$
OLF-MAE	$21.77\pm2.43_{B}$	$602.87\pm4.71_{E}$	$451.38\pm2.99_{D}$
OLD-MAE	$22.74\pm0.19_{AB}$	$176.16\pm1.43_{F}$	$3995.87\pm74.90_{A}$
OLF-SFE	0 ± 0	$129.76\pm10.66_{G}$	0 ± 0
OLD-SFE	0 ± 0	$116.07\pm10.16_{G}$	0 ± 0

Different letters indicate statistically significant differences between the different samples.

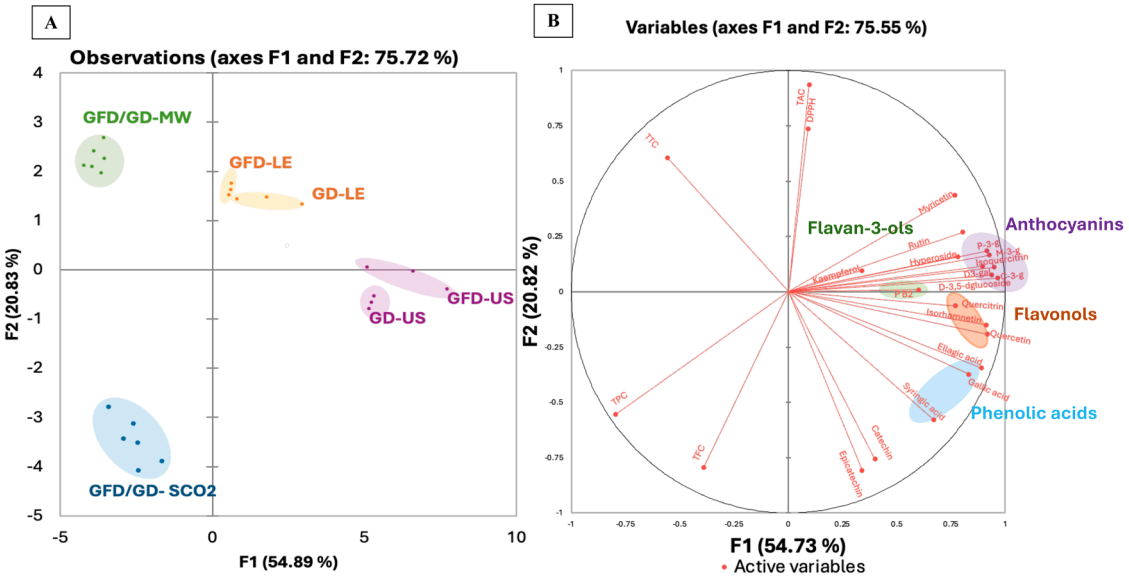

Fig. 4. (a) The PCA score plot representing the distribution of the different obtained grape pomace extracts. (b) The PCA loading plot demonstrating the correlation between the 29 individual phenolic compounds and spectrophotometric assays variables of the grape pomace matrix.

been observed, depending on the different extraction procedures. The highest yield, among the differently tested compounds, was observed for the oleuropein, in particular by using LSE and UAE as extraction methods, regardless of the pre-treatment applied, yielding from 630 to 758 mg/kg, respectively, for OLF-LSE and OLD-UAE extract. Lower amount of oleuropein was found in the extracts obtained by using SFE either using fresh leaves or dried ones (around 116 mg/kg). The latter results, by using HPLC-DAD, were not in agreement with the TPC, which revealed good retention by using SFE, probably ascribed to the non-specific and less sensitive spectrophotometric methodology. However, comparing these finding to the literature, lower content of oleuropein was obtained in olive leaves extract obtained by applied SFE at 250 bar and 40 °C with a flow rate of 20 mL/min by using ethanol as co-solvent (7.44 mg/kg) (d'Avanzo et al., 2024). Concerning the application of MAE, significant differences were observed between OLF and OLD extracts, showing higher content of oleuropein in OLF, due to the degradation of oleuropein in OLD probably through the consequent application of different temperature that could causes the activation of degradative enzyme. Di Meo et al. (2022) reported higher values of oleuropein in olive leaves from *Caizzana* cultivar harvested in march, air-dried at 20 °C for 10 days, and subsequently treated with MAE at 300 W for 2 min by using EtOH/H_2O 70:30 (57.3 mg/kg). Concerning the extraction yield of hydroxytyrosol higher value, around 1300 mg/kg was obtained for olive leave's extract obtained by the application of UAE in OLF. Instead, lower values were observed for extracts obtained by MAE for both OLF and OLD, while for the extracts achieved by SFE independently of the pre-treatment applied, this compound has not been detected.

Martín-García et al. (2022) reported lower values of hydroxytyrosol, ranged from around 50 mg/kg to 140 mg/kg in seven different olive leaf cultivars (*Arbequina, Arbosana, Changlot Real, Frantoio, Koroneiki, Picual, and Sikitita*) by applying respectively UAE sonotrode (mixture: 1/400 (w/v) with EtOH/H_2O (55:45, *v/v*) at 151 W for 8 min) and UAE bath (mixture: 1/100 (w/v) with EtOH/H_2O (80:20, *v/v*) at 35 kHz for 20 min). Similar results, in terms of hydroxytyrosol content, were obtained by using UAE with natural deep eutectic solvents, made with different mixture and molar ratio in olive leaves. The present study showed higher values of hydroxytyrosol compared to the cultivar *Caizzana, Leccino* (around 40 mg/kg), while similar results were observed in *Coratina, Ortice* and *Frantoio* by using MAE with EtOH:H_2O 70:30 in air-dried olive leaves (Di Meo et al., 2022).

The levels of hydroxytytosol recorded for the extract obtained by SFE at 30 MPa, 90 °C, and ethanol as co-solvent ranged around 2500 mg/kg (Kyriakoudi et al., 2024). Regarding the results of the tyrosol content, similar values were obtained for all olive leaves' extracts except for the ones obtained with SFE, where it was not detected. In olive leaves of *Manzanilla* variety, air-dried at different temperature 25 °C, 45 °C and 70 °C, the tyrosol was not observed probably due to its lower content. The release of tyrosol from olive leaves tissue is strongly dependent by specific factors related to the extraction system (Markhali & Teixeira, 2024).

Ghomari et al., 2019Ghomari et al., 2019Ghomari et al., 2019 reported a selective extraction of tyrosol by using respectively LSE and UAE procedures with distilled water as the extraction solvent, comparing the latter with 80 % EtOH and 20 % acetonitrile.

In Fig. 5 it was observed the distribution of different olive leaves extracts in relation to the measured variables. With the objective of first comparing the raw data between samples, unsupervised multivariate statistical analysis was carried out using PCA. A total 77.30 % of data variability was explained by the sum of the two principal components (PCs). Thus, OLD-UAE, OLD-LSE and OLF-MAE were positively correlated to hydroxytyrosol, tyrosol, and oleuropein, while OLD-MAE, OLD-SFE and OLF-SFE samples, grouped in the right side of the score plot, were not well characterized by the specific phenolic compounds but well represented to all the tested spectrophotometric assays. OLF-UAE and OLF-LSE constitute the group on the left lower side of the score plot.

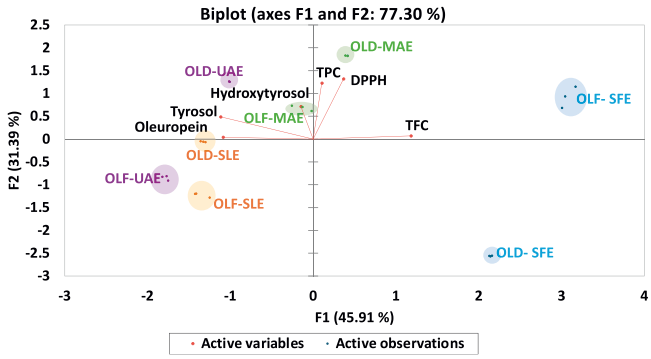

Fig. 5. The PCA biplot representing the distribution of the different obtained olive leaves' extracts according to the three analysed phenolic compounds and spectrophotometric assays variables of the olive leaves' matrix.

They were mostly characterized by high levels of oleuropein.

4. Conclusions

The results obtained for grape pomace and olive leaves extracts clearly demonstrate how the different extraction techniques can significantly influence the content of phenolic compounds, with a direct impact on antioxidant activity and, consequently, on potential health benefits. In particular, it can be asserted that, depending on the extraction method applied to the grape pomace, a selective extraction of bioactive molecules can be performed, while significant differences among the two pre-treatment applied were not found, thus the air-drying process at low temperature could be useful for the stabilization of the raw matrix. UAE revealed as the most promising technology for obtaining grape pomace extract enriched in phenolic compounds, including flavan-3-ols, phenolic acids, and anthocyanins, due to its efficiency and sustainable process. The UAE could be a promising extraction technique for large-scale application, which is an important consideration for the industry adoption. For olive leaves' extracts, there is not a clear distribution and selectivity of both pre-treatment and extraction procedures. The specific phenolic compounds were well extracted and preserved in OLD by using SLE and UAE, as for OLF by MAE. However, in both cases there are some limitations to be considered such as variability of the raw materials (cultivar, growing conditions, harvesting period), stability and bioavailability of the extracts, industrial feasibility and Life Cycle Assessment (LCA). Indeed, even if UAE appears to be the best extraction technology, the feasibility of its scaling-up should be further investigated. Moreover, other emerging pre-treatment and extraction procedures could be investigated in order to enhance the recovery of bioactive compounds taking into account the sustainability issues. In conclusion, the results confirm the potential reuse of both investigated food waste, due to their high antioxidant properties, able to carry out beneficial effects on the human body, contributing to ensure the waste management as well as the valorization and promotion of the local products. Thus, the obtained results could help to develop a new regulation concerning the use of grape pomace and olive leaves wastes as a valid opportunity for the formulation of functional foods.

CRediT authorship contribution statement

Francesca Pompei: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Laura Alessandroni:** Writing – review & editing, Visualization, Data curation. **Riccardo Marconi:** Writing – original draft, Investigation, Formal analysis, Data curation. **Giovanni Caprioli:** Writing – review & editing, Validation. **Gianni Sagratini:** Writing – review & editing, Visualization, Supervision. **Sauro Vittori:** Writing – review & editing, Supervision, Resources, Project

administration, Funding acquisition. **Cinzia Mannozzi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101084.

Data availability

Data will be made available on request.

References

Abouelenein, D., Mustafa, A. M., Caprioli, G., Ricciutelli, M., Sagratini, G., & Vittori, S. (2023). Phenolic and nutritional profiles, and antioxidant activity of grape pomaces and seeds from Lacrima di Morro d'Alba and Verdicchio varieties. *Food Bioscience, 53*, Article 102808. https://doi.org/10.1016/J.FBIO.2023.102808

Alessandroni, L., Bellabarba, L., Corsetti, S., & Sagratini, G. (2024). Valorization of Cynara cardunculus L. var. Scolymus processing by-products of typical landrace "Carciofo Di Montelupone" from Marche Region (Italy). *Gastronomy, 2*(4), 129–140. https://doi.org/10.3390/gastronomy2040010

Barba, F. J., Zhu, Z., Koubaa, M., Sant'Ana, A. S., & Orlien, V. (2016). Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review. *Trends in Food Science & Technology, 49*, 96–109. https://doi.org/10.1016/J.TIFS.2016.01.006

Boselli, E., Giomo, A., Minardi, M., & Frega, N. G. (2008). Characterization of phenolics in Lacrima di Morro d'Alba wine and role on its sensory attributes. *European Food Research and Technology, 227*(3), 709–720. https://doi.org/10.1007/s00217-007-0777-7

Caballero, A. S., Romero-García, J. M., Castro, E., & Cardona, C. A. (2020). Supercritical fluid extraction for enhancing polyphenolic compounds production from olive waste extracts. *Journal of Chemical Technology and Biotechnology, 95*(2), 356–362. https://doi.org/10.1002/jctb.5907

Carmona-Jiménez, Y., García-Moreno, M. V., & García-Barroso, C. (2018). Effect of drying on the phenolic content and antioxidant activity of red grape pomace. *Plant Foods for Human Nutrition, 73*(1), 74–81. https://doi.org/10.1007/s11130-018-0658-1

Casagrande, M., Zanela, J., Pereira, D., de Lima, V. A., Oldoni, T. L. C., & Carpes, S. T. (2019). Optimization of the extraction of antioxidant phenolic compounds from grape pomace using response surface methodology. *Journal of Food Measurement and Characterization, 13*(2), 1120–1129. https://doi.org/10.1007/s11694-018-00027-x

Chowdhary, P., Gupta, A., Gnansounou, E., Pandey, A., & Chaturvedi, P. (2021). Current trends and possibilities for exploitation of Grape pomace as a potential source for value addition. *Environmental Pollution, 278*, Article 116796. https://doi.org/10.1016/J.ENVPOL.2021.116796

Da Porto, C., & Natolino, A. (2024). Policosanols from grape marc: A new step towards a sustainable biorefinery for the wine industry by SC-CO2 extraction. *Journal of CO2 Utilization, 82*, Article 102762. https://doi.org/10.1016/J.JCOU.2024.102762

d'Avanzo, N., Mancuso, A., Mare, R., Silletta, A., Maurotti, S., Parisi, O. I., Cristiano, M. C., & Paolino, D. (2024). Olive leaves and citrus peels: From waste to potential resource for cosmetic products. *Cosmetics, 11*(2). https://doi.org/10.3390/cosmetics11020041

Di Meo, M. C., De Cristofaro, G. A., Imperatore, R., Rocco, M., Giaquinto, D., Palladino, A., Zotti, T., Vito, P., Paolucci, M., & Varricchio, E. (2022). Microwave-assisted extraction of olive leaf from five Italian cultivars: Effects of harvest-time and extraction conditions on phenolic compounds and In vitro antioxidant properties. *ACS Food Science and Technology, 2*(1), 31–40. https://doi.org/10.1021/acsfoodscitech.1c00227

Drevelegka, I., & Goula, A. M. (2020). Recovery of grape pomace phenolic compounds through optimized extraction and adsorption processes. *Chemical Engineering and Processing - Process Intensification, 149*, Article 107845. https://doi.org/10.1016/J.CEP.2020.107845

Fiori, L., de Faveri, D., Casazza, A. A., & Perego, P. (2009). Grape by-products: Extraction of polyphenolic compounds using supercritical CO2 and liquid organic solvent–a preliminary investigation subproductos de la uva: Extracción de compuestos polifenólicos usando CO2 supercrítico y disolventes orgánicos líquidos–una investigación preliminar. *CYTA - Journal of Food, 7*(3), 163–171. https://doi.org/10.1080/11358120902989715

Garcia-Garcia, G., Stone, J., & Rahimifard, S. (2019). Opportunities for waste valorisation in the food industry – A case study with four UK food manufacturers. *Journal of Cleaner Production, 211*, 1339–1356. https://doi.org/10.1016/J.JCLEPRO.2018.11.269

Ghomari, O., Sounni, F., Massaoudi, Y., Ghanam, J., Drissi Kaitouni, L. B., Merzouki, M., & Benlemlih, M. (2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. *Biotechnology Reports, 23*, Article e00347. https://doi.org/10.1016/J.BTRE.2019.E00347

Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., & Battino, M. (2022). Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chemistry, 378*, Article 131918. https://doi.org/10.1016/J.FOODCHEM.2021.131918

Hannachi, H., Hasnia, B., Elfalleh, W., Hannachi, H., Benmoussa, H., Saadaoui, E., Saanoun, I., & Negri, N. (2019). Optimization of ultrasound and microwave-assisted extraction of phenolic compounds from olive leaves by response surface methodology. *Article in Research Journal of Biotechnology, 28*(7). https://www.researchgate.net/publication/334083686.

Hoehn, D., Vázquez-Rowe, I., Kahhat, R., Margallo, M., Laso, J., Fernández-Ríos, A., Ruiz-Salmón, I., & Aldaco, R. (2023). A critical review on food loss and waste quantification approaches: Is there a need to develop alternatives beyond the currently widespread pathways? Resources. *Conservation and Recycling, 188*, Article 106671. https://doi.org/10.1016/J.RESCONREC.2022.106671

Iora, S. R. F., Maciel, G. M., Zielinski, A. A. F., da Silva, M. V., Pontes, P. V., de, A., Haminiuk, C. W. I., & Granato, D. (2015). Evaluation of the bioactive compounds and the antioxidant capacity of grape pomace. *International Journal of Food Science and Technology, 50*(1), 62–69. https://doi.org/10.1111/ijfs.12583

Irakli, M., Chatzopoulou, P., & Ekateriniadou, L. (2018). Optimization of ultrasound-assisted extraction of phenolic compounds: Oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. *Industrial Crops and Products, 124*, 382–388. https://doi.org/10.1016/J.INDCROP.2018.07.070

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology, 119*, 579–591. https://doi.org/10.1016/J.TIFS.2021.11.019

Khwaldia, K., Attour, N., Matthes, J., Beck, L., & Schmid, M. (2022). Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. *Comprehensive Reviews in Food Science and Food Safety, 21*(2), 1218–1253. https://doi.org/10.1111/1541-4337.12882

Kyriakoudi, A., Mourtzinos, I., Tyśkiewicz, K., & Milovanovic, S. (2024). An eco-friendly supercritical CO2 recovery of value-added extracts from Olea europaea leaves. *Foods (Basel, Switzerland), 13*(12). https://doi.org/10.3390/foods13121836

Liang, N. N., He, F., Bi, H. Q., Duan, C. Q., Reeves, M. J., & Wang, J. (2012). Evolution of flavonols in berry skins of different grape cultivars during ripening and a comparison of two vintages. *European Food Research and Technology, 235*(6), 1187–1197. https://doi.org/10.1007/s00217-012-1850-4

Manna, L., Bugnone, C. A., & Banchero, M. (2015). Valorization of hazelnut, coffee and grape wastes through supercritical fluid extraction of triglycerides and polyphenols. *The Journal of Supercritical Fluids, 104*, 204–211. https://doi.org/10.1016/J.SUPFLU.2015.06.012

Markhali, F. S., & Teixeira, J. A. (2024). Extractability of oleuropein, hydroxytyrosol, tyrosol, verbascoside and flavonoid-derivatives from olive leaves using ohmic heating (a green process for value addition). *Sustainable Food Technology, 2*(2), 461–469. https://doi.org/10.1039/d3fb00252g

Martín-García, B., De Montijo-Prieto, S., Jiménez-Valera, M., Carrasco-Pancorbo, A., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M. (2022). Comparative extraction of phenolic compounds from olive leaves using a sonotrode and an ultrasonic bath and the evaluation of both antioxidant and antimicrobial activity. *Antioxidants, 11*(3). https://doi.org/10.3390/antiox11030558

Milinčić, D. D., Stanisavljević, N. S., Kostić, A., Soković Bajić, S., Kojić, M. O., Gašić, U. M., Barać, M. B., Stanojević, S. P., Lj Tešić, Ž., & Pešić, M. B. (2021). Phenolic compounds and biopotential of grape pomace extracts from Prokupac red grape variety. *LWT, 138*, Article 110739. https://doi.org/10.1016/J.LWT.2020.110739

Negro, C., Aprile, A., Luvisi, A., De Bellis, L., & Miceli, A. (2021). Antioxidant activity and polyphenols characterization of four monovarietal grape pomaces from salento (Apulia, italy). *Antioxidants, 10*(9). https://doi.org/10.3390/antiox10091406

Onache, P. A., Geana, E. I., Ciucure, C. T., Florea, A., Sumedrea, D. I., Ionete, R. E., & Tița, O. (2022). Bioactive phytochemical composition of grape pomace resulted from different white and red grape cultivars. *Separations, 9*(12). https://doi.org/10.3390/separations9120395

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology, 57*(12), 4299–4315. https://doi.org/10.1007/s13197-020-04433-2

Pascual, O., González-Royo, E., Gil, M., Gómez-Alonso, S., García-Romero, E., Canals, J. M., Hermosín-Gutíerrez, I., & Zamora, F. (2016). Influence of grape seeds and stems on wine composition and astringency. *Journal of Agricultural and Food Chemistry, 64*(34), 6555–6566. https://doi.org/10.1021/acs.jafc.6b01806

Pattnaik, M., Pandey, P., Martin, G. J. O., Mishra, H. N., & Ashokkumar, M. (2021). Innovative technologies for extraction and microencapsulation of bioactives from

plant-based food waste and their applications in functional food development. In *In foods, 10* pp. 1–30). MDPI AG. https://doi.org/10.3390/foods10020279

Pazir, F., Koçak, E., Turan, F., & Ova, G. (2021). Extraction of anthocyanins from grape pomace by using supercritical carbon dioxide. *Journal of Food Processing and Preservation, 45*(8). https://doi.org/10.1111/jfpp.14950

Peixoto, C. M., Dias, M. I., Alves, M. J., Calhelha, R. C., Barros, L., Pinho, S. P., & Ferreira, I. C. F. R (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. *Food Chemistry, 253*, 132–138. https://doi.org/10.1016/J.FOODCHEM.2018.01.163

Putnik, P., Barba, F. J., Španić, I., Zorić, Z., Dragović-Uzelac, V., & Bursać Kovačević, D. (2017). Green extraction approach for the recovery of polyphenols from Croatian olive leaves (Olea europea). *Food and Bioproducts Processing, 106*, 19–28. https://doi.org/10.1016/J.FBP.2017.08.004

Ricciutelli, M., Marconi, S., Boarelli, M. C., Caprioli, G., Sagratini, G., Ballini, R., & Fiorini, D. (2017). Olive oil polyphenols: A quantitative method by high-performance liquid-chromatography-diode-array detection for their determination and the assessment of the related health claim. *Journal of Chromatography A, 1481*, 53–63. https://doi.org/10.1016/J.CHROMA.2016.12.020

Rockenbach, I. I., Gonzaga, L. V., Rizelio, V. M., Gonçalves, A. E., de, S. S., Genovese, M. I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. *Food Research International, 44*(4), 897–901. https://doi.org/10.1016/J.FOODRES.2011.01.049

Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry, 92*(2), 235–254. https://doi.org/10.1016/J.FOODCHEM.2004.08.004

Rosa, A. D., Junges, A., Fernandes, I. A., Cansian, R. L., Corazza, M. L., Franceschi, E., Backes, G. T., & Valduga, E. (2019). High pressure extraction of olive leaves (Olea europaea): bioactive compounds, bioactivity and kinetic modelling. *Journal of Food Science and Technology, 56*(8), 3864–3876. https://doi.org/10.1007/s13197-019-03856-w

Ruberto, G., Renda, A., Daquino, C., Amico, V., Spatafora, C., Tringali, C., & Tommasi, N. De (2007). Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. *Food Chemistry, 100*(1), 203–210. https://doi.org/10.1016/J.FOODCHEM.2005.09.041

Şahin, S., İlbay, Z., & Kırbaşlar, Ş.İ. (2015). Study on optimum extraction conditions for olive leaf extracts rich in polyphenol and flavonoid. *Separation Science and Technology, 50*(8), 1181–1189. https://doi.org/10.1080/01496395.2014.966203

Salvador, R., Barros, M. V., Donner, M., Brito, P., Halog, A., & De Francisco, A. C. (2022). How to advance regional circular bioeconomy systems? Identifying barriers, challenges, drivers, and opportunities. *Sustainable Production and Consumption, 32*, 248–269. https://doi.org/10.1016/J.SPC.2022.04.025

Sarker, A., Ahmmed, R., Ahsan, S. M., Rana, J., Ghosh, M. K., & Nandi, R. (2023). A comprehensive review of food waste valorization for the sustainable management of global food waste. *Sustainable Food Technology, 2*(1), 48–69. https://doi.org/10.1039/d3fb00156c

Staniškis, J. K. (2022). Socio-environmental-economic transformations towards sustainable development. *Transformation of business organization towards sustainability. world sustainability series*. Cham: Springer. https://doi.org/10.1007/978-3-030-93298-5_3

Tournour, H. H., Segundo, M. A., Magalhães, L. M., Barreiros, L., Queiroz, J., & Cunha, L. M. (2015). Valorization of grape pomace: Extraction of bioactive phenolics with antioxidant properties. *Industrial Crops and Products, 74*, 397–406. https://doi.org/10.1016/J.INDCROP.2015.05.055

Tseng, A., & Zhao, Y. (2012). Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). *Journal of Food Science, 77*(9). https://doi.org/10.1111/j.1750-3841.2012.02840.x

Vatai, T., Škerget, M., & Knez, Ž. (2009). Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. *Journal of Food Engineering, 90*(2), 246–254. https://doi.org/10.1016/J.JFOODENG.2008.06.028

Wojdyło, A., Figiel, A., & Oszmiański, J. (2009). Effect of drying methods with the application of vacuum microwaves on the bioactive compounds, color, and antioxidant activity of strawberry fruits. *Journal of Agricultural and Food Chemistry, 57*(4), 1337–1343. https://doi.org/10.1021/jf802507j

Xu, Y., Burton, S., Kim, C., & Sismour, E. (2016). Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four virginia-grown grape varieties. *Food Science and Nutrition, 4*(1), 125–133. https://doi.org/10.1002/fsn3.264

Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., & Gotoh, H. (2024). DPPH measurements and structure—Activity relationship studies on the antioxidant capacity of phenols. *Antioxidants, 13*, 309. https://doi.org/10.3390/antiox13030309

Zhang, C., Xin, X., Zhang, J., Zhu, S., Niu, E., Zhou, Z., & Liu, D. (2022). Comparative evaluation of the phytochemical profiles and antioxidant potentials of olive leaves from 32 cultivars grown in China. *Molecules, 27*(4), 1292. https://doi.org/10.3390/molecules27041292