



Intensification of grape marc (*Vitis vinifera*) exploitation by subcritical water-ethanol extraction: Effect on polyphenol bioactivities and kinetic modelling

A. Natolino^{*}, P. Passaghe, G. Brugnera, P. Comuzzo

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via Sondrio 2/A, 33100, Udine, Italy

ARTICLE INFO

Keywords:

Grape marc
Subcritical fluids
Polyphenols
Kinetics
Bioactivities

ABSTRACT

Subcritical solvent extraction (SSE) is an efficient and versatile technology for the recovery of bioactive compounds from food by-products. The present work aims to study the SSE efficiency and kinetics for the extraction of grape marc polyphenols. Water, ethanol, and a 50% water-ethanol mixture (EtOH 50%) were used as green solvents. The higher polyphenol content (4.05 ± 0.23 g_{GAE}/100 g_{d. m.}) and antioxidant activity (42.30 ± 0.68 mg α -toc/mL) were obtained with subcritical EtOH 50% at 120 °C and 10.3 MPa, respectively, 1.5- and 3-fold higher than the conventional extraction. According to kinetic modeling, SSE was four-fold faster and a degradation phenomenon of polyphenols was highlighted for times longer than 10 min. The two-site kinetic model and a second order exponential decay function suitably described the extraction kinetics and degradation phase, respectively ($R^2 > 0.97$). A remarkable effect was highlighted on the polymerization degree and galloylation percentage of proanthocyanidins by SSE, and an increase in their antioxidant activity was observed.

1. Introduction

The global food chain generates large amounts of by-products. The primary production and processing stages account for approximately 14% of total food losses in the food production cycle (FAO, 2019). Most of these biomasses are disposed in landfills, contributing to climate change through green-house gas emissions, water, and soil contamination (Sharma et al., 2020). Several strategies have been proposed to reduce their environmental impact and convert these biomasses into valuable economic resources (Castro-Muñoz et al., 2022; Gómez-García et al., 2021).

The extraction of valuable compounds with technological or healthful properties from food by-products represents a pioneering solution for reducing waste and generating new economic opportunities, as well as supporting the creation of circular economies (Liu et al., 2023). Several extraction methods are available and can be subdivided into two categories: conventional and innovative techniques. The conventional methods, such as Soxhlet, maceration, and hydro-distillation, have been extensively studied (Bitwell et al., 2023; Kaleem and Ahmad, 2018). However, their effectiveness for getting pure compounds and adding value to waste is quite limited due to the possible loss of multiple thermolabile solutes, the use of harmful solvents, and the massive

extraction waste generation (Bitwell et al., 2023). Moreover, the resulting bioactive extracts may contain residues of toxic and nonedible solvents, which makes the bioactive extracts incompatible for food and pharmaceutical applications (More et al., 2022). Conventional methods are considered time- and energy-consuming, and hence not economically viable (Rifna et al., 2023; Sagar et al., 2018).

Several innovative processes have been developed according to the principles of the Green Chemistry, to increase process efficiency and selectivity, to promote alternative and sustainable solvents, reduce energy consumption, and mitigate waste generation and contaminants (Chemat et al., 2012). Compressed fluids-based extraction techniques, specifically sub- and supercritical extraction methods, are among those able to better overcome the aforementioned drawbacks. Moreover, they may be upscaled and coupled to other techniques within a biorefinery strategy (Gallego et al., 2019). Supercritical fluid extraction (SFE) has undergone extensive studies and is generally perceived as a process with supercritical carbon dioxide (SC-CO₂) (Essien et al., 2020). SC-CO₂ is limited by its low polarity, and it must be aided by a co-solvent in order to extract more polar compounds. Subcritical solvents represent a valuable alternative to the co-solvent addition of SC-CO₂. The subcritical state is achievable by increasing the temperature above the boiling point and below the critical one. The pressure must be set to maintain the

^{*} Corresponding author.

E-mail address: andrea.natolino@uniud.it (A. Natolino).

solvent in the liquid state. The increase in temperature and pressure improves the extraction efficiency by several mechanisms, including increased diffusion rates, solvent penetration, mass transfer, disruption of solute-matrix interactions, and analytes solubilisation (Monrad et al., 2010).

Extraction with subcritical fluids (SSE), also known as pressurized liquid extraction (PLE) and hot-pressed extraction (HPE), allows the use of a wide variety of solvents with different polarities, like water, ethanol, methanol, hexane, ethyl acetate, and their mixtures. This gives a great adaptability to SSE, which can be used to extract a great range of compounds, from polar (e.g. polyphenols, carbohydrates, or proteins) to mid/non-polar components (e.g. lipids and carotenoids) (Gallego et al., 2019; Marcus, 2018). Moreover, the physical properties of subcritical solvent (such as density, ionic product, dielectric constant) can be finely modulated by varying temperature and pressure (Cocero et al., 2018). Subcritical fluids are also considered sustainable reaction mediums, promoting certain chemical reactions, such as hydrogenation, oxidation, polymerization, depolymerization, carbonylation, hydrolysis and Maillard reactions (Knez et al., 2018; Möller et al., 2011; Pavlović et al., 2013). As a result, the chemical composition of the extracts may be affected (Basak and Annature, 2022; Fan and Gao, 2022).

SSE has already been investigated for the extraction of different classes of bioactive compounds, especially polyphenols, from several agri-food by-products: defatted sesame seeds (Bodoira et al., 2017), olive oil filter cake (Lozano-Sánchez et al., 2014), apple pomace (Wijngaard and Brunton, 2009), citrus pomace (Cheigh et al., 2012; Kim et al., 2009), pomegranate peels and seeds (Çam and Hışıl, 2010; García et al., 2021), onion skins (Ko et al., 2011), mango peels (García-Mendoza et al., 2015), spent coffee ground (Xu et al., 2015), and grape marc (Otero-Pareja et al., 2015; Pedras et al., 2017; Pereira et al., 2019; Todd and Baroutian, 2017).

The grape marc is the most abundant solid by-product of the wine-making process. It has been estimated that 20–25 kg of grape marc is produced from 100 kg of grapes, and about 8–9 million tons of grape marc are produced worldwide each year (Beres et al., 2017). Approximately, 70% of the phenolic content in grapes is retained in the grape marc, making it a valuable resource of these bioactive compounds (González-Centeno et al., 2010).

In this study, three different green solvents at subcritical state were considered for the extraction of polyphenols from defatted grape marc: water, ethanol, and their mixture (50% v/v). A technological comparison between subcritical fluid extraction (SSE) and conventional solid-liquid extraction (CSLE) were carried out both on the overall extraction curves (OECs) and the kinetic parameters obtained from the mathematical modelling of the experimental data. The study also took into account the chemical effects induced by subcritical conditions on the polyphenol profile and certain analytical indices related to tannin composition.

2. Material and methods

2.1. Chemicals

Analytical grade (purity >99%) ethanol, methanol, butan-1-ol, and acetonitrile were purchased from Sigma Aldrich Co. (Milan, Italy). Analytical grade chemicals, including Folin-Ciocalteu reagent, sodium carbonate, sodium acetate, formic acid, hydrochloric acid, gallic acid, ascorbic acid, phloroglucinol, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), malvidin-3-glucoside, (+)- α -tocopherol, and (+)-catechin, were purchased from Sigma Aldrich Co. (Milan, Italy).

2.2. Raw material

Grape marc from Pinot Gris grapes was collected during 2022 vintage in the Friuli Venezia-Giulia region (Italy). The fresh grape marc was air dried at room temperature until to reach the final moisture content of

$6.8 \pm 0.3\%$ (w/w). Subsequently, the dried grape marc was ground by a domestic grinder, vacuum packed, and stored at refrigerated conditions ($+4\text{ }^{\circ}\text{C}$).

2.3. Defatting of the grape marc

Prior to polyphenol extraction, the grape marc was defatted by supercritical carbon dioxide (SC-CO₂) extraction at 280 bar, 45 °C, 10 kg/h of CO₂ flow, for 3 h (Da Porto and Natolino, 2017), using a SFE pilot-plant (SCF100 serie 3 PLC-GR-DLMP, Separeco S. r.l., Pinerolo, Italy) equipped with a 1 L extraction vessel (E1), two 0.3 L separators in series (S1, S2), and a tank (B1) in which CO₂ was stored and recycled.

2.4. Conventional solid-liquid extraction (CSLE)

Conventional solid-liquid extraction was performed by mixing 5 g of defatted grape marc with 50 mL of solvent. Three different green solvents were considered: water (H₂O), ethanol (EtOH) and their 50% mixture (EtOH 50%). The sample was continuously stirred at 40 °C for different times (5, 10, 15, 30, 45, 60, and 90 min) to assess several data points for the kinetic extraction curves. The samples were filtered through acetate cellulose filter paper (0.65 μm), using a Buchner funnel, and the liquid extract was recovered and stored at 4 °C. All extractions were triplicated.

2.5. Subcritical solvent extraction (SSE)

The extractions were performed with an ASE 350 Accelerated Solvent Extraction System (Thermo Scientific) equipped with 22 mL stainless steel cells and 60 mL collection vials. The extraction vessels were loaded with the dried and defatted grape marc (5.0 g), homogeneously mixed with glass balls (0.5 cm in diameter). A cellulose paper filter (Dionex) was placed at the bottom of the ASE cell.

Extraction conditions were set to standard operating pressure 10.3 MPa (1500 psi), the flush volume was 60%, the purge time was set at 60 s, and the press solvent saver mode was selected. All the extractions were performed at 120 °C and different times (1, 2, 3, 4, 5, 7, 10, 15, 30, 60, and 90 min) were considered. The same green solvents of conventional process were used: water (H₂O), ethanol (EtOH) and their 50% mixture (EtOH 50%). The liquid extracts were centrifuged (3000 rpm for 10 min) and stored at 4 °C. All extractions were triplicated.

2.6. Analytical determination

2.6.1. Total polyphenol content

Total polyphenols content (TPC) analysis was performed by a spectrophotometric method reported by (Nel, 2018), with slight modifications. The reaction mixture was prepared with 100 μL of extract, 500 μL of the Folin-Ciocalteu reagent, 4 mL of Milli-Q water and 2 mL of 15% (w/v) aqueous solution of Na₂CO₃. The reaction mixture was vigorously shaken and left in the dark for 2 h. Absorbance was determined at 750 nm using UV-vis spectrophotometer (Shimadzu UV-1650, Italy). TPC quantification was performed using a calibration curve created with standard solutions of gallic acid in the range of 50–500 ppm ($R^2 = 0.99$). Then results were expressed as grams of equivalent gallic acid per 100 g of dried matter (g GAE/100 g d.m.).

2.6.2. Anthocyanin content

Anthocyanin content was determined mixing 1 mL of sample, 1 mL of HCl/EtOH solution (0.1% v/v), and 20 mL of HCl/H₂O solution (2% v/v), as reported by (Nel, 2018). Subsequently, 2.5 mL of sample mixture was added to 1 mL of deionized H₂O and other 2.5 mL of sample mixture with 1 mL of potassium bisulphite solution (20% w/v). After 15 min of reaction time, the absorbance of each solution was measured at 520 nm using a UV-Vis spectrophotometer (Shimadzu UV 1650, Milano, Italy), using distilled water as a control. A calibration curve was prepared with

different solutions of malvidin-3-glucoside as a standard (0–500 mg/L). The results were expressed as milligrams of malvidin-3-glucoside equivalent per 100 g of dried matter (mg/100 g_{d.m.}).

2.6.3. Flavan-3-ols content

The flavan-3-ols content was determined according to the analytical protocol proposed by (Waterhouse et al., 2016). The chromogen reagent was prepared with 1 g of 4-(dimethylamino)-cinnamaldehyde (DAC) dissolved in 250 mL of 37% HCl and 750 mL of methanol. Then, 1 mL of diluted sample (1:25 v/v) was added to 5 mL of DAC solution. The absorbance was read at 640 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan) against a blank prepared by substituting the sample with 1 mL of water. A calibration curve was prepared with several standard solutions of (+)-catechin in the concentration range of 0–50 mg/L. Results were expressed as grams of (+)-catechin equivalents per 100 g of dried matter (g/100 g_{d.m.}).

2.6.4. Condensed tannins content

As reported by (Comuzzo et al., 2020), two reaction mixtures were prepared by mixing 2 mL of diluted sample and 6 mL of hydrochloric acid–butanol solution. The reaction mixture was divided into two equal volumes (4 mL): one of them was placed in a water bath at 100 °C for 20 min (A), the other at room temperature (B). After cooling, the absorbance of the two mixtures was measured at 550 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Tokyo, Japan). The concentration of condensed tannins was then calculated using the following equation:

$$TA = \Delta Abs_{550} \cdot DF \cdot 0.1736$$

where TA is the concentration of tannins, expressed as grams per 100 g of dried matter (g/100 g_{d.m.}), ΔAbs_{550} is the absorbance difference between mixture (A) and (B), and DF is the dilution factor.

2.6.5. Antioxidant activity

The antioxidant activity of the grape marc extracts was evaluated by the total free radical scavenger capacity (RSC), defined as the difference between the DPPH concentration before ($C_{DPPH,i}$) and after reaction with the sample ($C_{DPPH,f}$). The spectrophotometric methodology described by Natolino and Da Porto (2020) was performed. Briefly, 10 μ L of extract was mixed with 1990 L of fresh methanol DPPH solution (93 μ M). The reaction mixture was vigorously shaken and left in the dark for 60 min. Absorbance was measured at 515 nm using UV–vis spectrophotometer (Shimadzu UV-1650, Italy). Antioxidant activity was quantified using a calibration curve created with standard solutions of α -tocopherol in the range of 5.8×10^{-5} – 2.3×10^{-3} mol/L ($R^2 = 0.98$). The antioxidant activity of the samples was expressed as grams of α -tocopherol per mL of extract (g _{α -tocopherol/mL}).

2.6.6. HPLC analysis of tannins

The extracts were centrifugated at 3000 rpm for 10 min and filtered at 0.2 μ m. Subsequently, 1 mL of filtered sample was evaporated and redissolved in 1 mL of methanol (0.2 N HCl). A reaction mixture was prepared adding 0.8 mL of methanolic solution with 0.1 mL of 0.2 N HCl in methanol solution, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid. The phloroglucinol reaction was carried out in a water bath at 50 °C for 20 min, and stopped by adding 1 mL of a 200 mM sodium acetate solution.

HPLC analysis was performed on an LC-2010 AHT liquid chromatographic system (Shimadzu, Kyoto, Japan), equipped with an integrated autosampler and UV–Vis detector. The samples (10 μ L injection volume) were injected on an Atlantis dC18 column (250 \times 4.6 mm, 5 μ m packing). The elution conditions were as follows: 0.8 mL/min flow rate; oven temperature, 30 °C; solvent A, water/formic acid (98:2, v/v), and solvent B, acetonitrile/solvent A (80:20 v/v). Elution began with 0% B for 5 min, linear gradient from 0 to 10% B in 30 min and gradient from

10 to 20% in 35 min, followed by washing and re-equilibration of the column.

As reported by Busse-Valverde et al. (2010), proanthocyanidin cleavage products were estimated using their response factors relative to (\pm)-catechin, which was used as the quantitative standard. These analyses allowed determination of the total proanthocyanidin content, the apparent mean degree of polymerization (mDP) and the percentage of each constitutive unit. The mDP was calculated as the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all flavan-3-ol monomers (in moles).

2.7. Kinetic modelling

The CSLE and SSE extraction kinetics of polyphenols from grape marc were modelled using the so-called “two-site kinetic model” (Duba et al., 2015). The model assumes that the extraction process consists of two stages, washing and diffusion, and it is given by the following equation:

$$C_t = C_w [1 - e^{(-k_w t)}] + C_d [1 - e^{(-k_d t)}]$$

where C_t is the extractable substances content at a specific time t (min), C_w and C_d are the concentration of extractable substances recovered during the washing and diffusion stage, respectively, and k_w and k_d are coefficient of extraction during the two process stages.

Previous studies indicated decreases in experimental results due to thermal degradations of polyphenols contents (Pettersson et al., 2010; Shimanouchi et al., 2014). These degradations can be described by a two-term exponential decay function (Erim Kose, 2022; Scrob et al., 2022; Silva et al., 2023) as follows:

$$C_t = C_1 \bullet e^{-k_1 \bullet t} + C_2 \bullet e^{-k_2 \bullet t}$$

where C_t is the extractable substance content at a specific time t (min), C_1 and C_2 are the constants of the two degradation phases, and k_1 and k_2 are the degradation rates.

The values of the kinetic model parameters and graph plots were calculated by Matlab R2023b (MathWorks, Inc., USA). The fitting strategy was based on a robust least-squares method by minimizing the least absolute residuals between predicted values and experimental observations for each data point. The agreement between the experimental and modelled values was assessed by means of correlation coefficients (R^2 and R^2_{adj}): higher values indicate a better fit of the model to the experimental values. The predicted R squared (R^2_{pred}) was also calculate to estimate how the model predict responses for new observations.

2.8. Statistical analysis

The triplicates of each experimental set were expressed by their mean \pm standard deviation. Minitab 17 software (Minitab Inc., State College, Pennsylvania, US) was used for statistical analysis by one-way analysis of variance (ANOVA), with the level of significance set at p -value < 0.05 . Tukey’s honest significant difference (HSD) multiple comparison test was performed when ANOVA analysis revealed significant differences between groups (p -value < 0.05). A Tukey’s post-doc test was chosen to make every possible pairwise comparison, and the level of significance was set at p -value < 0.05 .

3. Results and discussion

3.1. Extraction kinetics – TPC

The experimental and modelled extraction curves of total polyphenol content by conventional (CSLE) and subcritical solvent extraction (SSE) with water, ethanol, and their mixture (EtOH 50%) are shown in Fig. 1. CSLE and SSE showed different extraction kinetics. CSLE commonly

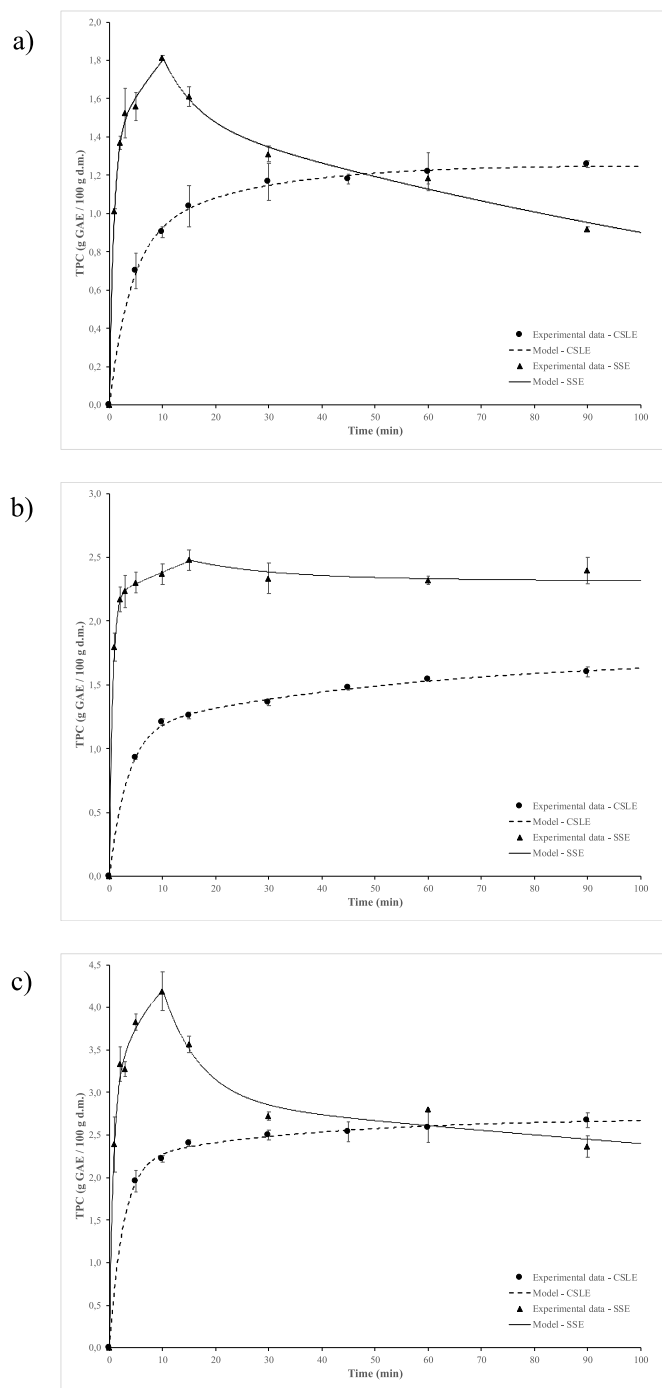


Fig. 1. Comparison between experimental (symbols) and modelled overall extraction curves (OECs) of total polyphenol content (TPC) of extracts obtained by CSLE and SSE from grape marc using water (a), ethanol (b), and their mixture EtOH 50% (c) as solvent.

involves two sequential extraction steps. The initial period is controlled by a desorption phenomenon of solutes at a higher rate, known as the washing phase. The second step, known as the diffusion stage, shows solute desorption at a lower rate due to the predominance of slow diffusion mechanisms inside the porous solid matrix (Duba et al., 2015).

Two process stages can also be identified for SSE, but the overall kinetic curves were different. After an initial and rapid extraction phase, a decrease in polyphenol content was noticed at process times longer than 10 min.

3.1.1. Extraction phase

The use of subcritical solvents led to a significant increase in polyphenol content, as depicted by the OECs in Figs. 1 and 2. The SSE allowed a mean 48% increase of TPC, compared to conventional extraction. The highest TPC content was 4.05 ± 0.23 g GAE/100 g d.m. with subcritical EtOH 50% at 100 bar, 120 °C, and 10 min of extraction. The subcritical EtOH 50% led to 2.2-fold and 1.6-fold increase of TPC compared to subcritical water or ethanol.

Polyphenols from grape by-products can be extracted more effectively using solvent mixtures (Bosso et al., 2016; Makris et al., 2007; Pinelo et al., 2005; Spigno et al., 2007) rather than a corresponding mono-component solvent system. Several solvents have been mixed with water in binary and ternary systems, but using ethanol is particularly interesting due to its potential to improve the sustainability of the extraction process. Ethanol can be obtained from the distillation of grape marc, and it may be considered as another by-product of winemaking (Makris et al., 2007).

A comparison of the extraction curves for SSE and CSLE (Figs. 1 and 2) also highlighted an increase in the slope of the initial linear part of the SSE curves, indicating an increase in the solute extraction rates. The maximum TPC contents can be achieved within 10 min of extraction using subcritical solvents. The improvement in extraction efficiency may be related to multiple effects. The increase in pressure and temperature induces several changes in the solvent and matrix properties. The rise in temperature reduces the viscosity of the solvent, thereby increasing its diffusion capacity in the porous solid matrix (Alvarez-Rivera et al., 2020). Moreover, temperature affects equilibria occurring at solid surfaces, in fact, it alters strong solute–matrix interactions due to van der Waals forces, hydrogen bondings, and dipole attractions. Thermal energy can overcome cohesive (solute–solute) and adhesive (solute–matrix) interactions by decreasing the activation energy required for desorption (Priego-Capote and Delgado De La Torre, 2013). On the other hand, pressure can also affect the extraction mechanisms. High pressures can enhance the extraction phenomenon by forcing the solvent into matrix areas that would normally not be accessible under atmospheric conditions (Mandal et al., 2015).

The TPC increase observed for SSE may also be due to the breaking of lignin–phenols bonds or the breakdown of lignin itself, resulting in the extraction of more phenolic compounds (Antony and Farid, 2022). The bounded phenols, also known as Non-Extractable Polyphenols (NEPs), remain insoluble in the conventional aqueous-organic solvents due to the capacity of their hydroxyl functionalities to form polymers or to link to macromolecules such as polysaccharides and proteins (Ding et al., 2020).

Modelling of extraction curves is of paramount importance for the process scaling-up. Several models can be adopted for solid-liquid extraction, such as the Power Law model, Peleg's model, Elovich's equation, Weibull equation, second order rate model, and others (Natolino and Da Porto, 2020; Peixoto et al., 2023). Among them, the two-site kinetic model is one of the most used, giving useful insights into the solutes' desorption mechanisms during two process stages.

The CSLE and the first part of the SSE curves, within 10 min, are well described by the two-site kinetic model, as indicated by the values of the correlation coefficients, R^2 (>0.9981) and R^2_{adj} (>0.9978), in Table 1. Furthermore, the model showed a good prediction level on new observations ($R^2_{pred} > 0.9889$). The kinetic parameters confirmed that the ethanol-water mixture was the best solvent for polyphenol extraction, at both conventional and subcritical conditions. The dissolution capacity of the solvent depends on its dielectric constant (ϵ), which affects its capacity to solubilize molecules with different degree of polarity. The binary 50% mixture ethanol/water have ϵ value ($\epsilon = 52$) intermediate between those of the pure ethanol ($\epsilon = 24.3$) and water ($\epsilon = 80.1$), expanding the range of extractable polyphenols and increasing the extraction yield (Bosso et al., 2016).

The washing and diffusion extraction stages considered by the model can also be identified for the SSE, despite the rapid TPC increase and the

Table 1

Kinetic parameters of the extraction and degradation phase of total polyphenols (TPC) from grape marc by conventional (CSLE) and subcritical solvent extraction (SSE) with different solvents.

Extraction phase	Kinetic parameters	CSLE			SSE		
		H ₂ O	EtOH	EtOH 50%	H ₂ O	EtOH	EtOH 50%
Extraction phase	C_w	0.8354	1.1411	2.1927	1.3878	2.2001	2.9644
	k_w	0.2587	0.3018	0.3897	1.2222	1.3553	1.4124
	C_d	0.4181	0.5821	0.5062	1.4997	2.4392	1.7624
	k_d	0.0458	0.0184	0.0283	0.0321	0.0078	0.0120
	R^2	0.9987	0.9996	0.9988	0.9981	0.9999	0.9989
	R^2 -adj.	0.9985	0.9995	0.9986	0.9978	0.9999	0.9987
	R^2 -pred.	0.9983	0.9986	0.9981	0.9889	0.9993	0.9984
Degradation phase	C_1	–	–	–	1.6262	0.3864	5.4871
	k_1	–	–	–	–0.1608	–0.0660	–0.1432
	C_2	–	–	–	1.5781	2.3392	2.9605
	k_2	–	–	–	–0.0056	–0.0001	–0.0021
	R^2	–	–	–	0.9881	0.9980	0.9701
	R^2 -adj.	–	–	–	0.9524	0.9731	0.8805
	R^2 -pred.	–	–	–	0.9304	0.9682	0.8242

beginning of the decrease phenomenon after only 10 min. The two orders of magnitude difference between the extraction constant rates (k_w and k_d) confirmed that the solute mass transfer is faster during the washing phase, instead of the diffusion one, for both extraction techniques. As assumed also by the so called “broken and intact cells model” (Sovová, 1994), which is largely used in the extraction of solutes from solid substrates, the solutes are contained in cells of the plant matrix and, as a result of mechanical milling pretreatment, some cells in the solids are broken and the remaining cells in the core of the particles are intact. The solute in the broken cell is directly exposed to the particle surface and can be easily extracted. The extraction rate depends on the washing rate constant k_w . Instead, the solute in the intact cells is much more difficult to extract due to the high mass transfer resistance inside the particle itself, and the extraction rate depends on the diffusion rate constant k_d . Moreover, the pressure and temperature increase at subcritical conditions allow an average four-fold enhancement of extraction rate (k_w) compared to CSLE, from 0.3167 min⁻¹ to 1.3300 min⁻¹. The rise in pressure and temperature allows higher solvent diffusibility into solid matrix, and faster solutes desorption. Same findings were reported for the extraction of polyphenols from grape seeds and skins (Duba et al., 2015), citrus unshiu peel (Kim and Lim, 2020), and malted quinoa (Mufari et al., 2021) by water or ethanol-water mixtures at subcritical conditions.

3.1.2. Degradation phase

The OECs highlighted a significant decrease in total polyphenols after 10 min of SSE, mainly with ethanol and the ethanol-water mixture (Figs. 1–2). A degradation phase on CSLE cannot be observed, as indicated by the asymptotic trend of kinetic curves for times longer than 40 min. The CSLE trials were carried out at 40 °C, which enabled the preservation of phenol compounds.

The conventional approach for studying polyphenol recovery involves using a maximum temperature of around 60–80 °C. Higher temperatures have not been explored due to the potential solvent losses and phenol degradation (Antony and Farid, 2022). Treatments at temperatures above 80 °C, for times longer than 10 min, can induce a significant decrease in polyphenol yields (Sólyom et al., 2014), however, SSE must be carried out at high pressures and temperatures, above 90–100 °C. The behaviour of polyphenols at higher temperatures and subcritical conditions is complex and can be attributed to three mechanisms: breaking of phenolic acids-lignin bonds, lignin degradation, and thermal degradation. The first two are mainly associated to the increase of phenolic content and antioxidant activity observed with an increase in extraction temperature, up to 180–200 °C (Antony and Farid, 2022). However, thermal degradation is the primary mechanism adopted to explain the decrease of polyphenol yield during extraction at high

temperatures, but it does not fully account for the behaviour of polyphenols. A multi-factorial evaluation should be considered. For example, the magnitude and speed of the degradation phenomenon can be affected by the extraction conditions and the polyphenol sub-classes (Casazza et al., 2012; Vergara-Salinas et al., 2012). The bioactive compounds degradation was reported also for the polyphenol extraction with subcritical water from chestnut peels, at temperatures above 150 °C and process time longer than 30 min. Additionally, the phenols can undergo many reaction mechanisms producing other compounds, such as pyrogallol from hydrolyzable tannins (Cravotto et al., 2022).

The experimental results pointed out that the magnitude of the degradation process is different among the subcritical solvents. As shown in Figs. 1 and 2, the decrease in TPC is higher with water and the ethanol-water mixture, compared to pure ethanol. In this study, a second order exponential decay function was chosen for the description of the degradation phase (Table 1). A good correlation was found between the model and the experimental values ($R^2 > 0.9701$; $R^2_{adj.} > 0.8805$), as a good prediction level of new observations ($R^2_{pred.} > 0.8103$). The model assumes that the degradation process is constituted by a first faster step, followed by a second phase characterized by a slower and smoother trend. The magnitude of the degradation constant rates (k_1 and k_2) confirmed that a faster decrease in TPC can be observed when water is used as a solvent, followed by the ethanol-water mixture. Indeed, the use of subcritical ethanol highlighted a TPC decrease in the first initial step (k_1), 2–2.5 folds lower (–0.0660 min⁻¹) compared to subcritical water (–0.1608 min⁻¹) or ethanol-water mixture (–0.1432 min⁻¹). Moreover, the k_2 value indicates that the second decrease phase with subcritical ethanol could be neglected.

It is well known that the hydrolysis reaction of biopolymers can be promoted in aqueous mediums at subcritical conditions (Zhang et al., 2020). The hydrolysis of phenol-lignin bonds and lignin structures enhances the polyphenol recovery observed in the initial period of SSE. Depolymerization may also occur on condensed tannins, resulting in a potential increase in small phenols content.

In contrast, the subcritical aqueous medium can promote oxidation and degradation reactions at high temperatures and for long times, particularly for small molecules, such as gallic acid, which can be more sensitive to high temperatures than condensed tannins (Cravotto et al., 2022).

3.2. Antioxidant activity

Grape marc polyphenols have many human-health benefits due to their well known antioxidant properties. The antioxidant activity of the extracts obtained during the SSE and CSLE trials are reported in the overall kinetic curves showed a similar trend to the TPC (Figs. 1 and 2).

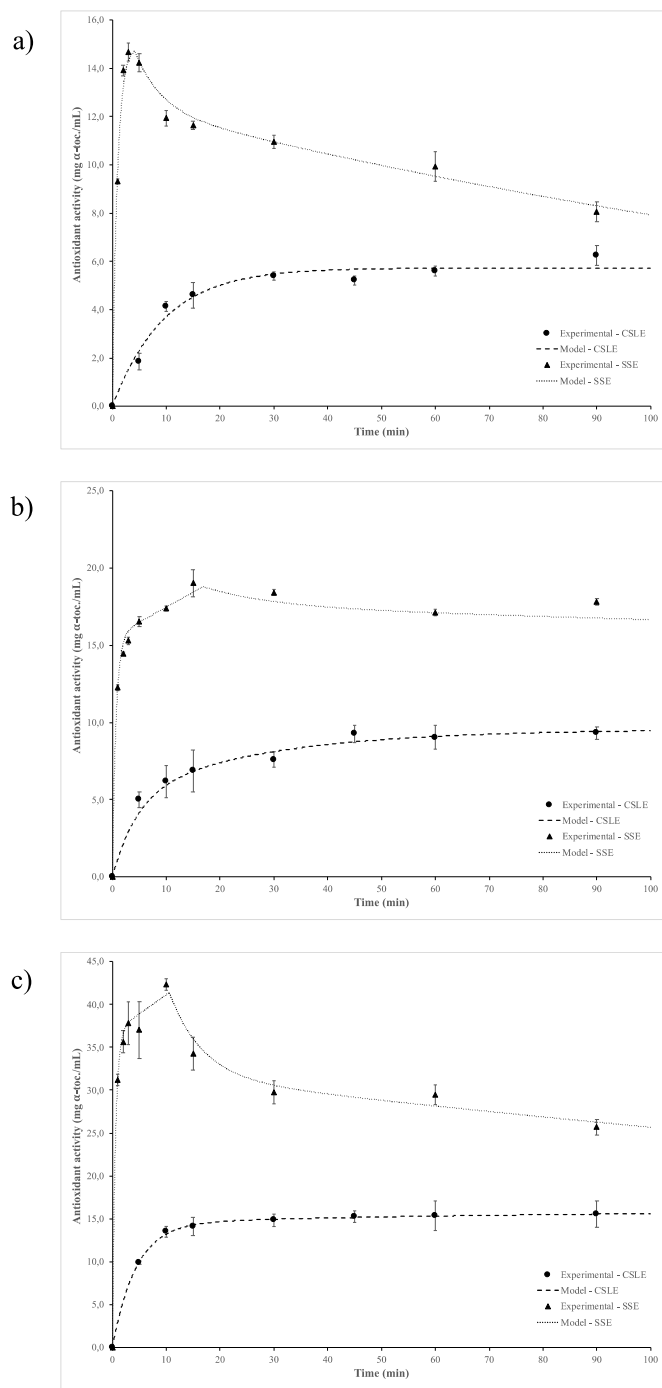


Fig. 2. Comparison between experimental (symbols) and modelled overall extraction curves (OECs) of antioxidant activity of extracts obtained by CSLE and SSE from grape marc using water (a), ethanol (b), and their mixture EtOH 50% (c) as solvent.

An initial increase period, followed by an asymptotic and constant trend was observed for CSLE. Instead, the SSE showed again an initial faster increase in the antioxidant activity, and a significant decrease for times longer than few minutes. The modelling of the experimental data using the two-site kinetic and the two terms exponential decay model allowed to mathematical evaluate the different CSLE and SSE process periods. The kinetic models fitted well the experimental data, as indicated by the correlation coefficients in Table 2 ($R^2 > 0.9734$; $R_{adj}^2 > 0.9690$), and they also able to well predict responses for new observations ($R_{pred}^2 > 0.8103$). The estimation of the extraction constant rates, k_w and k_d ,

confirmed that the EtOH 50% at subcritical conditions was the better solvents for the extraction of polyphenols from grape marc with the higher antioxidant activity. The highest antioxidant activity (42.30 ± 0.68 mg α -toc/mL) was achieved by SSE with EtOH 50% and 10 min. The results were 3 times higher than CSLE (15.54 ± 1.51 mg α -toc/mL). The starting point and the magnitude of SSE degradation phase vary among the different subcritical solvents. The AA decrease began early for water (3 min), compared to the ethanol-water mixture (10 min) or ethanol (15 min). Furthermore, the degradation constant rates indicated a more intense and faster degradation phenomena of polyphenols when subcritical water was used as a solvent ($k_1 = -0.2087$ min $^{-1}$; $k_2 = -0.0046$ min $^{-1}$).

Despite the similarity in kinetic curves, there was a moderate correlation found between the TPC and antioxidant activity ($R^2 = 0.79$). The evaluation of the ratio between antioxidant activity and TPC (AA-TPC ratio) is worthy of note. The AA-TPC ratio was 4.62, 4.10, and 4.32 mg α -toc/mg GAE, respectively for EtOH50%, ethanol, and water at the best SSE extraction conditions. Instead, the CSLE extracts showed a lower AA-TPC ratio: 4.04, 3.88, and 3.15 mg α -toc/mg GAE for EtOH50%, ethanol, and water.

The antioxidant activity is affected not only by the polyphenol content, but other factors should be considered, such as the degree of polymerization, functional groups, molecular conformation, and others (Olszowy, 2019). The experimental results suggested that subcritical conditions may also affect the chemical composition of the polyphenol extracts, as reported by other authors (Álvarez-Casas et al., 2014; Andrew et al., 2020; Casazza et al., 2012; Vergara-Salinas et al., 2012).

3.3. Polyphenol profile

The chemical composition of the extracts with the highest TPC and antioxidant activity, obtained using CSLE and SSE, can be compared in Table 3. The best results were obtained after 45 min using CSLE with all the solvents. In contrast, the best SSE results were obtained at 3 min with subcritical water, 15 min with subcritical ethanol, and 10 min with subcritical EtOH 50% mixture.

The TPC obtained with subcritical water (1.80 ± 0.01 g_{GAE}/100 g_{d.m.}) is similar to that reported by other authors (Pedras et al., 2017), who obtained polyphenol yields between 1.44 and 2.62 g/100 g_{d.m.} On the other hand, the SSE with ethanol and the EtOH 50% mixture resulted in a slightly lower phenolic content compared to the results reported by (Pereira et al., 2019), which were 3.67 and 6.57 g/100 g_{d.m.} by using ethanol and EtOH 50%, respectively. However, they had considered a grape marc from Syrah grapes, which has a higher phenolic content than the grape variety used in this study (cv. Pinot Gris). Extraction yields are affected not only by the extraction technology and conditions, but also by other factors, such as grape varieties, pedo-climatic conditions, agronomy practices, and winemaking technologies (Vatai et al., 2009).

Three classes of polyphenols were considered: anthocyanins, flavan-3-ols, and tannins. Low anthocyanin yields (1.76–28.75 mg/100 g_{d.m.}) were achieved for both extraction technologies. The Pinot Gris grapes used in this study had a low anthocyanin content, and were macerated for 12 h during the vinification process. Flavan-3-ols and tannins were the most abundant classes of polyphenols extracted using both CSLE and SSE.

SSE provided an average four-fold increase in anthocyanin yields, two-fold increase in flava-3-ols yields, and a 1.5-fold increase in total tannin yields, compared to CSLE. The ethanol-water mixture at subcritical conditions provided the highest yields of phenolic classes: 28.75 ± 0.19 mg/100 g_{d.m.} of anthocyanins, 1.16 ± 0.02 g/100 g_{d.m.} of flavan-3-ols, and 3.95 ± 0.55 g/100 g_{d.m.} of total tannins.

The intensification of polyphenols extraction by EtOH 50% mixture at subcritical conditions from winemaking byproducts was also reported by other authors (Monrad et al., 2010; Pereira et al., 2019). Moreover, the efficiency of subcritical EtOH 50% on phenols extraction was confirmed also for other biomasses, such as citrus or feijoca peels

Table 2

Kinetic parameters of the extraction and degradation phase of antioxidant activity (AA) from grape marc by conventional (CSLE) and subcritical solvent extraction (SSE) with different solvents.

Extraction phase	Kinetic parameters	CSLE			SSE			
		H ₂ O	EtOH	EtOH 50%	H ₂ O	EtOH	EtOH 50%	
	C _w	5.6663	5.0710	14.4358	8.4183	15.5133	36.6750	
	k _w	0.1039	0.2212	0.2316	1.0888	1.4961	1.8191	
	C _d	0.0615	4.4896	1.5480	6.4717	487.64	1021.11	
	k _d	0.1031	0.0378	0.0145	1.0890	0.0004	0.0004	
	R ²	0.9714	0.9912	0.9992	0.9917	0.9984	0.9951	
	R ² -adj.	0.9500	0.9847	0.9986	0.9669	0.9969	0.9879	
	R ² -pred.	0.9986	0.9628	0.9456	0.9766	0.9789	0.8633	
Degradation phase	Kinetic parameters	C ₁	–	–	–	5.6831	4.8397	54.9490
		k ₁	–	–	–	–0.2087	–0.0784	–0.1628
		C ₂	–	–	–	12.5610	17.6846	32.3271
		k ₂	–	–	–	–0.0046	–0.0006	–0.0023
		R ²	–	–	–	0.9831	0.9994	0.9838
		R ² -adj.	–	–	–	0.9576	0.9932	0.9352
		R ² -pred.	–	–	–	0.8648	0.8103	0.9320

Table 3

Polyphenol profile of extracts obtained by conventional (CSLE) and subcritical solvent (SSE) extraction with water, ethanol and their mixture (EtOH 50%) as green solvents.

TPC (Total polyphenols content)	g GAE/100 g d.m	CSLE			SSE		
		H ₂ O	EtOH	EtOH 50%	H ₂ O	EtOH	EtOH 50%
Antioxidant activity	mg α-toc./mL	6.25 ± 0.40 e	9.31 ± 0.42 d	15.54 ± 1.51 c	14.24 ± 0.37 c	19.03 ± 0.87 b	42.30 ± 0.68 a
Anthocyanins	mg/100 g d.m	1.76 ± 0.78 b	9.27 ± 2.21 b	6.02 ± 1.31 b	8.96 ± 2.64 b	26.87 ± 6.88 a	28.75 ± 0.19 a
Flavan-3-ols	g/100 g d.m	0.25 ± 0.02 e	0.33 ± 0.07 de	0.55 ± 0.10 bc	0.45 ± 0.04 cd	0.63 ± 0.08 b	1.16 ± 0.02 a
Tannins	g/100 g d.m	0.90 ± 0.07 c	1.38 ± 0.51 bc	1.96 ± 0.28 b	1.50 ± 0.20 bc	1.23 ± 0.29 bc	3.96 ± 0.55 a
Proanthocyanidins							
Total PAs	g/100 g d.m	0.42 ± 0.02 d	0.97 ± 0.01 c	1.07 ± 0.01 c	0.44 ± 0.02 d	1.65 ± 0.01 b	3.41 ± 0.03 a
mDP	–	4.09 ± 0.22 b	4.78 ± 0.07 a	3.45 ± 0.03 c	2.40 ± 0.10 e	2.25 ± 0.02 e	2.72 ± 0.03 d
% G	%	8.88 ± 0.49 d	22.26 ± 0.32 a	17.56 ± 0.15 b	8.72 ± 0.36 d	10.21 ± 0.07 c	9.14 ± 0.09 d
% t Cat	%	15.65 ± 0.96 d	14.06 ± 0.20 e	11.76 ± 0.10 f	25.90 ± 1.06 b	27.67 ± 0.18 a	22.97 ± 0.23 c
% tECat	%	13.11 ± 0.74 b	8.83 ± 0.13 c	8.70 ± 0.07 c	15.17 ± 0.62 a	16.20 ± 0.11 a	13.26 ± 0.13 b
% tECatG	%	0.18 ± 0.01 e	1.56 ± 0.02 a	0.45 ± 0.01 d	0.64 ± 0.03 b	0.62 ± 0.01 b	0.53 ± 0.01 c
% extCat	%	8.45 ± 0.46 b	5.12 ± 0.07 d	7.20 ± 0.06 c	8.40 ± 0.34 b	7.07 ± 0.05 c	9.40 ± 0.09 a
% extECat	%	53.90 ± 2.96 a	49.72 ± 0.72 b	54.76 ± 0.46 a	41.81 ± 1.71 cd	38.85 ± 0.26 d	45.23 ± 0.45 c
% extECatG	%	7.58 ± 0.42 d	20.16 ± 0.29 a	16.91 ± 0.14 b	7.59 ± 0.31 d	9.17 ± 0.06 c	8.24 ± 0.08 d

Different letters within line indicate significant differences ($p < 0.05$).

mDP, mean degree of poly-merization; % G, percentage of galloylation; % tCat, percentage of terminal (b)-catechin; % tECat, percentage of terminal (–)-epicatechin; % extCat, percentage of extension (b)-catechin; % extECat, per-centage of extension (–)-epicatechin; % extECatG, percentage of extension (–)- epigallocatechin; % extECatG, percentage of extension (–)-epicatechin gallate.

^a Each data represents the mean of three replicates ± standard deviation.

(Barrales et al., 2018; Santos et al., 2019).

Tannins are an important class of bioactive compounds that have gained popularity due to their beneficial effects on human health as antioxidant, anti-inflammatory, anti-allergic, anti-cancer, immunostimulating, anti-viral, cardio-protective, and antithrombotic features (Smeriglio et al., 2017; Unusan, 2020). Some of their properties, such as their antioxidant activity or their ability to interact with other macromolecules, are related to their chemical structure. In Table 3, the chemical composition of the grape proanthocyanidins extracted by CSLE and SSE is reported. The HPLC method enabled the estimation of the degree of polymerization (mDP) and the galloylation percentage (%G) of the proanthocyanidins extracted with water, ethanol, and the EtOH 50% mixture at conventional and subcritical conditions. The experimental results pointed out a significant effect of subcritical conditions also on chemical structure of proanthocyanins, with a significant decrease of their mDP and %G. The rise of temperature and pressure at subcritical state can affect some chemical properties, such as the ionic product, and hydrolysis and other depolymerization mechanisms can be promoted (Cocero et al., 2018).

The degree of polymerization and galloylation percentage are

important modulators that influence the antioxidant capacity of polyphenols. Lower mDP and %G were related to a higher antioxidant capacity and lower cytotoxic effect (Mitjans et al., 2011). The experimental results (Table 3) confirmed these findings, showing a higher antioxidant activity with a lower mDP and %G.

4. Conclusions

The subcritical solvent extraction significantly increased the extraction efficiency and polyphenols yield from grape marc. The best results were obtained using a subcritical 50% water-ethanol mixture at 120 °C, 100 bar, and 10 min. A 1.5-fold increase in polyphenol content and a three-fold increase in antioxidant activity were achieved at the best SSE conditions, compared to the conventional extraction.

However, a degradation phenomenon of polyphenols was highlighted at subcritical conditions and times longer than 10 min. The magnitude of the polyphenol content decrease was different among the solvents, and it was promoted in aqueous solvents.

The description of the extraction kinetics using suitable models is important for gaining valuable information for the process scale-up. The

two-site kinetic model and a two terms exponential decay model well described the experimental results of the two process stages identified for SSE ($R^2 > 0.99$). The mathematical modelling of the initial extraction period showed that SSE enables a polyphenol extraction that is 5 times faster than CSLE.

Significant effects on some chemical properties of the phenolic extracts were highlighted. Significant decreases in the degree of polymerization and the galloylation percentage of the proanthocyanidins were detected, that can be related to the antioxidant activity increase.

The subcritical solvent extraction can be considered an efficient and versatile green technology and controlling process conditions is crucial for improving both extraction yield and the quality of the resulting extracts. Additional research on the effect of SSE parameters, and more detailed kinetic models are necessary for process optimization and its potential scaling-up at an industrial scale.

Funding

This study was carried out within the Interconnected Nord-Est Innovation Ecosystem (iNEST) and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.5 – D.D. 1058 June 23, 2022, ECS00000043). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

A. Natolino: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Data curation, Conceptualization. **P. Passaghe:** Writing – review & editing, Writing – original draft, Visualization, Validation, Data curation, Conceptualization. **G. Brugnara:** Formal analysis, Data curation. **P. Comuzzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition.

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.

Data availability

Data will be made available on request.

References

- Álvarez-Casas, M., García-Jares, C., Llompарт, M., Lores, M., 2014. Effect of experimental parameters in the pressurized solvent extraction of polyphenolic compounds from white grape marc. *Food Chem.* 157, 524–532. <https://doi.org/10.1016/j.foodchem.2014.02.078>.
- Alvarez-Rivera, G., Bueno, M., Ballesteros-Vivas, D., Mendiola, J.A., Ibañez, E., 2020. Pressurized liquid extraction. In: *Liquid-Phase Extraction*. Elsevier, pp. 375–398. <https://doi.org/10.1016/B978-0-12-816911-7.00013-X>.
- Andrew, J., Masetlwa, J., Tesfaye, T., Sithole, B., 2020. Beneficiation of eucalyptus tree barks in the context of an integrated biorefinery – optimisation of accelerated solvent extraction (ASE) of polyphenolic compounds using response surface methodology. *Sustainable Chemistry and Pharmacy* 18, 100327. <https://doi.org/10.1016/j.scp.2020.100327>.
- Antony, A., Farid, M., 2022. Effect of temperatures on polyphenols during extraction. *Appl. Sci.* 12, 2107. <https://doi.org/10.3390/app12042107>.
- Barrales, F.M., Silveira, P., Barbosa, P.D.P.M., Ruviano, A.R., Paulino, B.N., Pastore, G.M., Macedo, G.A., Martínez, J., 2018. Recovery of phenolic compounds from citrus by-products using pressurized liquids — an application to orange peel. *Food Bioprod. Process.* 112, 9–21. <https://doi.org/10.1016/j.fbp.2018.08.006>.
- Basak, S., Annappure, U.S., 2022. The potential of subcritical water as a “green” method for the extraction and modification of pectin: a critical review. *Food Res. Int.* 161, 111849. <https://doi.org/10.1016/j.foodres.2022.111849>.
- Beres, C., Costa, G.N.S., Cabezedo, I., Da Silva-James, N.K., Teles, A.S.C., Cruz, A.P.G., Mellinger-Silva, C., Toton, R.V., Cabral, L.M.C., Freitas, S.P., 2017. Towards integral utilization of grape pomace from winemaking process: a review. *Waste Manag.* 68, 581–594. <https://doi.org/10.1016/j.wasman.2017.07.017>.
- Bitwell, C., Indra, S.S., Luke, C., Kakoma, M.K., 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African* 19, e01585. <https://doi.org/10.1016/j.sciaf.2023.e01585>.
- Bodoira, R., Velez, A., Andreatta, A.E., Martínez, M., Maestri, D., 2017. Extraction of bioactive compounds from sesame (*Sesamum indicum* L.) defatted seeds using water and ethanol under sub-critical conditions. *Food Chem.* 237, 114–120. <https://doi.org/10.1016/j.foodchem.2017.05.102>.
- Bosso, A., Guaita, M., Petrozziello, M., 2016. Influence of solvents on the composition of condensed tannins in grape pomace seed extracts. *Food Chem.* 207, 162–169. <https://doi.org/10.1016/j.foodchem.2016.03.084>.
- Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J.M., Gil-Muñoz, R., Fernández-Fernández, J.I., Bautista-Ortín, A.B., 2010. Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. *J. Agric. Food Chem.* 58, 11333–11339. <https://doi.org/10.1021/jf102265c>.
- Çam, M., Hişil, Y., 2010. Pressurized water extraction of polyphenols from pomegranate peels. *Food Chem.* 123, 878–885. <https://doi.org/10.1016/j.foodchem.2010.05.011>.
- Casazza, A.A., Aliakbarian, B., Sannita, E., Perego, P., 2012. High-pressure high-temperature extraction of phenolic compounds from grape skins. *Int. J. Food Sci. Technol.* 47, 399–405. <https://doi.org/10.1111/j.1365-2621.2011.02853.x>.
- Castro-Muñoz, R., Díaz-Montes, E., Gontarek-Castro, E., Boczkaj, G., Galanakis, C.M., 2022. A comprehensive review on current and emerging technologies toward the valorization of bio-based wastes and by products from foods. *Compr. Rev. Food Sci. Food Saf.* 21, 46–105. <https://doi.org/10.1111/1541-4337.12894>.
- Cheigh, C.-I., Chung, E.-Y., Chung, M.-S., 2012. Enhanced extraction of flavanones hesperidin and narirutin from Citrus unshiu peel using subcritical water. *J. Food Eng.* 110, 472–477. <https://doi.org/10.1016/j.jfoodeng.2011.12.019>.
- Chemat, F., Vian, M.A., Cravotto, G., 2012. Green extraction of natural products: concept and principles. *IJMS* 13, 8615–8627. <https://doi.org/10.3390/ijms13078615>.
- Cocero, M.J., Cabeza, Á., Abad, N., Adamovic, T., Vaquerizo, L., Martínez, C.M., Pazo-Cepeda, M.V., 2018. Understanding biomass fractionation in subcritical & supercritical water. *J. Supercrit. Fluids* 133, 550–565. <https://doi.org/10.1016/j.supflu.2017.08.012>.
- Comuzzo, P., Voce, S., Grazioli, C., Tubaro, F., Marconi, M., Zanella, G., Querzè, M., 2020. Pulsed electric field processing of red grapes (cv. Rondinella): modifications of phenolic fraction and effects on wine evolution. *Foods* 9, 414. <https://doi.org/10.3390/foods9040414>.
- Cravotto, C., Grillo, G., Binello, A., Gallina, L., Olivares-Vicente, M., Herranz-López, M., Micol, V., Barrajón-Catalán, E., Cravotto, G., 2022. Bioactive antioxidant compounds from chestnut peels through semi-industrial subcritical water extraction. *Antioxidants* 11, 988. <https://doi.org/10.3390/antiox11050988>.
- Da Porto, C., Natolino, A., 2017. Supercritical fluid extraction of polyphenols from grape seed (*Vitis vinifera*): study on process variables and kinetics. *J. Supercrit. Fluids* 130, 239–245. <https://doi.org/10.1016/j.supflu.2017.02.013>.
- Ding, Y., Morozova, K., Scampicchio, M., Ferrentino, G., 2020. Non-extractable polyphenols from food by-products: current knowledge on recovery, characterisation, and potential applications. *Processes* 8, 925. <https://doi.org/10.3390/pr8080925>.
- Duba, K.S., Casazza, A.A., Mohamed, H.B., Perego, P., Fiori, L., 2015. Extraction of polyphenols from grape skins and defatted grape seeds using subcritical water: experiments and modeling. *Food Bioprod. Process.* 94, 29–38. <https://doi.org/10.1016/j.fbp.2015.01.001>.
- Erim Kose, Y., 2022. Degradation kinetic modeling of bioactive compounds and enzyme activity in wheat germ during stabilization. *LWT* 153, 112501. <https://doi.org/10.1016/j.lwt.2021.112501>.
- Essien, S.O., Young, B., Baroutian, S., 2020. Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials. *Trends Food Sci. Technol.* 97, 156–169. <https://doi.org/10.1016/j.tifs.2020.01.014>.
- Fan, R., Gao, Y., 2022. Maillard and hydrolytic reactions in subcritical water extraction of bioactive compounds from licorice. *Molecules* 27, 6851. <https://doi.org/10.3390/molecules27206851>.
- FAO, 2019. In: *Moving Forward on Food Loss and Waste Reduction, the State of Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome*.
- Gallego, R., Bueno, M., Herrero, M., 2019. Sub- and supercritical fluid extraction of bioactive compounds from plants, food-by-products, seaweeds and microalgae – an update. *TrAC, Trends Anal. Chem.* 116, 198–213. <https://doi.org/10.1016/j.trac.2019.04.030>.
- García, P., Fedes, C., Cea, I., Lozano-Sánchez, J., Leyva-Jiménez, F.J., Robert, P., Vergara, C., Jimenez, P., 2021. Recovery of bioactive compounds from pomegranate (*punica granatum* L.) peel using pressurized liquid extraction. *Foods* 10, 203. <https://doi.org/10.3390/foods10020203>.
- García-Mendoza, M.P., Paula, J.T., Paviani, L.C., Cabral, F.A., Martínez-Correa, H.A., 2015. Extracts from mango peel by-product obtained by supercritical CO₂ and pressurized solvent processes. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft - Technol.)* 62, 131–137. <https://doi.org/10.1016/j.lwt.2015.01.026>.
- Gómez-García, R., Campos, D.A., Aguilar, C.N., Madureira, A.R., Pintado, M., 2021. Valorisation of food agro-industrial by-products: from the past to the present and perspectives. *J. Environ. Manag.* 299, 113571. <https://doi.org/10.1016/j.jenvman.2021.113571>.
- González-Centeno, M.R., Rosselló, C., Simal, S., Garau, M.C., López, F., Femenia, A., 2010. Physico-chemical properties of cell wall materials obtained from ten grape varieties and their byproducts: grape pomaces and stems. *LWT - Food Sci. Technol.*

- (Lebensmittel-Wissenschaft -Technol.) 43, 1580–1586. <https://doi.org/10.1016/j.lwt.2010.06.024>.
- Kaleem, M., Ahmad, A., 2018. Flavonoids as nutraceuticals. In: Therapeutic, Probiotic, and Unconventional Foods. Elsevier, pp. 137–155. <https://doi.org/10.1016/B978-0-12-814625-5.00008-X>.
- Kim, D.-S., Lim, S.-B., 2020. Kinetic study of subcritical water extraction of flavonoids from citrus unshiu peel. *Separ. Purif. Technol.* 250, 117259. <https://doi.org/10.1016/j.seppur.2020.117259>.
- Kim, J.-W., Nagaoka, T., Ishida, Y., Hasegawa, T., Kitagawa, K., Lee, S.-C., 2009. Subcritical water extraction of nutraceutical compounds from citrus pomaces. *Separ. Sci. Technol.* 44, 2598–2608. <https://doi.org/10.1080/01496390903014375>.
- Knez, Ž., Leitgeb, M., Primožič, M., 2018. Chemical reactions in subcritical and supercritical fluids. In: Meyers, R.A. (Ed.), *Encyclopedia of Sustainability Science and Technology*. Springer, New York, New York, NY, pp. 1–21. https://doi.org/10.1007/978-1-4939-2493-6_1004-1.
- Ko, M.-J., Cheigh, C.-I., Cho, S.-W., Chung, M.-S., 2011. Subcritical water extraction of flavonol quercetin from onion skin. *J. Food Eng.* 102, 327–333. <https://doi.org/10.1016/j.jfoodeng.2010.09.008>.
- Liu, Z., De Souza, T.S.P., Holland, B., Dunshea, F., Barrow, C., Suleria, H.A.R., 2023. Valorization of food waste to produce value-added products based on its bioactive compounds. *Processes* 11, 840. <https://doi.org/10.3390/pr11030840>.
- Lozano-Sánchez, J., Castro-Puyana, M., Mendiola, J., Segura-Carretero, A., Cifuentes, A., Ibáñez, E., 2014. Recovering bioactive compounds from olive oil filter cake by advanced extraction techniques. *IJMS* 15, 16270–16283. <https://doi.org/10.3390/ijms150916270>.
- Makris, D.P., Boskou, G., Andrikopoulos, N.K., 2007. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *J. Food Compos. Anal.* 20, 125–132. <https://doi.org/10.1016/j.jfca.2006.04.010>.
- Mandal, S.C., Mandal, V., Das, A.K., 2015. Classification of extraction methods. In: *Essentials of Botanical Extraction*. Elsevier, pp. 83–136. <https://doi.org/10.1016/B978-0-12-802325-9.00006-9>.
- Marcus, Y., 2018. Extraction by subcritical and supercritical water, methanol, ethanol and their mixtures. *Separations* 5, 4. <https://doi.org/10.3390/separations5010004>.
- Mitjans, M., Ugartondo, V., Martínez, V., Touriño, S., Torres, J.L., Vinardell, M.P., 2011. Role of galloylation and polymerization in cytoprotective effects of polyphenolic fractions against hydrogen peroxide insult. *J. Agric. Food Chem.* 59, 2113–2119. <https://doi.org/10.1021/jf1025532>.
- Möller, M., Nilges, P., Harnisch, F., Schröder, U., 2011. Subcritical water as reaction environment: fundamentals of hydrothermal biomass transformation. *ChemSusChem* 4, 566–579. <https://doi.org/10.1002/cssc.201000341>.
- Monrad, J.K., Howard, L.R., King, J.W., Srinivas, K., Maoumoustakos, A., 2010. Subcritical solvent extraction of procyanidins from dried red grape pomace. *J. Agric. Food Chem.* 58, 4014–4021. <https://doi.org/10.1021/jf9028283>.
- More, P.R., Jambak, A.R., Arya, S.S., 2022. Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization. *Trends Food Sci. Technol.* 128, 296–315. <https://doi.org/10.1016/j.tifs.2022.08.016>.
- Mufari, J.R., Rodríguez-Ruiz, A.C., Bergesse, A.E., Miranda-Villa, P.P., Nepote, V., Velez, A.R., 2021. Bioactive compounds extraction from malted quinoa using water-ethanol mixtures under subcritical conditions. *LWT* 138, 110574. <https://doi.org/10.1016/j.lwt.2020.110574>.
- Natolino, A., Da Porto, C., 2020. Kinetic models for conventional and ultrasound assisted extraction of polyphenols from defatted fresh and distilled grape marc and its main components skins and seeds. *Chem. Eng. Res. Des.* 156, 1–12. <https://doi.org/10.1016/j.cherd.2020.01.009>.
- Nel, A.P., 2018. Tannins and anthocyanins: from their origin to wine analysis – a review. *SAJEV* 39. <https://doi.org/10.21548/39-1-1503>.
- Olszowy, M., 2019. What is responsible for antioxidant properties of polyphenolic compounds from plants? *Plant Physiol. Biochem.* 144, 135–143. <https://doi.org/10.1016/j.plaphy.2019.09.039>.
- Otero-Pareja, M., Casas, L., Fernández-Ponce, M., Mantell, C., Ossa, E., 2015. Green extraction of antioxidants from different varieties of red grape pomace. *Molecules* 20, 9686–9702. <https://doi.org/10.3390/molecules20069686>.
- Pavlović, I., Knez, Ž., Škerget, M., 2013. Hydrothermal reactions of agricultural and food processing wastes in sub- and supercritical water: a review of fundamentals, mechanisms, and state of research. *J. Agric. Food Chem.* 61, 8003–8025. <https://doi.org/10.1021/jf401008a>.
- Pedras, B., Salema-Oom, M., Sá-Nogueira, I., Simões, P., Paiva, A., Barreiros, S., 2017. Valorization of white wine grape pomace through application of subcritical water: analysis of extraction, hydrolysis, and biological activity of the extracts obtained. *J. Supercrit. Fluids* 128, 138–144. <https://doi.org/10.1016/j.supflu.2017.05.020>.
- Peixoto, F.B., Raimundini Aranha, A.C., Oliveira Defendi, R., Tonin, L.T.D., Sipoli, C.C., Fragoso, Á.G., Düsman, E., Suzuki, R.M., 2023. Optimization of extraction of bioactive compounds with antiproliferative activity from goji berry and assessment of kinetic models. *J. Chem. Technol. Biotechnol.* 98, 2888–2903. <https://doi.org/10.1002/jctb.7498>.
- Pereira, D.T.V., Tarone, A.G., Cazarin, C.B.B., Barbero, G.F., Martínez, J., 2019. Pressurized liquid extraction of bioactive compounds from grape marc. *J. Food Eng.* 240, 105–113. <https://doi.org/10.1016/j.jfoodeng.2018.07.019>.
- Petersson, E.V., Liu, J., Sjöberg, P.J.R., Danielsson, R., Turner, C., 2010. Pressurized Hot Water Extraction of anthocyanins from red onion: a study on extraction and degradation rates. *Anal. Chim. Acta* 663, 27–32. <https://doi.org/10.1016/j.aca.2010.01.023>.
- Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., Núñez, M.J., 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J. Agric. Food Chem.* 53, 2111–2117. <https://doi.org/10.1021/jf0488110>.
- Priego-Capote, F., Delgado De La Torre, M.D.P., 2013. Accelerated liquid extraction. In: Rostagno, M.A., Prado, J.M. (Eds.), *Green Chemistry Series*. Royal Society of Chemistry, Cambridge, pp. 157–195. <https://doi.org/10.1039/9781849737579-00157>.
- Rifna, E.J., Misra, N.N., Dwivedi, M., 2023. Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: a review. *Crit. Rev. Food Sci. Nutr.* 63, 719–752. <https://doi.org/10.1080/10408398.2021.1952923>.
- Sagar, N.A., Pareek, S., Sharma, S., Yahia, E.M., Lobo, M.G., 2018. Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. *Compr. Rev. Food Sci. Food Saf.* 17, 512–531. <https://doi.org/10.1111/1541-4337.12330>.
- Santos, P.H., Baggio Ribeiro, D.H., Micke, G.A., Vitali, L., Hense, H., 2019. Extraction of bioactive compounds from feijoa (*Acca sellowiana* (O. Berg) Burret) peel by low and high-pressure techniques. *J. Supercrit. Fluids* 145, 219–227. <https://doi.org/10.1016/j.supflu.2018.12.016>.
- Scrob, T., Varodi, S.M., Vintilă, G.A., Casoni, D., Cimpoiu, C., 2022. Estimation of degradation kinetics of bioactive compounds in several lingonberry jams as affected by different sweeteners and storage conditions. *Food Chem. X* 16, 100471. <https://doi.org/10.1016/j.fochx.2022.100471>.
- Sharma, P., Gaur, V.K., Sirohi, R., Larroche, C., Kim, S.H., Pandey, A., 2020. Valorization of cashew nut processing residues for industrial applications. *Ind. Crop. Prod.* 152, 112550. <https://doi.org/10.1016/j.indcrop.2020.112550>.
- Shimanouchi, T., Ueno, S., Yang, W., Kimura, Y., 2014. Extraction of reducing sugar with anti-oxidative scavengers from peels of *Carya cathayensis* Sarg.: use of subcritical water. *Environmental Engineering Research* 19, 41–45. <https://doi.org/10.4491/eer.2014.19.1.041>.
- Silva, E.T.D.V., Queiroz, A.J.M.D., Figueirêdo, R.M.F.D., Moura, H.V., Santos, F.S.D., Silva, A.P.D.F., Cavalcanti, C.F., Gregório, M.G., Galdino, P.O., Gomes, J.P., 2023. Dynamic modelling of degradation kinetics of phenolic compounds, phenolic profiles, mineral content, and overall antioxidant capacity of germinated peanut flours. *LWT* 183, 114927. <https://doi.org/10.1016/j.lwt.2023.114927>.
- Smeriglio, A., Barreca, D., Bellocco, E., Trombetta, D., 2017. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* 174, 1244–1262. <https://doi.org/10.1111/bph.13630>.
- Sólyom, K., Solá, R., Cocero, M.J., Mato, R.B., 2014. Thermal degradation of grape marc polyphenols. *Food Chem.* 159, 361–366. <https://doi.org/10.1016/j.foodchem.2014.03.021>.
- Sovová, H., 1994. Rate of the vegetable oil extraction with supercritical CO₂—I. Modelling of extraction curves. *Chem. Eng. Sci.* 49, 409–414. [https://doi.org/10.1016/0009-2509\(94\)87012-8](https://doi.org/10.1016/0009-2509(94)87012-8).
- Spigno, G., Tramelli, L., De Faveri, D.M., 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.* 81, 200–208. <https://doi.org/10.1016/j.jfoodeng.2006.10.021>.
- Todd, R., Baroutian, S., 2017. A techno-economic comparison of subcritical water, supercritical CO₂ and organic solvent extraction of bioactives from grape marc. *J. Clean. Prod.* 158, 349–358. <https://doi.org/10.1016/j.jclepro.2017.05.043>.
- Unusan, N., 2020. Proanthocyanidins in grape seeds: an updated review of their health benefits and potential uses in the food industry. *J. Funct. Foods* 67, 103861. <https://doi.org/10.1016/j.jff.2020.103861>.
- 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. *J. Food Eng.* 90, 246–254. <https://doi.org/10.1016/j.jfoodeng.2008.06.028>.
- Vergara-Salinas, J.R., Pérez-Jiménez, J., Torres, J.L., Agosin, E., Pérez-Correa, J.R., 2012. Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*). *J. Agric. Food Chem.* 60, 10920–10929. <https://doi.org/10.1021/jf3027759>.
- Waterhouse, A.L., Sacks, G.L., Jeffery, D.W., 2016. *Understanding Wine Chemistry*. John Wiley & Sons, Ltd, Chichester, UK. <https://doi.org/10.1002/9781118730720>.
- Wijngaard, H., Brunton, N., 2009. The optimization of extraction of antioxidants from apple pomace by pressurized liquids. *J. Agric. Food Chem.* 57, 10625–10631. <https://doi.org/10.1021/jf902498y>.
- Xu, H., Wang, W., Liu, X., Yuan, F., Gao, Y., 2015. Antioxidative phenolics obtained from spent coffee grounds (*Coffea arabica* L.) by subcritical water extraction. *Ind. Crop. Prod.* 76, 946–954. <https://doi.org/10.1016/j.indcrop.2015.07.054>.
- Zhang, J., Wen, C., Zhang, H., Duan, Y., Ma, H., 2020. Recent advances in the extraction of bioactive compounds with subcritical water: A review. *Trends Food Sci. Technol.* 95, 183–195. <https://doi.org/10.1016/j.tifs.2019.11.018>.