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Effect of cold pressing and supercritical CO_2 extraction assisted with pulsed electric fields pretreatment on grape seed oil yield, composition and antioxidant characteristics

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ABSTRACT

The aim of this study was to valorize the oil fraction of grape wine pomace by improving oil yield and sustaining quality. Two "green" extraction methods were applied: supercritical CO_2 (SC CO_2) extraction, as well as pulsed electric fields (PEF) assisted SC CO_2 extraction; and compared with conventional cold pressing. Optimal SC CO_2 parameters supporting maximum yield and/or antioxidant capacity were (i) 35 MPa, 45 °C, and (ii) 50 MPa, 35 °C, both at 45 g CO_2/min. Two PEF pretreatments at 5 kV/cm and 120 Hz during (i) 5 min and (ii) 1 min were applied. Cold pressing, despite the lower extraction yield (67.1 ± 0.2 g/kg), extracted significantly higher concentrations of tocochromanols, hydrophilic antioxidants and major fatty acid, linoleic acid. Both parameters of PEF pretreatments and SC CO_2 extraction had a crucial role in increasing extraction yield (up to 81.8 ± 1.0 g/kg) and offering possibilities for more selective extractions, particularly of sterols and nonflavonoids (phenolic acids and *trans*-resveratrol) compared to cold pressing. The highest concentrations of these compounds were extracted with the longer PEF pretreatment followed by the extraction at 35 MPa and 45 °C, amounting up to 5347.0 ± 0.6 mg/kg and 1378 ± 6 mg/kg for sterols and nonflavonoids, respectively.

1. Introduction

Seed fraction of the grape pomace represents a very valuable wine waste by-product, not only as a source of various polyphenolic compounds, but also containing 7.3–22.4% of grape seed oil (Dabetic et al., 2020; Martin, Grao-Cruces, Millan-Linares, & Montserrat De la Plaz, 2020; Matthäus, 2008). Grape seed oil is an important alternative vegetable oil, a rich source of essential unsaturated fatty acids (around 90%), particularly linoleic acid, as well as a source of other bioactive compounds with strong antioxidant activity: vitamin E active compounds (tocopherols and tocotrienols), phytosterols and polyphenolic compounds (Dimić et al., 2020; Martin, Grao-Cruces, Millan-Linares, & Montserrat-De la Paz, 2020). Also, this oil is interesting from sensory perspective, for vinous and fruity aromas with raisins and nutty notes and specific taste (Matthäus, 2008). Recently, interest for grape seed oil production has risen in food industry, due to trends in wine sector to promote and implement sustainable wine production, to reduce negative environmental impacts and create new value-added food products (Martin et al., 2020).

The chemical composition and sensory characteristics of the grape seed oil depend on cultivar characteristics, agricultural practices and seed maturity, but also on the oil extraction technique (Martin et al., 2020; Matthäus, 2008). Most commonly, grape seed oil is extracted by cold pressing or with organic solvents (Duba & Fiori, 2015). Cold pressing is known to result in lower yields (Crews et al., 2006), but cold pressed/virgin oils, in contrast to high yield solvent-extracted/refined oils, have higher content of antioxidants and aromatic complexity with diverse acid, alcohol and ester composition (Bail, Stuebiger, Krist, Unterweger, & Buchbauer, 2008; Sevindik, Kelebek, Rombolà, & Selli, 2022).

To conquer limitations of conventional extractions (low yield, long time, high solvent consumption, toxic residues) various innovative techniques such as supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, have been investigated in

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the last decade (Dimić et al., 2020; Martin et al., 2020). Among the presented techniques, supercritical fluid extraction with carbon dioxide (SC CO_2) has been recognized as green, low-cost, and nontoxic technique using nonflammable CO_2 as solvent (Dimić et al., 2020). Since grape seeds are rich in temperature sensitive compounds (Saykova, Iatcheva, & Stoylov, 2022), low processing temperatures make this technique suitable for production of high-quality oil (de Souza et al., 2020). Moreover, SC CO_2 offers selectivity towards extraction of valuable compounds (Beveridge, Girard, Kopp, & Drover, 2005; de Souza et al., 2020; Dimić et al., 2020; Mohamed et al., 2016). The efficacy of SC CO_2 extraction depends primarily on the proper selection of temperature, pressure, flow rate, extraction time and sample size (Casquete et al., 2022; Dimić et al., 2020; Duba & Fiori, 2015; Jokić, Bijuk, Aladić, Bilić, & Molnar, 2016). However, to maintain high quality and yield of grape seed oil, optimization of extraction parameters is a crucial factor. In addition, new trends are moving towards combined techniques (e.g. ultrasound along with SC CO_2), or pretreatments of seeds, with the aim to additionally increase oil yield and quality, as well as to ensure safety and reduce time of the extraction (de Souza et al., 2020). In this context, pulsed electric field (PEF) should be considered as a novel pretreatment technique for enhanced extraction. This technique is based on the application of short pulses (μs to ms) at moderate electrical voltage (0.5–20 kV/cm), which provokes electroporation of the cell membrane (Kumari, Tiwari, Hossain, Brunton, & Rai, 2018). Such mechanism improves mass transfer of intracellular compounds through enhanced diffusion, and in this way increases efficiency of the extraction (Kumari et al., 2018). PEF treatments have been used to extract oil (including tocopherols, antioxidants, phytosterols and other functional components) from various oilseeds (Bakhshabadi, Mirzaei, Ghodsvali, Jafari, & Ziaiifar, 2018; Guderjan, Elez-Martínez, & Knorr, 2007; Haji-Moradkhani, Rezaei, & Moghimi, 2019; Sarkis, Boussetta, Tessaro, Marczak, & Vorobiev, 2015; Shorstkii, Mirshekarloo, & Koshevoi, 2017) resulting in higher extraction yields without adverse effects on oil quality. However, to the best of our knowledge, no studies have investigated the effects of PEF pretreatment on grape seed oil SC CO_2 extraction yield as well as oil composition.

The present research explores, for the first time, the effects of SC CO_2 and PEF assisted SC CO_2 oil extraction from the Graševina (*Vitis vinifera* L.) white grape seeds, in comparison with cold pressing (CP). After considering SC CO_2 extraction kinetics, optimal parameters of SC CO_2 pressure, temperature and flow rate were defined by response surface methodology (RSM) based on extraction yield and antioxidant capacity and coupled with PEF pretreatment. Finally, the effects of different extraction technologies on grape seed oil yield, chemical composition (sterols, tocopherols, tocotrienols, polyphenols and fatty acids) and antioxidant characteristics (H-ORAC and L-ORAC) were investigated.

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile, *n*-hexane, 2-propanol were HPLC grade and were purchased from J.T. Baker (Deventer, Netherlands). Folin Ciocalteu's phenol reagent was purchased from Reagecon (Shannon, Ireland), and 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH) from Acros (Gell, Belgium). Fatty acids methyl esters (FAME) standards (C8–C22) were purchased from Supelco (Bellefonte, PA, USA). Silicagel F254 plates, N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane and α-tocopherol were purchased from Merck (Darmstadt, Germany). Ethanol, diethyl ether, isooctane, sodium carbonate, sodium dihydrogen phosphate, sodium hydrogen sulfate monohydrate, disodium hydrogen phosphate, potassium hydroxide, fluorescein, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), methyl-β-cyclodextrin, 5-α-cholestanol, gallic acid, hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, procyanidin dimer B1, quercetin and myricetin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Materials

This study was carried out with white grape seed pomaces from *Vitis vinifera* cv. Graševina, grown in Kutjevo, Croatia. Grapes were harvested in their technological maturity in September 2021, since Graševina is mid-late ripening cultivar. After the pressing, seeds were separated from grape pomace skin, manually cleaned, and directly transported to laboratory for further oil extraction. The crude fat content in grape seed fraction, determined by Soxhlet extraction with hexane using standard ISO method 659 (International Organization for Standardization, 2009) amounted up to 85.0 ± 0.4 g/kg.

2.3. Extraction methods of grape seed oils

2.3.1. Cold pressing

Cold pressed grape seed oil was extracted using laboratory expeller press (Monforts & Reiners, Rheydt, Germany). Seeds were first dried in oven dryer (35 °C, 6 h) to obtain moisture content of 80 g/kg, and then further pressed without grinding or conditioning (Cecchi et al., 2019). One kg of seeds was pressed in each extraction cycle, extraction was conducted in triplicate. Oils were further clarified by sedimentation; the oil yield was 67.1 ± 0.2 g/kg. Extracted grape seed oil was stored in dark glass bottles under nitrogen at −18 °C until further analysis.

2.3.2. Supercritical CO_2 (SC CO_2) extraction

SC CO_2 extraction was conducted using laboratory scale extractor (Extratex, Neuves-Maisons, France) consisting of CO_2 container; chiller (1300 W, 20 °C); high-pressure CO_2 pump (up to 100 g/min; 100 MPa); distributer; stainless steel cylindrical extraction vessel (up to 100 mL; 100 MPa, 250 °C); oven; manual back pressure regulator; cyclonic separator (0.3 L; 20 MPa, 150 °C); valves; heat and cold exchangers; manometers, temperature and flow meters.

Prior to SC CO_2 extraction, grape pomace seeds were first dried (35 °C, 48 h) and further ground using grinder with integrated cooling system (IKA, Staufen, Germany). To obtain homogeneous sample in all SC CO_2 experiments, the batch amount of 2.5 kg was used. The average particle diameter size (d_p) of batch after grinding was analyzed according to ICC standard method (1998), using vibratory sieve shaker (Bühler, Uzwil, Switzerland) and calculated to be 554 ± 5 μm. A portion of 50 g of ground seeds were placed into 100 mL stainless steel cylindrical extractor. SC CO_2 extraction was performed for 90 min at different operating conditions. Namely, extraction experiments were first conducted according to Box-Behnken design (BBD) which included three independent variables at three factorial levels (Table 1), selected according to literature data (Dimić et al., 2020; Duba & Fiori, 2015): pressure (X_1) at 30, 40 and 50 MPa; temperature (X_2) at 35, 45 and 55 °C and flow rate (X_3) at 15, 30 and 45 g/min. Each SC CO_2 extraction was conducted in duplicate. In addition, optimal combinations of process parameters determined by response surface methodology (RSM) were further selected and applied in PEF pretreated and non-pretreated samples. Extracted grape seed oil was stored in dark glass bottles under nitrogen at −18 °C until further analysis.

2.3.3. Pulsed electric fields (PEF) pretreatment following SC CO_2 extraction

Pulsed electric fields pretreatments were conducted on HVG60/1 PEF equipment (Impel d. o.o., Zagreb, Croatia) previously described (Bebek Markovinović et al., 2022). PEF treatment chamber (500 mL capacity) was equipped with two parallel stainless-steel electrodes (68 mm of diameter) with inter electrode gap distance of 50 mm. For the pretreatments, 70 g of homogenized sample (dried and ground) was added to chamber with distilled water (1:2) (Sarkis et al., 2015). Electric field strength was set at 5 kV/cm with pulse frequency of 120 Hz and applied during 5 min (pretreatment PEF1) and 1 min (pretreatment PEF2). The estimated specific energy inputs (Raso et al., 2016) were

Table 1
Box–Behnken experimental design for grape seed oil supercritical CO_2 extraction and experimental results for oil extraction yield (EY), hydrophilic ORAC (H-ORAC) and lipophilic ORAC (L-ORAC).

Run	Independent variables levels			Dependent variables responses		
	X_1	X_2	X_3			
	Pressure (MPa)	Temperature (° C)	Flow rate (g/min)	EY (g/kg)	H-ORAC (μmol TE/g)	L-ORAC (μmol TE/g)
1	30 (−1)	35 (−1)	30 (0)	74.9±0.8	2.83±0.14	1.36±0.08
2	50 (+1)	35 (−1)	30 (0)	72.4±0.3	2.92±0.05	0.92±0.05
3	30 (−1)	55 (+1)	30 (0)	76.0±0.3	2.52±0.07	1.61±0.11
4	50 (+1)	55 (+1)	30 (0)	66.3±0.4	2.84±0.20	1.07±0.08
5	30 (−1)	45 (0)	15 (−1)	73.5±0.6	3.27±0.09	1.54±0.10
6	50 (+1)	45 (0)	15 (−1)	72.7±0.3	3.91±0.06	1.06±0.06
7	30 (−1)	45 (0)	45 (+1)	74.4±0.4	3.60±0.17	2.55±0.12
8	50 (+1)	45 (0)	45 (+1)	73.0±1.2	4.36±0.06	1.88±0.07
9	40 (0)	35 (−1)	15 (−1)	78.4±0.5	3.22±0.05	1.75±0.11
10	40 (0)	55 (+1)	15 (−1)	73.1±0.2	3.63±0.02	1.81±0.09
11	40 (0)	35 (−1)	45 (+1)	78.4±1.0	4.05±0.14	2.34±0.05
12	40 (0)	55 (+1)	45 (+1)	76.2±1.1	2.92±0.08	2.16±0.11
13	40 (0)	45 (0)	30 (0)	80.0±0.4	2.98±0.01	1.95±0.04
14	40 (0)	45 (0)	30 (0)	79.5±0.2	2.90±0.01	1.86±0.04
15	40 (0)	45 (0)	30 (0)	80.3±0.6	2.90±0.03	1.83±0.05
16	40 (0)	45 (0)	30 (0)	79.5±0.5	2.92±0.03	1.82±0.05
17	40 (0)	45 (0)	30 (0)	79.8±0.1	2.98±0.03	1.82±0.05

Data of EY are presented as mean value ± standard deviation over two replicates. Data of H-ORAC and L-ORAC are presented as mean value ± standard deviation over three replicates. Replicated central points (run 13–17): EY = 79.8 ± 0.4 g/kg; H-ORAC = 2.93 ± 0.04 μM TE/g; L-ORAC = 1.86 ± 0.06 μM TE/g.

24.00 kJ/kg and 4.79 kJ/kg for PEF1 and PEF2, respectively. Initial conductivity and temperature of samples were 1.13 ± 0.05 ms/cm and 25.2 ± 0.3 °C. Conductivity and temperature measured after the pretreatment were 1.66 ± 0.05 mS/cm and 28.1 ± 0.4 °C for PEF1, and 1.57 ± 0.04 mS/cm and 25.4 ± 0.2 °C for PEF2, respectively. After the pretreatment, samples were freeze dried to remove water, and further subjected to SC CO_2 extraction of oil according to the protocol described above (2.3.2). Each PEF pretreatment followed by SC CO_2 extraction was conducted in triplicate.

2.4. Extraction yield

Grape seed oil extraction yields (g/kg) for all the extraction methods were calculated according to the given Eq. (1).

$$\text{EY (g/kg)} \frac{\text{mass of extracted oil}}{\text{mass of grape seed pomace}} \quad (1)$$

2.5. Oil chemical analysis

2.5.1. Total phenols

Total phenols were determined using Folin-Ciocalteu method (Singleton & Rossi, 1965). The extraction of phenolic compounds was conducted using aqueous methanol (100 mL/L) according to procedure of Bail et al. (2008). Analyzes were conducted in triplicate. Results were expressed in mg gallic acid equivalents per kg of oil sample (mg GAE/kg).

2.5.2. Antioxidant capacity

Oxygen radical absorbance capacity (ORAC) was measured according to Ou, Hampsch-Woodill, and Prior (2001). Both, hydrophilic (H-ORAC) and lipophilic (L-ORAC) methods were carried out using procedures earlier described (Ou, Chang, Huang, & Prior, 2013; Shinagawa, Santana, Araujo, Purgatto, & Mancini-Filho, 2017). Analyzes were conducted in triplicate. Results were expressed as μmol Trolox equivalents per g of oil sample (μmol TE/g).

2.5.3. GC-FID/MS analysis of sterols

Sterols were analyzed by standard ISO method 12228–1 (International Organization for Standardization, 2014) using α-cholestanol as an internal standard. Saponification was conducted with ethanolic potassium hydroxide solution, while separation of unsaponified compounds was performed by glass column chromatography and elution with diethyl ether. Sterol fraction was separated by silica gel chromatography, while silylation reaction was performed to obtain trimethylsilyl derivatives. Analyses, identification, and quantification were conducted on Agilent Technologies 6890N Network GC system (Agilent, Santa Clara, CA, USA) equipped with flame ionization detector (FID) and mass detector (MS) as earlier precisely described (Balbino et al., 2021). The capillary column used was DB-17MS (30 m × 0.32 mm, 0.25 μm) (Agilent, Santa Clara, CA, USA). The temperature of the injector was set at 290 °C, while split ratio was 13.3:1. Helium at flow rate 1.5 mL was used as carrier gas. The oven temperature was set to increase from 180 to 270 °C at rate of 6 °C/min, and further kept on this temperature for 30 min. Temperature of transfer line was set at 280 °C, MS source at 230 °C and quadrupole at 150 °C. Sterols were quantified by using the internal standard method. Analyzes were conducted in triplicate. Results were expressed in mg per kg of oil sample (mg/kg).

2.5.4. HPLC-FLUO analysis of tocopherols and tocotrienols

Tocopherols and tocotrienols were determined according to standard ISO method 9936 (International Organization for Standardization, 2016) on Agilent Technologies 1290 Infinity II LC System (Agilent, Santa Clara, CA, USA) coupled FLUO detector. Separation was conducted on LiChroCART Silica 60 (250 mm × 4.6 mm, 5 μm) column (Phenomenex, Torrance, CA, USA) by isocratic chromatography using propan-2-ol in *n*-hexane (7 mL/L) as mobile phase at 0.9 mL/min as described by Kraljić et al. (2018). Detection was performed at 295 nm excitation and 330 nm emission wavelengths. Tocopherols and tocotrienols were quantified by using the standard calibration curve of α-tocopherol. Analyzes were conducted in triplicate. Results were expressed in mg per kg of oil sample (mg/kg).

2.5.5. HPLC-DAD/MS analysis of polyphenols

Analysis of phenolic acids (gallic, hydroxybenzoic, *p*-coumaric and ferulic acids), *trans*-resveratrol, flavan-3-ols [(+)-catechin, (−)-epicatechin and procyanidin dimer B1 and flavonols (quercetin and myricetin) was performed on Agilent Technologies 1290 Infinity II LC System (Agilent, Santa Clara, CA, USA) coupled to DAD and MS detector. Separation was conducted on Gemini C18 (250 mm × 4.6 mm, 5 μm) column (Phenomenex, Torrance, CA, USA) by using aqueous solution of formic acid (30 mL/L) as mobile phase A and methanol as solvent B according to the method previously described (Lukić et al., 2020), with

small modifications in gradient conditions: 2–25% B linear from 0 to 15 min, 25–30% B linear from 15 to 20 min; 30–40% B linear from 20 to 40 min, 40–50% B linear from 40 to 50 min, 50% B isocratic from 50 to 65 min, 50-2% B linear from 65 to 68 min, with re-equilibration of the column from 68 to 70 min under initial gradient conditions. Identification was performed with DAD at 280, 320 and 360 nm by comparison with the retention times of standards and mass spectral data, while quantification was done with calibration curves of external standards listed above. Analyzes were conducted in triplicate. Results were expressed in μg per kg of oil sample (μg/kg).

2.5.6. GC-FID/MS analysis of fatty acid profile

Methyl esters of fatty acids were obtained by transmethylation according to the standard ISO method 12,966–2 (International Organization for Standardization, 2017). The prepared methyl esters were injected (1 μL) into an Agilent Technologies 6890N Network GC system (Agilent, Santa Clara, CA, USA) equipped with flame ionization detector (FID) and mass detector (MS). Fatty acid methyl esters were separated on a DB-23 capillary (60 m × 0.25 mm × 0.25 μm) column (Agilent, Santa Clara, CA, USA) according to the method earlier briefly described by Kraljić et al. (2018). Fatty acid methyl esters were identified by comparison of their retention times with those of the FAME commercial standards. Analyzes were conducted in triplicate. The content of each fatty acid is expressed in g per kg of total fatty acids (g/kg).

2.6. Data analysis and modeling

2.6.1. Descriptive statistic and analysis of variance

Results of extraction yield, H-ORAC and L-ORAC were expressed as mean values ± standard deviation. Results of sterols, tocochromanols, polyphenolic compounds and fatty acids are presented as mean values, and the reproducibility of the results was expressed as pooled standard deviation values (pooled SD). Pooled standard deviations were calculated using the sum of individual variances divided by the individual degrees of freedom of each series of replicates. The statistical analyses of oil chemical data were carried out using the Analysis of Variance (ANOVA). Prior to ANOVA analysis data distribution was tested using Shapiro-Wilks test and uniformity of the variance was tested using Leven's test. Tukey's HSD test was used as a comparison test when samples were significantly different after ANOVA ($p < 0.05$) using Statistica v.14 software (Tibco Software Inc., Palo Alto, CA, USA).

2.6.2. Response surface modeling and extraction optimization

Regression analysis was performed on experimental data of dependent variables: extraction yield (EY), H-ORAC and L-ORAC; and fitted to an empirical second order polynomial model (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008) using Statistica v.14 software (Tibco Software Inc., Palo Alto, CA, USA). Optimal SC CO_2 extraction conditions were further estimated based on the proposed RSM models. Optimization of multiple responses was performed using the desirability function proposed by Derringer and Suich (1980). This methodology is based on constructing on transformation of predicted values obtained from each response into a dimensionless individual scale (d_i), while overall desirability (D) is defined as geometric mean of individual desirabilities. The scale of desirability function values ranges between 0 (completely undesirable response) to 1 (fully desired response). The main aim of the optimization was to maximize the dependent variables, that was conducted by transformation function described by Bezerra et al. (2008).

2.6.3. Modeling extraction kinetics of SC CO_2 extraction

The extraction curves of grape seed oil were adjusted using empirical model developed by Kandiah and Spiro (1990) (Eq. (2)):

$$Y_{EY} = x_0\left[1 - \left(f_1 e^{-k_1 t} + f_2 e^{-k_2 t}\right)\right] \quad (2)$$

where x_0 is initial amount of soluble compound, f_1 and f_2 are the fractions of solute extracted with rate constants k_1 and k_2. Coefficients of the model were estimated using nonlinear regression implemented in software WR Mathematica 10.0 (Wolfram Research, Champaign, IL, USA).

3. Results and discussion

3.1. Grape seed oil supercritical CO_2 (SC CO_2) extraction kinetics

In order to optimize SC CO_2 extraction of grape seed oil, experiments according to Box-Behnken experimental design (BBD) were performed (Table 1). Prior to optimization, the effects of these extraction parameters (independent variables) on extraction kinetics (oil yield) were also studied (Fig. 1 and Table 2). Considering the importance of mathematical modeling in supercritical fluid extraction, the kinetics of the supercritical CO_2 extraction of grape seed oil was investigated by modeling the extraction curves using the model described by Kandiah and Spiro (1990). Used model is based on the assumption that the internal resistance to mass transfer has a significant effect on the process from the beginning of extraction. Model also proposes two extraction phases: the first one relatively fast and the second one relatively slow. Mathematical modeling can aid in better understanding the extraction mechanism, rapid optimization/calculation of extraction conditions for scale up, and simulation of overall extraction curves (Fiori et al., 2014; Zeković, Filip, Vidović, Jokić, & Svilović, 2014).

Analyzing the extraction curves given at Fig. 1 it can be noticed all 13 of them follow the same trend. The curves exhibit a typical linear trend at the beginning due to free oil extraction, followed by a decrease in extraction rate due to slower tied oil extraction. The extraction curves mostly overlap at the end of the process, while there are differences between them at the beginning of the process, indicating the significant influence of the test process variables.

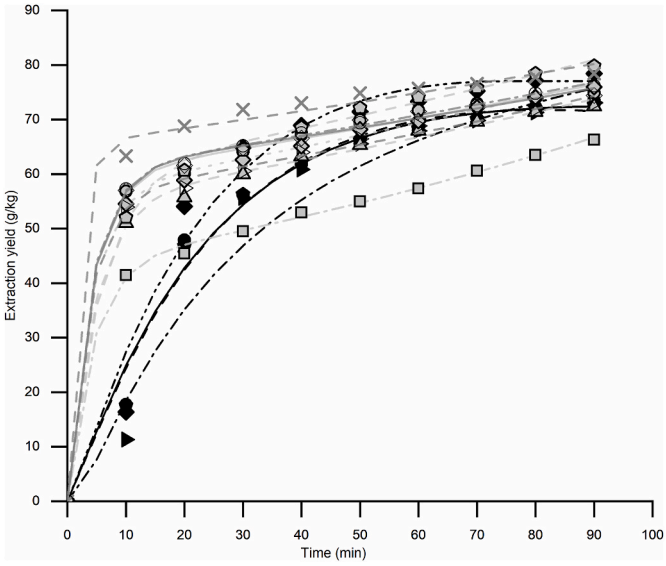

Fig. 1. Effect of different supercritical CO_2 extraction parameters (pressure, temperature and CO_2 flow rate) on grape seed oil extraction kinetics (experimental and model curves): 30 MPa, 35 °C, 30 g/min exp — model; 50 MPa, 35 °C, 30 g/min exp — model; 30 MPa, 55 °C, 30 g/min exp — model; 50 MPa, 55 °C, 30 g/min exp — model; 30 MPa, 45 °C, 15 g/min exp — model; 50 MPa, 45 °C, 15 g/min exp — model; 30 MPa, 45 °C, 45 g/min exp — model; 50 MPa, 45 °C, 45 g/min exp — model; 40 MPa, 35 °C, 15 g/min exp — model; 40 MPa, 55 °C, 15 g/min exp — model; 40 MPa, 35 °C, 45 g/min exp — model; 40 MPa, 55 °C, 45 g/min exp — model; 40 MPa, 45 °C, 30 g/min exp — model.

Table 2
Estimated model kinetics parameters for grape seed oil supercritical CO_2 extraction.

Run	Supercritical CO_2 extraction	f_1 (p value)	k_1 (1/min) (p value)	f_2 (p value)	k_2 (1/min) (p value)	R^2	R^2_{adj}
1	30 MPa, 35 °C, 30 g/min	59.39 ± 1.84 (<0.001)	0.25 ± 0.04 (<0.001)	58.41 ± 1.32 (<0.001)	0.0028 ± 0.0002 (<0.001)	0.9996	0.9995
2	50 MPa, 35 °C, 30 g/min	54.59 ± 1.57 (<0.001)	0.22 ± 0.02 (<0.001)	53.62 ± 1.04 (<0.001)	0.0034± 0.0003 (<0.001)	0.9997	0.9996
3	30 MPa, 55 °C, 30 g/min	56.66 ± 1.18 (<0.001)	0.25 ± 0.03 (<0.001)	55.67 ± 0.76 (<0.001)	0.0034 ± 0.0002 (<0.001)	0.9998	0.9997
4	50 MPa, 55 °C, 30 g/min	42.57 ± 1.16 (<0.001)	0.25 ± 0.03 (<0.001)	41.58 ± 0.73 (<0.001)	0.0051 ± 0.0002 (<0.001)	0.9997	0.9996
5	30 MPa, 45 °C, 15 g/min	83.75 ± 26.59 (<0.001)	0.04 ± 0.01 (<0.001)	80.55 ± 27.33 (<0.001)	0.0011 ± 0.0003 (<0.001)	0.9972	0.9964
6	50 MPa, 45 °C, 15 g/min	83.75 ± 26.59 (<0.001)	0.04 ± 0.01 (<0.001)	80.55 ± 27.33 (<0.001)	0.0011 ± 0.0003 (<0.001)	0.9972	0.9962
7	30 MPa, 45 °C, 45 g/min	60.36 ± 1.44 (<0.001)	0.25 ± 0.03 (<0.001)	59.37 ± 0.97 (<0.001)	0.0026 ± 0.0002 (<0.001)	0.9997	0.9996
8	50 MPa, 45 °C, 45 g/min	55.59 ± 1.47 (<0.001)	0.27 ± 0.04 (<0.001)	54.59 ± 0.95 (<0.001)	0.0028 ± 0.0002 (<0.001)	0.9997	0.9996
9	40 MPa, 35 °C, 15 g/min	104.42 ± 34.13 (0.003)	0.04 ± 0.02 (0.002)	99.87 ± 31.97 (0.004)	0.0027 ± 0.0003 (0.004)	0.9954	0.9944
10	40 MPa, 55 °C, 15 g/min	68.24 ± 2.63 (0.004)	0.01 ± 0.01 (0.003)	67.58 ± 2.35 (0.003)	0.0027 ± 0.0004 (0.006)	0.9922	0.9911
11	40 MPa, 35 °C, 45 g/min	62.39 ± 3.15 (<0.001)	0.34 ± 0.01 (<0.001)	67.76 ± 3.48 (<0.001)	0.0021 ± 0.0002 (<0.001)	0.9932	0.9921
12	40 MPa, 55 °C, 45 g/min	60.12 ± 1.04 (<0.001)	0.25 ± 0.02 (<0.001)	59.13 ± 0.98 (<0.001)	0.0028 ± 0.0001 (<0.001)	0.9998	0.9998
13–17	40 MPa, 45 °C, 30 g/min	62.36 ± 1.26 (<0.001)	0.16 ± 0.01 (<0.001)	58.41 ± 0.92 (<0.001)	0.0029 ± 0.0002 (<0.001)	0.9998	0.9997

Data representing estimated model value ± standard deviation over two replicates.

To define the specific effect of the extraction process conditions on the grape seed oil extraction dynamics, each extraction curve was analyzed individually based on the estimated extraction kinetic parameters (Table 2). The applicability of the used model for description of the extraction dynamics was estimated based on the R^2 and R^2_{adj} values. It can be noticed that for all experiments both R^2 and R^2_{adj} values were above 0.99 indicating that the extraction process was described with an appropriate mechanism. The lowest R^2 was obtained for experiment 10 probably due to slow extraction in the first phase. The estimated values of extraction rate are according to theory. For all analyzed experiments, k_1 was for an order of magnitude higher than the k_2 as presented by Krulj et al. (2021). The nominally highest values for k_1 were estimated for the experiment 11 ($k_1 = 0.34 \pm 0.01$/min) followed by experiment 8 ($k_1 = 0.27 \pm 0.04$/min) both performed with the highest CO_2 flow rate of 45 g/min. On the other hand, the nominally lowest value for k_1 was estimated for experiment 10, as well for the experiments 5, 6 and 9, where the CO_2 flow rate was 15 g/min. Furthermore, comparing the k_2 values it can be noticed that they were in the range from 0.0011 ± 0.0003/min for experiments 5 and 6 up to 0.0051 ± 0.0002/min as indicated for experiments 4. Obtained result show that higher CO_2 flow rate contributed to faster extraction process. As described by Wei, Wang, Wei, and Yang (2021) the contact area and collision opportunity frequency between the solvent and the plant sample were increased by increasing the flow rate, which contributes to higher extraction yield.

3.2. Optimization of grape seed oil SC CO_2 extraction and effect of pulsed electric fields (PEF) pretreatment on SC CO_2 extraction kinetics

Effects of three independent variables (pressure, temperature, and flow rate) on the extraction yield (EY), H-ORAC and L-ORAC were studied by RSM with the aim to define best optimal conditions of SC CO_2 extraction (Table 1). Obtained experimental data were strongly affected by extraction parameters. Multiple regression analysis of these data was employed to gain final predictive 2nd order polynomial equations (Eqs. (3)–(5)), that are presented below with significant coefficients marked bold:

$$Y_{EY} = \mathbf{-66} + \mathbf{4.9}X_1 + \mathbf{2.3}X_2 + 0.014X_3 \mathbf{- 0.018}X_1X_2 - 0.001X_1X_3 + 0.0052X_2X_3 \mathbf{- 0.053}X_1^2 \mathbf{- 0.022}X_2^2 \mathbf{- 0.0050}X_3^2 \quad (3)$$

$$Y_{H\text{-}ORAC} = \mathbf{-0.31} \mathbf{-0.77}X_1 + \mathbf{0.3}X_2 \mathbf{-0.088}X_3 + 0.0055X_1X_2 + 0.00019X_1X_3 \mathbf{- 0.0026}X_2X_3 + 0.00085X_1^2 \mathbf{- 0.0024}X_2^2 + \mathbf{0.0034}X_3^2 \quad (4)$$

$$Y_{L\text{-}ORAC} = \mathbf{-8.5} + \mathbf{0.34}X_1 + 0.19X_2 \mathbf{- 0.037}X_3 - 0.0021X_1X_2 - 0.0033X_1X_3 - 0.00038X_2X_3 \mathbf{- 0.0044}X_1^2 \mathbf{- 0.0018}X_2^2 + \mathbf{0.0015}X_3^2 \quad (5)$$

In addition, response surface plots representing polynomial equations were obtained for all possible combinations of independent variables with EY, H-ORAC and L-ORAC (Fig. 2).

Both linear and quadratic terms of pressure ($p < 0.001$) and temperature ($p < 0.001$), interaction among pressure and temperature ($p < 0.001$), as well as quadratic term of flow rate ($p < 0.05$), have significant effects on oil EY (Table S1). Earlier findings also indicate that pressure and temperature play important roles in the SC CO_2 extraction (Casquete et al., 2022). An increase in EY at constant temperature, as well as at constant flow rate was noticed up to 40 MPa (Fig. 2A and B), as proposed by Jokić et al. (2016), while further decrease of EY up to 50 MPa could be due to the decreased diffusivity of oil at high pressures (Obregón, Huayta, Cárdenas, & Chuquilin, 2020). Similarly, an increase in EY in the range of 30 to 45 °C and decrease from 45 to 60 °C, at constant pressure and flow was observed (Fig. 2C). As previously suggested (Duba & Fiori, 2015), the positive effect of pressure (at constant temperature) on the extraction rate is a result of increased solvent power due to the increased CO_2 density. In addition, the same authors pointed out that an increase in temperature leads to a decrease in SC CO_2 density, but solubility can still increase due to the enhanced solute vapor pressure.

Furthermore, regression analysis of H-ORAC and L-ORAC data indicated significant effects of linear terms of pressure ($p < 0.001$), while its quadratic term ($p < 0.001$) was significant only for L-ORAC (Table S1). Also, quadratic term of temperature was significant for both H-ORAC and L-ORAC ($p < 0.001$), but linear term was significant only for the first ($p < 0.01$), indicating greater importance of the temperature for the H-ORAC (Table S1). In addition, both H-ORAC and L-ORAC values were significantly affected by linear ($p < 0.01$ and $p < 0.001$, respectively) and quadratic terms ($p < 0.001$) of flow rate, but interaction among temperature and flow rate ($p < 0.001$) significantly affected

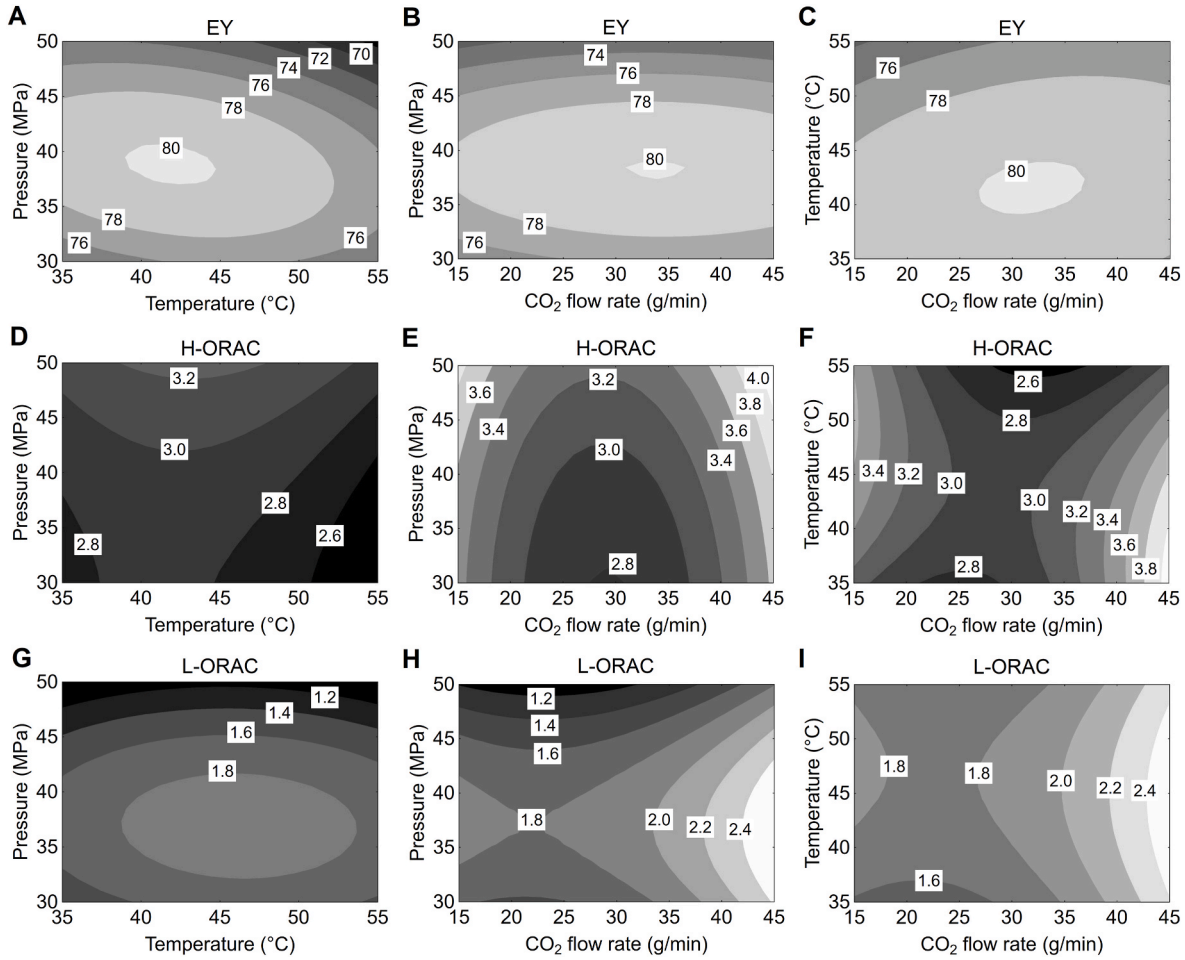

Fig. 2. Response surface 2-D contour plots showing effects of independent variables (pressure and temperature, pressure and CO_2 flow rate, temperature and CO_2 flow rate) on the extraction yield (EY) (A–C); hydrophilic ORAC (H-ORAC) (D–F); and lipophilic ORAC (L-ORAC) (G–I).

only H-ORAC values (Table S1). The importance of pressure as the main variable influencing the antioxidant capacity of SC CO_2 extracts has been confirmed previously (Casquete et al., 2022; Jokić et al., 2016). Different trends among H-ORAC (Fig. 2C–F) and L-ORAC (Fig. 2G–I) considering the impact of temperature and flow rate were also expected, due to the distinctive chemical differences among compounds contributing to hydrophilic and lipophilic fraction. As mentioned earlier, phenolic compounds were found to contribute mainly to the hydrophilic antioxidant capacity, while tocopherols, chlorophylls, and carotenoids contribute mostly to lipophilic antioxidant capacity (Mohamed et al., 2016).

The analysis of variance (ANOVA) of the quadratic model, indicating statistical significance and goodness fit of the models for EY, H-ORAC and L-ORAC, are presented in Table S1. The *F*-values (30, 40 and 39) and *p*-values ($p < 0.0001$) confirmed statistical significance of models. The R^2 and R^2_{adj} (0.9369 and 0.9132 for EY; 0.9368 and 0.9132 for H-ORAC; 0.9358 and 0.9117 for L-ORAC) indicated good fit to the experimental data, while non-significant lack of fit further validated the obtained models.

Finally, the optimal SC CO_2 process variables to achieve maximum extraction yield and antioxidant capacity were selected based on desirability function as follows: (i) 35 MPa, 45 °C, 45 g CO_2/min sustaining EY and L-ORAC (D = 0.9237) and (ii) 50 MPa, 35 °C and 45 g CO_2/min (D = 0.9898) sustaining H-ORAC, named SC1 and SC2, respectively. Validity of the predicted optimal values for SC1 and SC2 was experimentally confirmed, and the obtained data were not significantly different from the predicted ones. In addition, two PEF pretreatments (PEF1 and PEF2) were applied prior to optimal SC CO_2 extractions (SC1 and SC2).

Dynamic of the extraction process at optimal condition with PEF pretreatment was also analyzed based on the model described by Kandiah and Spiro (1990). Similar to the SC CO_2 optimization extraction experiments, all obtained curves follow the same trend. Small differences between the curves are already visible in the first 20 min of extraction, but thereafter these differences become even more pronounced (Fig. 3). The estimated values of kinetic parameters showed that PEF has positive effect on the extraction rate (Table 3). Increase in k_1 value can be observed between experiments with PEF and those without PEF. The kinetic values also showed the fast first phase and slow second phase of extraction. Moreover, SC CO_2 extraction (with values ranging from 76.3 ± 0.9 g/kg up to 78.6 ± 0.6 g/kg), as well as PEF assisted SC CO_2 extraction (with values ranging from 78.4 ± 0.6 g/kg up to 81.7 ± 1.0 g/kg) contributed to significantly higher yields compared to cold pressing (67.1 ± 0.2 g/kg). In addition, PEF pretreatments were more efficient when combined with extraction at 35 MPa and 45 °C, than 50 MPa and 35 °C. Namely, both 5 min and 1 min PEF pretreatments contributed to significantly higher extraction yields compared to SC CO_2 extracted oils (SC1 and SC2) ($p < 0.05$). Other authors also found that the extraction efficiency was improved by applying PEF as a pretreatment before extraction process, as it leads to electrical decomposition of cells and their higher permeability, resulting in better extraction of oil from seeds (Bakhshabadi et al., 2018; Guderjan et al., 2007; Haji-Moradkhani et al., 2019; Sarkis et al., 2015; Shorstkii et al., 2017).

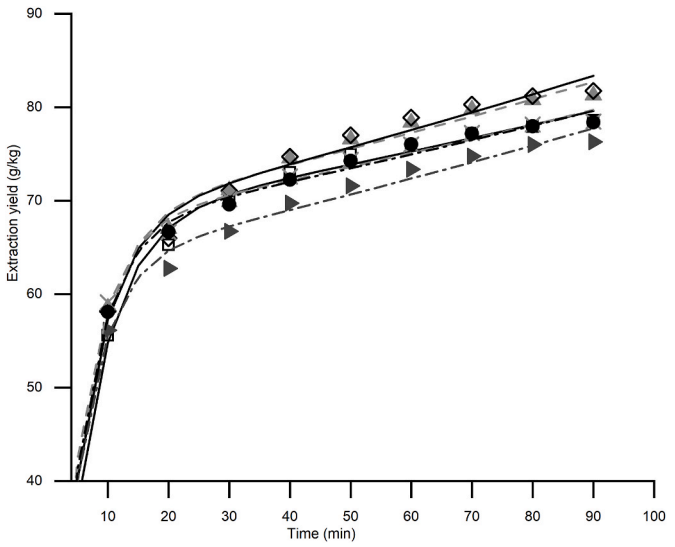

Fig. 3. Effect of pulsed electric field pretreatments and optimal supercritical CO_2 extraction parameters on grape seed oil extraction kinetics (experimental and model curves): SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min. SC1 □ exp ——— model; PEF1_SC1 ◇ exp ——— model; PEF2_SC1 ▲ exp – – – model; SC2 ▶ exp —·— model; PEF1_SC2 × exp – – – model; PEF2_SC2 ● exp —·— model.

3.3. *Effect of cold pressing, SC CO_2 extraction and PEF assisted SC CO_2 extraction on grape seed chemical composition and antioxidant characteristics*

Extraction technique showed significant impact on the concentration of sterols, as presented in Table 4. In accordance with literature data, the main sterol found in all grape seed oils was β-sitosterol, followed by stigmasterol and campesterol (Crews et al., 2006; Shinagawa et al., 2017). Compared with cold pressing, SC CO_2 extractions, as well as PEF assisted SC CO_2 extractions contributed to significantly higher concentrations of total sterols, particularly campesterol, stigmasterol, β-sitosterol and Δ5-avenasterol ($p < 0.05$). Moreover, both pretreatment and extraction parameters affected the concentrations of these compounds. For instance, application of lower pressure and higher temperature (35 MPa, 45 °C in sample SC1) extracted significantly higher concentrations of all sterols ($p < 0.05$) than application of higher pressure at lower temperature (50 MPa, 35 °C in sample SC2) under similar flow rate (45 g CO_2/min). Other studies, conducted on grape or plant seeds, also showed superiority of supercritical CO_2 extraction over conventional methods for the extraction of sterols, as well as the importance of the extraction conditions, primarily pressure and temperature (Beveridge et al., 2005; Nyam, Tan, Lai, Long, & Man, 2010). Furthermore, application of PEF pretreatments, additionally significantly increased concentrations of total sterols, stigmasterol, β-sitosterol, Δ5-avenasterol and Δ7-avenasterol ($p < 0.05$), which can be clearly seen when pretreated samples were compared with their non-pretreated counterparts (Table 4). Moreover, longer pretreatments (PEF1) were found to be more effective compared to shorter pretreatments (PEF2), because these treatments, beside the aforementioned sterols, also extracted significantly higher concentrations of campesterol and Δ5,24-stigmastadienol ($p < 0.05$). Since sterols are integral components of seed cell membranes, PEF-induced cell membranes electroporation and increased porosity (Kumari et al., 2018), probably contributed to these results, while higher intensity and/or longer duration could lead to greater cell disintegration, and consequently improve the release of these compounds. In addition, effect of PEF pretreatment to increase the extraction of sterols was earlier reported on maize and rapeseed oils produced by conventional methods (Guderjan et al., 2007; Guderjan, Töpfl, Angersbach, & Knorr, 2005). Nevertheless, as it can be noticed form Table 4, concentrations of sterols were more affected by extraction than pretreatment parameters, since all pretreated samples extracted at lower pressure and higher temperature compared to ones extracted at higher pressure and lower temperature showed significantly higher concentrations of all determined sterols, except stigmasterol and Δ5,24-stigmastadienol ($p < 0.05$).

As earlier proposed (Crews et al., 2006; Matthäus, 2008; Sabir, Unver, & Kara, 2012) the most abundant tocochromanols in grape seed oil were found to be γ-tocotrienol and α-tocotrienol, followed by α-tocopherol (Table 4). Furthermore, significantly higher concentrations of total tocochromanols and afore mentioned major compounds were obtained by cold pressing ($p < 0.05$). Although the extraction of tocochromanols was not encouraged by SC CO_2, some differences among two SC CO_2 extraction methods were found, resulting in significantly higher concentrations of γ-tocotrienol and total tocochromanols when lower pressure and higher temperature were applied (as previously noted for sterols). The literature does not always appear to be consistent regarding the effects of SC CO_2 grape seed oil extraction versus conventional methods on tocochromanol concentrations. Namely, grape variety and extraction parameters must be considered when interpreting these results, since in some cases a significant contribution was found (Agostini et al., 2012), while in others no significant effect was detected (Fiori et al., 2014). More importantly, PEF pretreatment resulted in significantly higher concentrations of all tocochromanols ($p < 0.05$). Similar observations regarding effect of PEF pretreatment on tocopherols were

Table 3
Estimated model kinetics parameters for optimal grape seed oil supercritical CO_2 extractions coupled with pulsed electric field pretreatments.

Sample name	f_1 (p value)	k_1 (1/min) (p value)	f_2 (p value)	k_2 (1/min) (p value)	R^2	R^2_{adj}
SC1	67.28 ± 1.61 (<0.001)	0.16 ± 0.01 (<0.001)	66.36 ± 1.19 (<0.001)	0.002 ± 0.0003 (<0.001)	0.9998	0.9997
SC2	62.73 ± 1.64 (<0.001)	0.19 ± 0.01 (<0.001)	61.77 ± 1.14 (<0.001)	0.002 ± 0.0002 (<0.001)	0.9998	0.9997
PEF1_SC1	67.09 ± 2.13 (<0.001)	0.18 ± 0.02 (<0.001)	66.17 ± 1.54 (<0.001)	0.002 ± 0.0003 (<0.001)	0.9999	0.9998
PEF2_SC1	67.47 ± 1.71 (<0.001)	0.18 ± 0.01 (<0.001)	66.52 ± 1.23 (<0.001)	0.002 ± 0.0003 (<0.001)	0.9998	0.9997
PEF1_SC2	66.67 ± 1.56 (<0.001)	0.2 ± 0.02 (<0.001)	65.7 ± 1.11 (<0.001)	0.002 ± 0.0003 (<0.001)	0.9999	0.9998
PEF2_SC2	66.48 ± 1.39 (<0.001)	0.19 ± 0.01 (<0.001)	65.51 ± 0.99 (<0.001)	0.002 ± 0.0003 (<0.001)	0.9999	0.9998

Data representing estimated model value ± standard deviation over three replicates. Abbreviations: SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min.

Table 4
Effect of cold pressing, supercritical CO_2 extraction (SC CO_2) and pulsed electric fields (PEF) assisted SC CO_2 extraction on the concentration of sterols and tocochromanols.

Sample name	Sterols (mg/kg)									Tocochromanols (mg/kg)					
	Campesterol	Campestanol	Stigmasterol	β-Sitosterol	Sitostanol	Δ5-Avenasterol	Δ5,24-Stigmastadienol	Δ7-Stigmastenol	Δ7-Avenasterol	Total sterols	α-Tocopherol	γ-Tocopherol	α-Tocotrienol	γ-Tocotrienol	Plastochromanol-8	Total T, T3, P8
CP	313.0^{e}	14.4^{a}	416.4^{d}	2854.7^{f}	18.1^{c}	91.4^{f}	17.9^{de}	100.5^{ab}	48.1^{d}	3874.7^{g}	75.9^{a}	12.6^{c}	124.1^{a}	144.5^{a}	7.6^{a}	364.7^{a}
SC1	384.9^{bc}	11.4^{b}	506.7^{b}	3727.4^{d}	22.0^{ab}	108.9^{cd}	20.4^{cd}	93.0^{abc}	52.1^{c}	4926.7^{e}	27.1^{e}	11.8^{c}	36.8^{e}	80.4^{e}	1.5^{d}	157.6^{e}
SC2	362.7^{d}	8.0^{c}	473.5^{c}	3586.3^{e}	19.8^{c}	100.9^{e}	16.6^{e}	79.7^{d}	32.9^{f}	4680.4^{f}	24.4^{e}	11.4^{c}	33.4^{e}	74.5^{f}	0.9^{d}	144.5^{f}
PEF1_SC1	395.6^{a}	14.5^{a}	527.2^{a}	3973.9^{a}	22.9^{ab}	151.5^{a}	39.4^{a}	101.4^{a}	120.6^{a}	5347.0^{a}	69.8^{bc}	19.7^{a}	69.5^{c}	99.9^{c}	4.7^{b}	263.7^{c}
PEF2_SC1	391.5^{ab}	11.6^{b}	526.4^{a}	3963.7^{a}	24.9^{a}	114.0^{b}	28.3^{b}	93.6^{abc}	114.5^{b}	5268.5^{b}	63.1^{d}	16.1^{b}	60.9^{d}	88.2^{d}	3.0^{c}	231.3^{d}
PEF1_SC2	379.4^{c}	8.8^{c}	523.6^{a}	3862.7^{b}	20.3^{bc}	109.7^{c}	21.6^{c}	91.2^{bc}	49.8^{d}	5067.0^{c}	73.6^{ab}	20.5^{a}	77.8^{b}	108.1^{b}	7.8^{a}	287.8^{b}
PEF2_SC2	370.0^{d}	8.2^{c}	508.8^{b}	3784.4^{c}	21.0^{bc}	105.6^{d}	19.2^{cde}	88.5^{c}	38.1^{e}	4943.9^{d}	65.6^{cd}	20.4^{a}	71.0^{c}	107.9^{b}	7.7^{a}	272.7^{c}
Pooled Std	2.1	0.5	1.2	3.0	0.8	0.9	0.8	2.6	0.5	2.8	1.4	0.3	1.1	1.0	0.2	2.5

Data are presented as mean value and pooled standard deviation (Pooled Std) over three replicates. ANOVA to compare data; different lowercase letters in the same column indicate statistical differences between samples (Tukey's test, $p < 0.05$). Abbreviations: T, tocopherols; T3, tocotrienols; P8, plastochromanol-8; CP, cold pressing; SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min.

obtained for other plant oils, such as rapeseed and black cumin seed oil (Bakhshabadi et al., 2018; Guderjan et al., 2007). In addition, outcomes of extraction were found to be dependent on pretreatment parameters/intensity, and following parameters of SC CO_2 extraction (Table 4). Longer pretreatment resulted in significantly higher concentrations of all tocochromanols when extraction at 35 MPa, 45 °C was applied, as well as significantly higher concentrations of α-tocopherol, α-tocotrienol and total tocochromanols at 50 MPa, 35 °C ($p < 0.05$). However, effect of longer pretreatment was particularly efficient when extraction at 50 MPa and 35 °C was further applied, resulting in the significantly higher concentrations of total tocochromanols among all pretreated samples ($p < 0.05$).

Effect of different extraction method on grape seed oil phenolic composition are given in Table 5. Among analyzed phenolic compounds, quantitatively the most abounded was the group of flavan-3-ols [with dominant procyanidin B1, (+)-catechin and (−)-epicatechin, followed by the group of phenolic acids (with dominant *p*-hydroxybenzoic, gallic and *p*-coumaric acid). Previous studies also confirmed a similar profile and concentrations of phenolic compounds in grape seed oils from different cultivars (Bjelica, Vujasinović, Rabrenović, & Dimić, 2019; Cecchi et al., 2019; de Souza et al., 2020). As it can be seen from Table 5, cold pressing resulted in significantly higher concertation of total and certain individual phenolics [*p*-hydroxybenzoic acid, (+)-catechin, (−)-epicatechin, procyanidin B1 and myricetin ($p < 0.05$). Fiori, De Faveri, Casazza, and Perego (2009) showed that SC CO_2 had low efficiency in extracting phenolic compounds from grape seeds when compared to organic solvents, while no differences among SC CO_2 and conventional solvent extraction were established by Mohamed et al. (2016). Some small differences among two SC CO_2 extractions were found, due to the significantly higher concentrations of gallic acid, procyanidin B1 and quercetin extracted at 50 MPa, 35 °C. Furthermore, PEF pretreatments contributed to the significantly higher concentrations of all phenolic acids, *trans*-resveratrol, and quercetin compared to non-pretreated counterparts ($p < 0.05$), as well as significantly higher concentrations of (+)-catechin, procyanidin B1 and total phenols ($p < 0.05$) for the extraction at 35 MPa and 45 °C. In addition, intensity of the PEF pretreatment was an important parameter affecting the extraction at 35 MPa and 45 °C, but not the extraction at 50 MPa and 35 °C. Recently, Saykova et al. (2022) showed that PEF (0.86 kV/cm, 13.3 Hz, 900 pulses of 100 μs duration) assisted solid-liquid extraction was able to extract higher concentration of total phenolics from grape seeds compared to single solid-liquid extraction. Same authors also highlighted that some of phenolic compounds with low (gallic acid) or medium (catechins monomers) molecular weight are capable to pass through PEF generated micropores in cell membranes, indicating the relevance of PEF process parameters for achieving effective electroporation. Favorable effects of PEF pretreatment prior to conventional extraction on the concentration of phenolics were earlier reported in oil seeds like rapeseeds or cannabis seeds (Guderjan et al., 2007; Haji--Moradkhani et al., 2019). Interestingly, even though cold pressing extracted the highest concentrations of total phenols and flavonoids (and hence overall phenolic compounds), the highest concentrations of nonflavonoids (gallic, *p*-coumaric and ferulic acids, *trans*-resveratrol) were obtained by longer pretreatment employing SC CO_2 extraction at 35 MPa and 45 °C.

Antioxidant capacity was determined by H-ORAC and L-ORAC assays, and the results were consistent with the literature (Shinagawa et al., 2017; Zhao, Yagiz, Xu, Fang, & Marshall, 2017). Cold pressed oil had the highest value of H-ORAC ($p < 0.05$), while differences among samples considering the L-ORAC value were not as much pronounced (Fig. 4). The application of PEF pretreatments resulted in oils with significantly higher H-ORAC for extraction at 35 MPa and 45 °C. This can be explained by the release of phenolics and other antioxidant compounds in the extracted oils. Guderjan et al. (2007) also found that oil antioxidant activity increased after rapeseeds PEF pretreatment (5 kV/cm, 60 pulses). In addition, H-ORAC values were very highly

Table 5
Effect of cold pressing, supercritical CO_2 extraction (SC CO_2) and pulsed electric fields (PEF) assisted SC CO_2 extraction on the concentration of phenolic compounds.

Sample name	Phenolic compounds										
	Nonflavonoids (μg/kg)					Flavonoids (μg/kg)					Total phenols (mg/kg)
	Gallic acid	Hydroxybenzoic acid	*p*-Coumaric acid	Ferulic acid	*trans*-Resveratrol	(+)-Catechin	(−)-Epicatechin	Procyanidin dimer B1	Quercetin	Myricetin	
CP	219.7^{b}	472.8^{a}	139.2^{d}	84.7^{d}	84.8^{d}	671.7^{a}	279.5^{a}	1514.5^{a}	64.9^{d}	70.3^{a}	39.7^{a}
SC1	33.3^{f}	173.4^{e}	74.1^{e}	60.5^{f}	69.0^{e}	281.8^{d}	30.2^{b}	772.9^{e}	63.2^{d}	45.1^{b}	23.9^{d}
SC2	53.7^{e}	174.6^{e}	85.0^{e}	63.1^{f}	65.8^{f}	292.5^{d}	39.9^{b}	899.6^{d}	75.8^{c}	45.3^{b}	24.0^{d}
PEF1_SC1	582.5^{a}	267.4^{b}	282.0^{a}	135.2^{a}	111.2^{a}	450.3^{b}	40.2^{b}	1338.0^{b}	106.7^{a}	47.2^{b}	35.4^{b}
PEF2_SC1	210.9^{c}	226.8^{c}	183.3^{c}	100.5^{b}	110.1^{b}	417.0^{c}	39.6^{b}	1001.3^{c}	104.6^{a}	46.6^{b}	29.0^{c}
PEF1_SC2	129.8^{d}	191.5^{d}	201.3^{b}	93.7^{c}	100.5^{c}	296.6^{d}	40.9^{b}	939.1^{d}	80.8^{b}	45.5^{b}	24.9^{d}
PEF2_SC2	130.0^{d}	197.0^{d}	130.6^{d}	76.3^{e}	100.9^{c}	293.8^{d}	40.2^{b}	912.1^{d}	81.3^{b}	45.4^{b}	25.0^{d}
Pooled Std	1.2	3.0	3.1	1.6	0.2	6.5	4.9	13.2	0.9	0.7	0.7

Data are presented as mean value and pooled standard deviation (Pooled Std) over three replicates. ANOVA to compare data; different lowercase letters in the same column indicate statistical differences between samples (Tukey's test, $p < 0.05$). Abbreviations: CP, cold pressing; SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min.

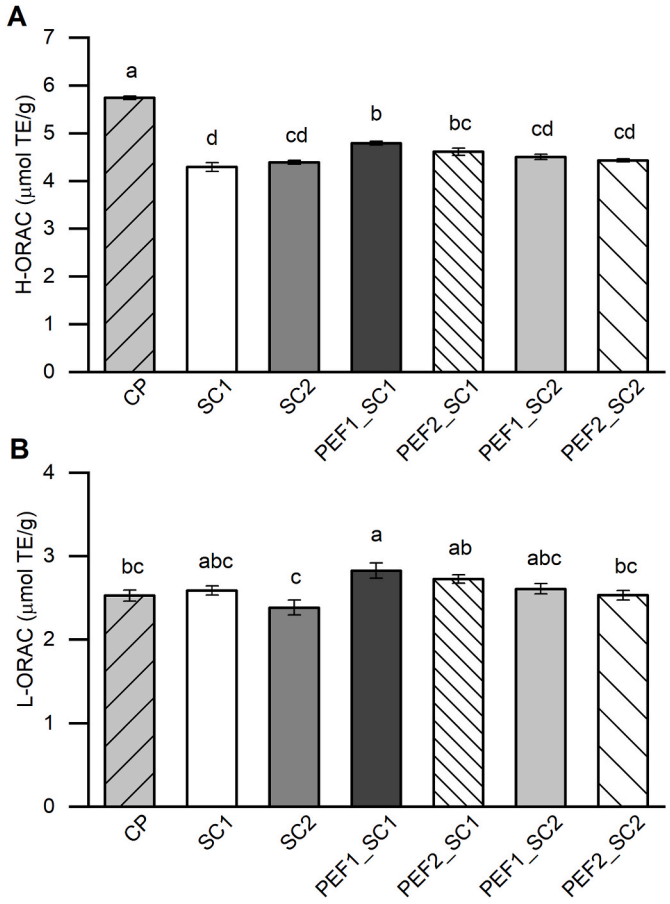

Fig. 4. Effect of cold pressing, supercritical CO_2 extraction (SC CO_2) and pulsed electric fields (PEF) assisted SC CO_2 extraction on hydrophilic ORAC (H-ORAC) (A) and lipophilic ORAC (L-ORAC) (B). Abbreviations: CP, cold pressing; SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min.

positively correlated with total (r = 0.917; $p < 0.05$) and overall individual phenols (r = 0.904; $p < 0.05$) and negatively correlated with overall sterol concentrations (r = −0.714; $p < 0.05$), in accordance with earlier findings (Kozłowska, Gruczyńska, Ścibisz, & Rudzińska, 2016; Mohamed et al., 2016).

As presented in Table 6, linoleic acid, followed by oleic acid, were the most abundant grape seed oil fatty acids, as previously proposed (Dabetic et al., 2020; Lachman et al., 2015). Cold pressed grape seed oil showed significantly higher values of polyunsaturated fatty acids (PUFAs), primarily due to the linoleic acid ($p < 0.05$); as well as significantly lower portion of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) ($p < 0.05$). Contrary, SC CO_2 extraction produced oils with significantly lower values of PUFAs and significantly higher ones for SFAs and MUFAs primarily due to the palmitic, stearic and oleic acids ($p < 0.05$). Namely, SC CO_2 is known to dissolve non-polar or slightly polar compounds, while solvent power of low molecular weight compounds is high and decreases with increasing molecular weight, while pressure-temperature combination of SC CO_2 significantly affects the extraction results (Da Porto, Decorti, & Tubaro, 2012; Sahena et al., 2009). Moreover, PEF pretreatments significantly enhanced the extraction of linoleic acid, and consequently PUFAs compared to non-pretreated counterparts, but the portions obtained were still significantly lower than in cold pressed oil ($p < 0.05$). Contrary, the portions of SFAs and MUFAs in PEF pretreated samples were lower than in non-pretreated ones, and higher than in cold pressed oil ($p < 0.05$).

4. Conclusions

Oil extraction methods combining pulsed electric fields (PEF) pretreatment and SC CO_2 extraction were applied for the first time on grape seed pomace. Even though both PEF and SCO_2 extraction require higher equipment investments, when combined, they offer high extraction yield comparable with Soxhlet extraction, but represent "green" alternative. More importantly, selection of pretreatment and extraction parameters allows more selective extraction toward target compounds, while maintaining good oil quality that further should be supported by oil sensory characteristics.

CRediT authorship contribution statement

Natka Ćurko: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Katarina Lukić:** Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Visualization. **Ana Jurinjak Tušek:** Formal analysis, Investigation, Writing – original draft, Data curation. **Sandra Balbino:** Methodology, Formal analysis, Investigation, Visualization. **Tomislava Vukušić Pavičić:** Methodology, Investigation. **Marina Tomašević:** Methodology, Formal analysis, Investigation. **Ivana Radojčić**

Table 6
Effect of cold pressing, supercritical CO_2 extraction (SC CO_2) and pulsed electric fields (PEF) assisted SC CO_2 extraction on the concentration of fatty acids.

Sample name	Fatty acids (g/kg)											
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	SFAs	MUFAs	PUFAs
CP	0.6a	76.1c	3.0 b	44.4bc	205.7c	661.7a	5.0 b	1.8a	1.7a	122.9c	210.4c	666.7a
SC1	0.9 b	88.8a	3.8a	44.9a	212.9a	640.0c	5.1 ab	1.8a	1.7a	136.4a	218.4a	645.1c
SC2	0.9 b	88.4a	3.8a	45.2a	214.1a	639.1c	5.2 ab	1.7a	1.7a	136.1a	219.6a	644.2c
PEF1_SC1	0.8 b	85.8 b	3.7a	44.0c	210.9 b	646.1 b	5.2 ab	1.8a	1.6a	132.4 b	216.2 b	651.3 b
PEF2_SC1	0.8 b	85.8 b	3.6a	44.5 b	211.1 b	645.5 b	5.2 ab	1.7a	1.7a	132.9 b	216.4 b	650.7 b
PEF1_SC2	0.8 b	86.2 b	3.6a	44.5 b	211.0 b	645.2 b	5.2 ab	1.8a	1.7a	133.3 b	216.3 b	650.4 b
PEF2_SC2	0.8 b	86.1 b	3.7a	44.3bc	210.8 b	645.4 b	5.3a	1.8a	1.7a	133.1 b	216.1 b	650.7 b
Pooled Std	0.0	0.5	0.2	0.1	0.6	0.8	0.1	0.1	0.1	0.6	0.7	0.8

Data are presented as mean value and pooled standard deviation (Pooled Std) over three replicates. ANOVA to compare data; different lowercase letters in the same column indicate statistical differences between samples (Tukey's test, $p < 0.05$). Abbreviations: C14:0, myristic acid; C16:0, palmitic acid; C16:1, pamitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid, SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids, CP, cold pressing; SC1, supercritical CO_2 extraction applying 35 MPa at 45 °C and 45 g CO_2/min; SC2, supercritical CO_2 extraction applying 50 MPa at 35 °C and 45 g CO_2/min; PEF1, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 5 min; PEF2, pulsed electric field pretreatment of 5 kV/cm, at 120 Hz during 1 min.

Redovniković: Methodology, Resources, Funding acquisition. **Karin Kovačević Ganić:** Conceptualization, Methodology, Visualization, Supervision.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114974.

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