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Evaluation of Pulsed Electric Field Polyphenol Extraction from *Vitis vinifera*, *Sideritis scardica* and *Crocus sativus*

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Abstract: This study exploited the application of pulsed electric field (PEF) on the recovery of polyphenols from aerial parts of *Sideritis scardica*, tepals of *Crocus sativus*, and fruits of *Vitis vinifera*. Short pulses of 10 μs in a period of 1 ms were applied to the plant material, while different electric field intensities, 1.2 to 2.0 kV/cm were tested to optimize the procedure. The content in total polyphenols and the polyphenolic profile of the plant extracts were evaluated. Along with PEF samples, control samples were prepared for comparison. PEF treatment enhanced the recovery in total polyphenols for all the three plants examined. A significant increase was noticed in each plant tested and PEF condition applied, though lower electric field intensities up to 1.4 kV/cm proved to be more effective. Under the optimum electric field intensities, 1.4 kV/cm for *V. vinifera* and 1.2 kV/cm for *S. scardica* and *C. sativus*, increases of 49.15%, 35.25%, and 44.36% in total polyphenol content, respectively, were achieved. Additionally, an 85% increase of quercetin 3-rutinoside for *V. vinifera*, a 56% of apigenin 7-O-glucoside for *S. scardica*, and a 64% increase for kaempferol 3-O-glucoside for *C. sativus* were obtained.

Keywords: pulsed electric field; green extraction; polyphenols; medicinal plants; HPLC

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1. Introduction

Since antiquity, herbal medicines have been used to maintain health and treat various diseases. Nowadays herbal formulations, being safer, less expensive, and often more biocompatible than their synthetic analogs, have gained an important position and great demand globally [1,2]. According to the World Health Organization (WHO) [1], 60% of the world's population still rely on herbal medicine and about 80% of the population in developing countries depend almost totally on it for their primary health care needs.

Vitis vinifera, *Crocus sativus*, and *Sideritis scardica* are medicinal plants, well known for their beneficial bioactivities. The pharmacological properties of *V. vinifera* fruit, grape, as well as the active compounds in different parts of the fruit, including skin, seeds, pomace, and stems, have been extensively described in the literature [3–5]. Among grapes' pharmacological effects, skin protection, antioxidant, antibacterial, anticancer, anti-inflammatory, and antidiabetic activities, as well as hepatoprotective, cardioprotective, and neuroprotective effects are included. *C. sativus* is one of the most studied species from the Crocus genus due to the production of the world's most expensive spice, saffron, from its stigmas. Several parts of the plant, specifically tepals, stigmas, leaves, and corns, possess biologically active constituents that have shown health-promoting effects. Therapeutic potential of *C. sativus* L. and its main constituents have been evaluated against different diseases like cancer, Alzheimer's, erectile dysfunction, diabetes, and cardiovascular disease [6,7]. *S. scardica*, known as "mountain tea", has been used in traditional medicine of the Balkan countries against a broad spectrum of disorders, including coughs, asthma, emphysema, and bronchitis. Nowadays, pharmacological investigations attribute to the plant, among others, anti-inflammatory, gastroprotective, antiglioma, cytotoxic, antimicrobial, and antioxidant activities [8,9].

Aforementioned bioactivities are nowadays attributed, to a significant extent, to the bioactive phytoconstituents that belong to the chemical group of polyphenols [10–13]. Despite their beneficial properties, polyphenols are not systematically isolated nowadays from natural sources, due to significant drawbacks of conventional extraction methods. The major drawbacks of conventional extraction methods include the use of expensive and toxic organic solvents in a large quantity, thermal energy and/or substantial mechanical force, the application of long extraction times, the low extraction selectivity, and the decomposition of thermo labile phytochemicals [14–18].

Pulse electric field (PEF) extraction is a green, non-thermal, and selective extraction technique that does not require substantial energy demand and has proven to be effective in short extraction durations [14,19]. The applied electric field causes electroporation to the cell membranes, leading to the formation of pores in weak areas of the cell membrane. As a result, cell membrane permeability is increased, resulting in a more efficient diffusion of the bioactive compounds from the plant tissue. The main factors implicated in PEF's treatment efficiency are the electric field strength, pulse shape, pulse width, number of pulses, pulse specific energy, and frequency [15,20,21]. These factors can be optimized to release only the desired compounds from the cell, adjusting extraction selectivity in addition to extraction efficiency. PEF effectiveness in ambient temperatures, short extraction durations, and minimized quantities of cheap green solvents leads to reduced energy consumption, financial costs, and environmental impact, as well as reduced degradation of heat-sensitive compounds. The above-mentioned properties, in combination with its selectivity, make PEF a promising green alternative extraction technique, which could enhance the recovery of bioactive compounds from plants and food industry waste, leading to the production of new drugs precursors, bio-functional foods, and food supplements [14,22].

PEF has been applied mostly in the food industry for the reduction of microbial growth/pasteurization and for the enhanced extraction of phytochemicals from fruit and vegetable industry wastes [15,17,18,20,23–27]. Bobinaitė et al. (2015) [23] investigated the influence of PEF pretreatment on the recovery of bioactive compounds from by-products (press cake) of blueberry fruits (*Vaccinium myrtillus* L.). PEF treatments carried out at field strengths of 1, 3, and 5 kV/cm and an energy input of 10 kJ/kg led to higher amounts of total phenolics (+63%), total anthocyanins (+78%), and antioxidant activity (+65%). Pataro et al. (2019) [18] applied PEF pretreatment at different field strengths (E = 0.5–5 kV/cm) and total specific energy input (W_T = 0.5–20 kJ/kg) to recover carotenoids from tomato peels. Peels pretreated with PEF at 5 kV/cm showed significantly higher total carotenoid content (47.3%) and antioxidant power (68%).

Fewer are the reports on the use of PEF as a primary extraction method for the recovery of bioactive compounds from food and vegetable industry wastes, while sporadic but promising are the very recent reports that speak for the use of PEF in the direct extraction of bioactive compounds from plants. Bozinou et al. (2019) [14] enhanced the extraction of polyphenols from the leaves of the *Moringa oleifera* tree by using PEF as the primary and only extraction method. PEF of 7 kV/cm proved to be effective, using water as extraction medium for a total extraction time of 40 min. Ntourtoglou et al. (2020) [28] also reached a 20% increase in the extraction rate of the alpha acids of bitter hop using a pulsed electric field of 1.15 to 2.5 kV/cm as the primary extraction method, while Tsapou et al. (2020) [29] achieved phenolic flavor enhancement in beer with a production efficiency in 4-vinylguaiacol (target compound) up to 120% (mg L^{-1}) by applying PEF extraction of 1 kV/cm during a 15 min treatment.

To further evaluate the PEF's potential as a green extraction method of bioactive compounds from plant material, the present study dealt with its application in the recovery of polyphenols from the plants *V. vinifera*, *C. sativus*, and *S. scardica*. Such an extraction process of these plants has not been previously reported (to our knowledge). Water was used as extraction medium, in order to produce edible extracts (no need for solvent evaporation) and minimize the cost and environmental impact. Among aforementioned PEF processing factors (electric field intensity, pulse shape, pulse width, number of pulses,

pulse-specific energy, and frequency) electric field intensity was chosen to be varied as a preliminary effort to optimize the procedure. The content of produced extracts in total polyphenols was evaluated using the Folin–Ciocalteu method and the polyphenolic profile of the produced extracts was additionally determined using high performance liquid chromatography (HPLC).

2. Materials and Methods

2.1. Chemicals

Acetonitrile, HPLC grade, used for chromatography and formic acid (99%) was from Carlo Erba (Val de Reuil, France). Sodium carbonate anhydrous (99%) and gallic acid monohydrate were from Penta (Prague, Czech Republic). The standard reference compounds used for chromatographic characterization were from Sigma Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent was from Panreac (Barcelona, Spain).

2.2. Plant Material

Aerial parts of *S. scardica*, tepals (not separated petals and sepals) of *C. sativus* and fruits of *V. vinifera*, each collected in different seasons of 2019, were air-dried in the dark, then grounded using a mixer and extracted immediately.

2.3. PEF Apparatus

The PEF system used was the static bench-scale apparatus previously reported by Bozinou et al. [14]. A PEF generator that could provide a maximum voltage of 25 kV (Leybold, LD Didactic GmbH, Huerth, Germany); a digital oscilloscope (Rigol DS1052E, Rigol Technologies, Inc, Beaverton, OR, USA) to monitor the signals of the voltage, the current frequency, and pulse waveform; a pulse generator (UPG100, ELV Elektronik AG, Leer, Germany); and a treatment chamber (Val-Electronic, Athens, Greece) were included. The electric field strength, E, was calculated as E = U/d, where U is the applied voltage and d is the distance between the two electrodes (d = 1 cm).

2.4. PEF and Non-PEF Assisted Extraction

An amount of about 1.25 g of ground material of each plant examined was mixed with 25 mL of double distilled water outside the treatment chamber for better homogenization and hydration (liquid to solid ratio 20:1 mL/g) for 10 min. Then, the mixture was added to the treatment chamber where PEF was applied for a total extraction time of 20 min. The processing factors selected to achieve PEF-assisted extraction are described in detail in Table 1. Pulse duration time was set at 10 μs in a period of 1 ms, while electric field intensity varied from 1.2 to 2.0 kV/cm. The period of the phenomena was 1 ms (frequency = 1000 Hz) and the total extraction time of 20 min results in $N = 1.2 \times 10^6$ total number of pulse cycles. The total PEF treatment time ("$t_{Pulse} \times N$") within the 20 min interval of each extraction trial was 12 s. Produced extracts were tested for possible temperature increase immediately after the end of the extraction using an infrared thermometer (GM300, Benetech, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China). Significant temperature increase was not observed ($\Delta t < 1$ °C). Extraction was followed by centrifugation for 10 min at 4500× *g* and the supernatant was collected in a suitable Falcon tube for immediate analysis. Control samples for each plant examined were prepared in the same way as PEF samples, but without the application of PEF.

Table 1. Electric field intensity of extractions.

Extraction Condition	Electric Field Intensity (kV/cm)
PEF 1	2.0
PEF 2	1.7
PEF 3	1.4
PEF 4	1.2
Control	-

2.5. Total Polyphenol Content of Extracts

The content of the extracts in total polyphenols was determined by the Folin–Ciocalteu assay, described by Lakka et al. [30]. A calibration curve using gallic acid as standard (10–80 mg L^{-1}) was used to determine the total polyphenol concentration (C_{TP}), and the yield in total polyphenols (Y_{TP}) was calculated as mg gallic acid equivalents (GAE) per gram of dry weight (dw) according to the following equation:

$$Y_{TP}\ (\text{mg GAE/g of dw}) = \frac{C_{TP} \times V}{w}$$

where C_{TP} is the total polyphenol concentration of the extract (mg L^{-1}), V is the volume of the extraction medium (L), and w is the dry weight (g) of the plant material.

2.6. High-Performance Liquid Chromatography Diode Array (HPLC-DAD)

A methodology previously described by Kaltsa et al. (2020) [31] was used. Chromatographic analyses were carried out using a Shimadzu CBM-20A liquid chromatograph (Shimadzu Europa GmbH, Duisburg, Germany), coupled to a Shimadzu SPD-M20A detector, and interfaced by Shimadzu LC solution software. The column used was a Phenomenex Luna C18(2) (100 Å, 5 μm, 4.6 × 250 mm) (Phenomenex, Inc., Torrance, CA, USA). In addition, 0.5% aqueous formic acid (A) and 0.5% formic acid in acetonitrile/water (6:4) (B) were used as eluents to perform chromatography according to the following elution program: 100% A to 60% A in 40 min; 60% A to 50% A in 10 min; and 50% A to 30% A in 10 min, which was kept constant for another 10 min. The column temperature was maintained at 40 °C, the flow rate was 1 mL min^{-1}, and the injected volume 20 μL.

Detection was performed by scanning from 190 to 800 nm. Identification of individual polyphenols was obtained by comparing their retention times and spectra with those of known standards, while calibration curves for each standard were constructed for quantification. The results are expressed as milligram per gram of dry weight (mg g^{-1}dw).

2.7. Statistical Analysis

The extraction procedures and all determinations were performed in triplicate. Statistical significance (at $p < 0.05$) of the differences between mean values was assessed by ANOVA test using SPSS (SPSS Inc., Chicago, IL, USA) software.

3. Results and Discussion

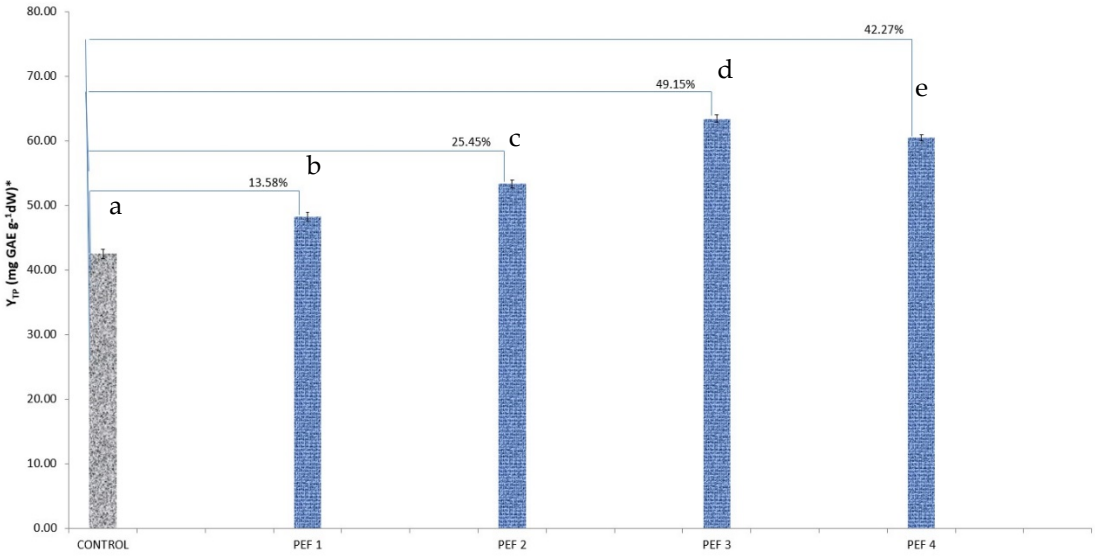
Among PEF's processing factors that influence efficiency and selectivity, electric field intensity and pulse duration are considered to possess a key role [15,16]. The electroporation of the cell membrane depends on the electric field intensity. Higher electric intensities create more significant levels of electroporation and mass transfer of cellular substance. The required electric intensity is differentiated according to the dimension of the target cell. The pulse duration of the applied high voltage pulses is related to the magnitude of the membranes' disturbance that affects the cells' efficiency in disintegrating and expelling intracellular substances. Generally, short pulses (micro or milliseconds) of high electric intensity (E > 1.5 KV/cm) are believed to be the most effective way in extracting phytochemicals using PEF.

In this framework, relatively high electric field intensities from 1.2 to 2.0 kV/cm and short pulses of 10 μs (pulse duration) were selected to achieve enhanced polyphenol extraction from plants using PEF as the primary and only extraction method. In order to estimate the procedure, produced plant extracts were evaluated determining their content in total polyphenols and their polyphenolic profile (by HPLC).

3.1. Total Polyphenol Content of the Extracts

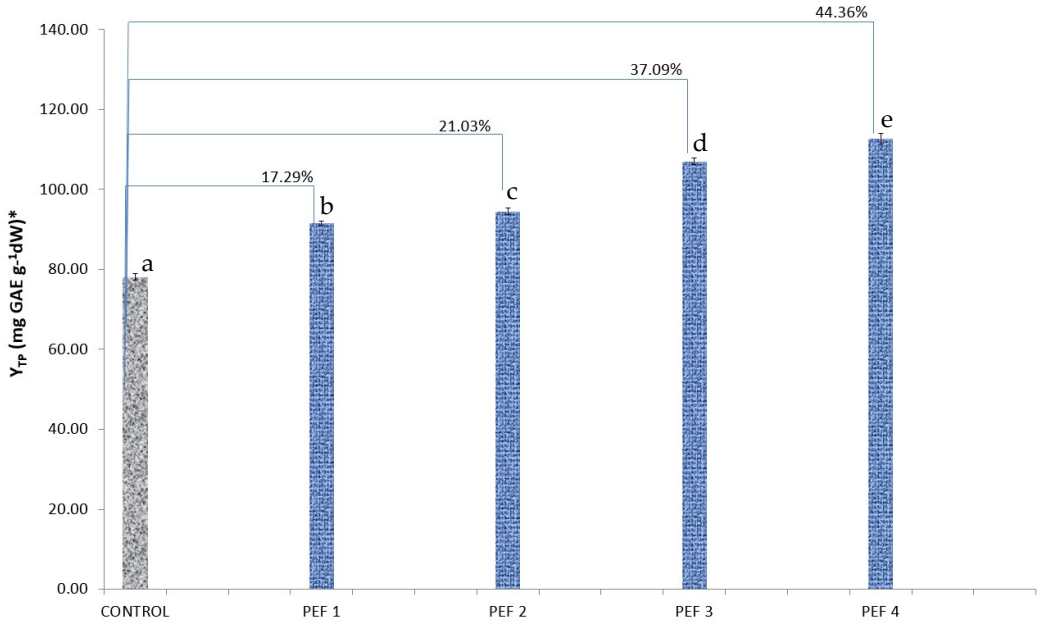
The Folin–Ciocalteu method was applied to determine the content of the produced extracts in total polyphenols. According to the results presented in Figures 1–3, PEF was always more efficient as a primary extraction technique to achieve direct enhanced recovery

of bioactive compounds from the examined plant material. The selection of water as extraction solvent seemed to be suitable while the intervals of 20 min as total extraction time and 10 μs as pulse duration appeared to be also adequate. Applied electric field from 1.2 to 2.0 kV/cm led to different percentages of increase for each plant examined, though, through the results it appeared that lower electric field intensities up to 1.4 kV/cm (conditions PEF 3 and PEF 4) could be more effective.



*mg gallic acid equivalents (GAE) per gram of dry weight (dw).

Figure 1. Yield in total polyphenols (Y_{TP}) for *Vitis vinifera* fruit PEF extracts in four different PEF conditions, in comparison to control extract. The reported percentages indicate the percentage increase in the Y_{TP} of the PEF sample in reference to the control sample. Bars designated with different letters indicate statistically different values ($p < 0.05$).



*mg gallic acid equivalents (GAE) per gram of dry weight (dw).

Figure 2. Yield in total polyphenols (Y_{TP}) for *Crocus sativus* tepal PEF extracts in four different PEF conditions, in comparison to control extract. The reported percentages indicate the percentage increase in the Y_{TP} of the PEF sample in reference to the control sample. Bars designated with different letters indicate statistically different values ($p < 0.05$).

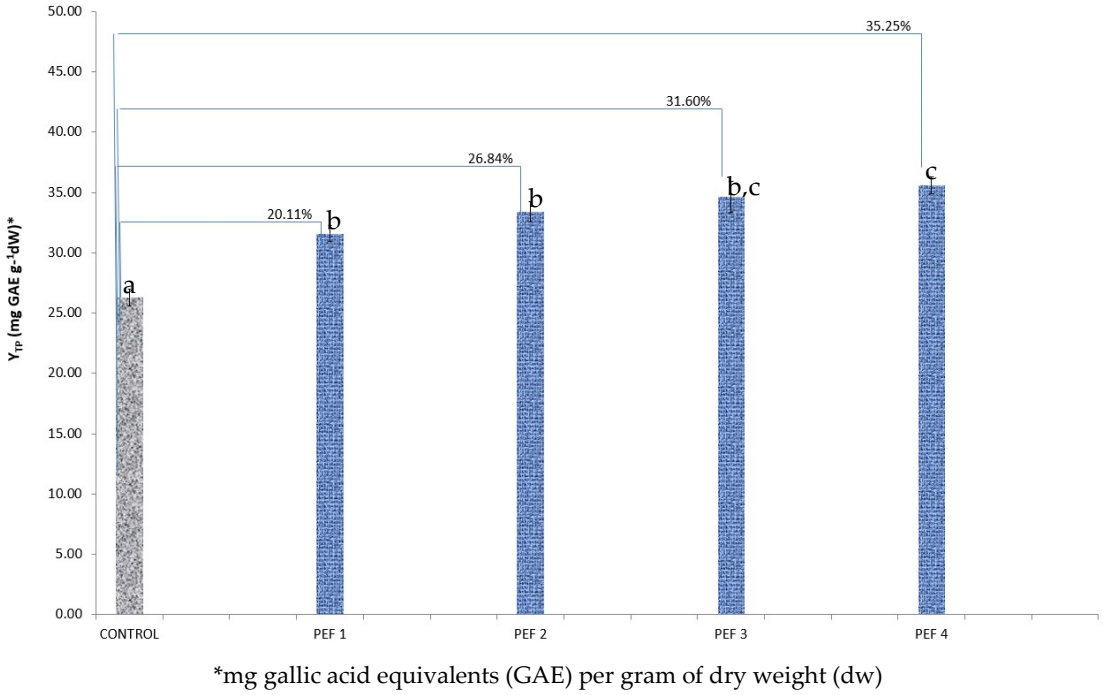


Figure 3. Yield in total polyphenols (Y_{TP}) for *Sideritis scardica* aerial part PEF extracts in four different PEF conditions, in comparison to control extract. The reported percentages indicate the percentage increase in the Y_{TP} of the PEF sample in reference to the control sample. Bars designated with different letters indicate statistically different values ($p < 0.05$).

More specifically, regarding *V. vinifera* fruits, the most effective condition (in comparison to the control) was PEF 3 followed by PEF 4 (Figure 1). The two conditions led to significant ($p < 0.05$) increases of 49.15% and 42.27%, respectively. Conditions PEF 1 and PEF 2 of higher electric intensities brought lower but also significant changes, with condition PEF 2 reaching an increase of 25.45%. Between all electric intensities, the differences in yield of total polyphenols were significant ($p < 0.05$).

Similar were the results in the case of *C. sativus* (Figure 2). There was a significant ($p < 0.05$) difference in all conditions, in comparison to the control. The condition PEF 4 turned out to be the most effective (44.36% increase). Again, between all applied electric intensities, the differences in yield of total polyphenols were significant ($p < 0.05$).

Finally, in the case of *S. scardica*, the maximum increase (significant at $p < 0.05$) was reached with the condition PEF 4 (35.25%) in comparison to the control (Figure 3). However, for this plant there was no significant difference between PEF3 and PEF4 or PEF1 and PEF2.

3.2. Polyphenol Composition

Further to the content in total polyphenols, produced extracts were also characterized by their composition in individual polyphenols. The metabolites contained were determined both qualitatively and quantitatively using HPLC-DAD. Results concerning qualitative determination were in line with those reported in relevant literature [30,32–34]. More specifically in *V. vinifera* fruits, quercetin 3-glucoside and quercetin 3-glucuronide were identified as the predominant metabolites, followed by kaempferol 3-glucoside, gallic acid, and quercetin 3-rutinoside. Chlorogenic acid, verbascoside, 5-caffeoylquinic acid, and apigenin 7-O-glucoside were the polyphenolic compounds identified in *S. scardica* aerial part extracts, while analysis of *C. sativus* tepal extract revealed the existence of kaempferol 3-O-sophoroside-7-glucoside, quercetin 3-O-sophoroside, kaempferol 3-O-sophoroside, and kaempferol 3-O-glucoside. Polyphenols in *C. sativus* extracts were also tentatively identified in our previous work [30].

The content of examined plants (mg g^{-1}dw) in each metabolite determined, as well as the percentage increase in its recovery, are described in the Tables 2–4. Chromatographic results follow phenol content determination results, indicating that electric field intensities up to 1.4 kV/cm were more effective. The optimum condition PEF 3, 1.4 kV/cm for *V. vinifera* fruit extracts increased the recovery of the predominant glycosides, quercetin 3-glucoside and quercetin 3-glucuronide, at a percentage of about 50%. Higher were corresponding increases for the secondary metabolites, quercetin 3-rutinoside, kaempferol 3-glucoside, and gallic acid, reaching 85%, 66%, and 63%, respectively. The results for the rest of the plants were similar. In *S. scardica* aerial part extracts, the optimum condition PEF 4, 1.2 kV/cm, led to a percentage increase of 54% for chlorogenic acid, 48% for verbascoside, 45% for 5-caffeoylquinic acid, and 56% for apigenin 7-O-glucoside. In *C. sativus* tepals, extracts treated under the same condition resulted in an increase of 25% for the main metabolite kaempferol 3-O-sophoroside. Increases for the secondary metabolites of *C. sativus* ranged from 52% to 64%. Significant increases ($p < 0.05$) were also found for the rest of the applied conditions for both plants, with condition PEF 3, 1.4 kV/cm, being more efficient compared to conditions PEF 1 and PEF 2.

Table 2. Polyphenolic profile of *Vitis vinifera* (fruit) extracts.

Extraction Condition	Individual Polyphenol Content, mg g^{-1}dw † (Mean Values of Three Replicates, RSD ‡ = 0.05–1%)									
	Quercetin 3-Rutinoside		Quercetin 3-Glucoside		Kaempferol 3-Glucoside		Quercetin 3-Glucuronide		Gallic Acid	
	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)
PEF 4	0.069 ± 0.0006	54	0.586 ± 0.0028	42	0.133 ± 0.0012	45	0.517 ± 0.0006	41	0.116 ± 0.0009	52
PEF 3	0.083 ± 0.0007	85	0.624 ± 0.0025	51	0.153 ± 0.0009	66	0.543 ± 0.0003	48	0.124 ± 0.0011	63
PEF 2	0.061 ± 0.0006	35	0.529 ± 0.0032	28	0.121 ± 0.0011	32	0.459 ± 0.0032	25	0.104 ± 0.0008	37
PEF 1	0.054 ± 0.0005	21	0.496 ± 0.0044	20	0.113 ± 0.0008	23	0.440 ± 0.0042	20	0.094 ± 0.0008	24
Reference (Control)	0.045 ±0.0004	-	0.413 ± 0.0036	-	0.092 ± 0.0007	-	0.367 ± 0.0009	-	0.076 ± 0.0005	-

† dw = dry weight, ‡ RSD = relative standard deviation, § SD = standard deviation.

Table 3. Polyphenolic profile of *Sideritis scardica* (aerial part) extracts.

Extraction Condition	Individual Polyphenol Content, mg g^{-1}dw † (Mean Values of Three Replicates, RSD ‡ = 0.05–1%)							
	Chlorogenic Acid		Verbascoside		5-Caffeoylquinic Acid		Apigenin 7-O-Glucoside	
	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)
PEF 4	1.096 ± 0.0092	54	1.366 ± 0.0120	48	0.844 ± 0.0081	45	1.017 ± 0.0088	56
PEF 3	1.054 ± 0.0079	48	1.218 ± 0.0089	32	0.786 ± 0.0074	35	0.913 ± 0.0072	40
PEF 2	0.854 ± 0.0061	20	1.126 ± 0.0110	22	0.698 ± 0.0066	20	0.782 ± 0.0076	20
PEF 1	0.862 ± 0.0028	21	1.145 ± 0.0088	24	0.704 ± 0.0053	21	0.802 ± 0.0069	23
Reference (Control)	0.712 ± 0.0047	-	0.923 ± 0.0077	-	0.582 ± 0.0052	-	0.652 ± 0.0065	-

† dw = dry weight, ‡ RSD = relative standard deviation, § SD = standard deviation.

Table 4. Polyphenolic profile of *Crocus Sativus* (tepal) extracts.

Extraction Condition	Individual Polyphenol Content, mg g^{-1}dw † (Mean Values of Three Replicates, RSD ‡ = 0.05–1%)							
	Kaempferol 3-O-Sophoroside-7-Glucoside		Quercetin 3-O-Sophoroside		Kaempferol 3-O-Sophoroside		Kaempferol 3-O-Glucoside	
	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)	Average ± SD §	Increase (%)
PEF 4	3.865 ± 0.0321	52	3.313 ± 0.0238	56	25.565 ± 0.1563	25	1.496 ± 0.0131	64
PEF 3	3.662 ± 0.0283	44	3.207 ± 0.0259	51	24.542 ± 0.2354	20	1.350 ± 0.0091	48
PEF 2	3.357 ± 0.0315	32	2.676 ± 0.0123	26	23.520 ± 0.0171	15	1.140 ± 0.0112	25
PEF 1	3.077 ± 0.0128	21	2.591 ± 0.0204	22	22.906 ± 0.0228	12	1.094 ± 0.0078	20
Reference (Control)	2.543 ± 0.0229	-	2.124 ± 0.0163	-	20.452 ± 0.0189	-	0.912 ± 0.0089	-

† dw = dry weight, ‡ RSD = relative standard deviation, § SD = standard deviation.

Values regarding the content in total polyphenols and individual metabolites, in the optimum for each plant PEF condition, are comparable with those achieved with different extraction methods, using mainly aqueous ethanol or methanol, in a percentage of 50% to 80%. It appears that possible losses in yield, related to the use of water as an extraction solvent, can be earned from the use of PEF. More precisely, in the case of *V. vinifera* fruits, the content in total polyphenols and the basic metabolites, quercetin 3-glucoside and quercetin 3-glucuronide, reached 63.45, 0.624, and 0.543 mg g^{-1}dw (in optimum condition PEF 3), respectively. Jara-Palacios et al. (2014b) [35] evaluated the total phenolic content, as well as the detailed phenolic composition of white grape pomaces from nine different varieties. They reached up to 31.13, 0.557, and 0.522 mg g^{-1}dw in total polyphenols, quercetin 3-glucoside, and quercetin 3-glucuronide, correspondingly, in 75% methanol extracts. For *S. scardica* aerial parts we achieved a total polyphenol content of 35.58 mg g^{-1}dw (in the optimum condition PEF 4). Ibraliu et al. (2014) [36] reported the results of the direct phytochemical comparison of samples of *S. scardica* (among others) from different locations with regards to the polyphenolic total content. They reached a content in total polyphenols up to 21.9 mg g^{-1}dw after extraction with 70% aqueous ethanol. Finally, treatment with condition PEF 4 of *C. sativus* tepals led to contents in kaempferol 3-O-sophoroside-7-glucoside, quercetin 3-O-sophoroside, and kaempferol 3-O-sophoroside of 3.865, 3.313, and 25.565 mg g^{-1}dw, correspondingly. The above results are in line with Serrano-Diaz et al. (2014) [37], who gave values of 5.601, 4.011, and 30.342 mg g^{-1}dw for kaempferol 3-O-sophoroside-7-glucoside, quercetin 3-O-sophoroside, and kaempferol 3-O-sophoroside, respectively, in water extracts of saffron floral bio-residues.

According to the results of the current work, PEF is an alternative and effective technique to be used as a primary extraction method for the direct recovery of bioactive compounds from *V. vinifera*, *S. scardica*, and *C. sativus*, offsetting several of the disadvantages of conventional extraction methods. Future studies could focus on the optimization of PEF processing factors and the application of them in real food and cosmetic preparations. Such an approach would certainly have a significant industrial prospect for large-scale application of PEF.

4. Conclusions

The study presented herein demonstrated the effectiveness of PEF in extracting polyphenols from the medicinal plants *V. vinifera*, *S. scardica*, and *C. sativus*. In order to develop a "green" extraction process and produce edible extracts, water was used as ex-

traction solvent, while the total extraction time was minimized at 20 min. Different electric field intensities from 1.2 to 2.0 kV/cm were tested as a partial optimization of the process. PEF treatment significantly enhanced the recovery in total polyphenols for all the three plants examined. The percentage increase in recovery was important in each case of plant and PEF condition examined, though lower electric field intensities up to 1.4 kV/cm proved to be more effective. Under the optimum electric field intensities, 1.4 kV/cm for *V. vinifera* and 1.2 kV/cm for *S. scardica* and *C. sativus*, increases of 49.15%, 35.25%, and 44.36% in total polyphenol content, respectively, were achieved. Important were also the increases regarding the individual polyphenols of each plant. An 85% increase of quercetin 3-rutinoside for *V. vinifera*, a 56% increase of apigenin 7-O-glucoside for *S. scardica*, and a 64% increase for kaempferol 3-O-glucoside for *C. sativus* were obtained. The choice of water as extraction solvent also seemed to be suitable, which is particularly important as it makes the method less costly and more applicable, compared to methods employing conventional organic solvents. Produced extracts are edible and can be directly applied to food and/or cosmetics as there is no need for additional energy-demanding and time-consuming downstream steps of extract purification.

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