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ORIGINAL PAPER

High Voltage Electrical Discharges, Pulsed Electric Field, and Ultrasound Assisted Extraction of Protein and Phenolic Compounds from Olive Kernel

Elena Roselló-Soto · Francisco J. Barba · Oleksii Parniakov · Charis M. Galanakis · Nikolai Lebovka · Nabil Grimi · Eugène Vorobiev

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Abstract The study was aimed at improvement of recovery of intracellular valuable compounds from olive kernels (*Olea europaea*). High voltage electrical discharges (HVED), pulsed electric field (PEF), and ultrasound (US) were applied as pretreatments before extraction. The influence of HVED energy input (0–109 kJ/kg), pH (2.5–12), and ethanol (0–50 %) on the efficiency of the extraction was studied. The extracts obtained immediately after pretreatments were analyzed for total phenolic compounds, antioxidant activity, proteins, and pigments. HVED treatment was demonstrated to be more effective than ultrasound and pulsed electric field in terms of energy input and effective treatment time to extract phenolic compounds and proteins. Moreover, the application of HVED increased significantly the aqueous and hydro-ethanolic extractions of total phenolic content (TPC), and proteins of the recovered extracts when energy input was augmented. pH and ethanol percentage had also a significant influence in TPC, protein, and antioxidant recovery. The interesting observation is that pH 2.5 resulted in the optimum conditions to recover TPC and antioxidant capacity. However, the higher protein content was found when pH 12 was used. Multiple response optimization showed that TPC, content of proteins, and antioxidant capacity (Trolox equivalent antioxidant capacity (TEAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) values) of the sample were further maximized after HVED pretreatment at energy input 66 kJ/kg at pH 2.5 followed by extraction in 49 % ethanol. TPC, content of proteins, TEAC, and DPPH values under such conditions of extraction were 626.6 mg GAE/L, 0.225 mg/mL, 9.80 mM TE, and 7.61 mM TE, respectively.

Keywords Olive kernel · Extraction · High voltage electrical discharges · Pulsed electric fields · Ultrasound

E. Roselló-Soto · F. J. Barba
Nutrition and Food Science Area, Universitat de València, Avda. Vicent Andrés Estellés, s/n. 46100, Burjassot, Spain

O. Parniakov · N. Grimi (✉) · E. Vorobiev
Laboratoire Transformations Intégrées de la Matière Renouvelable (TIMR EA 4297), Centre de Recherche de Royallieu, Université de Technologie de Compiègne, B.P. 20529-60205 Compiègne Cedex, France
e-mail: nabil.grimi@utc.fr

O. Parniakov · N. Lebovka
Institute of Biocolloidal Chemistry named after F. D. Ovcharenko, NAS of Ukraine, 42, blvr. Vernadskogo, Kyiv 03142, Ukraine

C. M. Galanakis
Department of Research and Innovation, Galanakis Laboratories, Skalidi 34, GR-73131, Chania, Greece

Introduction

The valuable compounds from agricultural by-products are nowadays recovered using the so-called 5-Stages Universal Recovery Process that include the following steps: (i) macroscopic pretreatment, (ii) separation of macro- and micro-molecules, (iii) extraction, (iv) purification, and (v) product formation (Galanakis 2012). Conventional processing techniques (membrane separation, alcohol precipitation, solvent extraction, etc.) that meet the demands of each recapture step were developed. On the other hand, non-thermal emerging technologies (i.e., high voltage electrical discharges, ultrasound, or pulsed electric field) have recently been proposed to shorten the processing time, increase recovery yield, control the Maillard reactions, improve the product quality, and enhance functionality of extracts (Galanakis 2013).

For instance, high voltage electric discharge (HVED) disrupts cell tissues in liquid samples and subsequently enhances extraction of valuable compounds from plant food materials and by-products. It happens because of the direct energy

release into the medium through the plasma channel formed by a HVED between two submerged electrodes (Boussetta and Vorobiev 2014). If the by-product is in solid form, a dielectric liquid such as water is added into the chamber (Vorobiev and Lebovka 2010). HVED was applied for enhancement of extraction of solutes from soybeans, potato, or fennelas as well as polyphenols from white grape pomace (Boussetta et al. 2009; Liu et al. 2011). Ultrasound (US) is also known to accelerate heat and mass transfer during extraction processes, as far as its cavitation effects disrupt the plant cell walls and thus release bioactive compounds. This technology has been successfully applied in different occasions, e.g., for recovery of polyphenols from citrus peel and coconut shells or for recapture of hemicellulose from wheat straw (Chemat et al. 2011; Sun and Tomkinson 2002). Pulsed electric field (PEF) is another non-thermal food processing technology of high potentiality. In the case of PEF treatment, accelerated mass transfer, tissue breakdown, and enhancement of tissue permeability are induced by application of the critical electrical potential to cell membranes. PEF has been applied to increase the extraction yield of polyphenols from grape seeds, pectin from apple pomace, and betalains from red beetroot (Liu et al. 2011; Vorobiev and Lebovka 2010).

Olive fruit (*Olea europaea*) is known to contain an appreciable amount of polyphenols with advanced antioxidant properties, dietary fibers with promising gelling properties, and other valuable organics, such as nitrogenous compounds (mainly proteins) and sugars (Galanakis et al. 2010a, b; Niaounakis and Halvadakis 2004). High amounts of these compounds are lost in the by-products of olive oil industry. For instance, production of olive oil from olive fruit using the popular three-phase continuous process generates two by-products: olive mill wastewater and olive kernel (solid waste product). Olive mill wastewater is known to concentrate high amounts of olive fruit polyphenols, whereas olive kernel contains both antioxidants (vitamin E, polyphenols, chlorophylls, carotenoids) and proteins, which are found in different parts of olive (Ghanbari et al. 2012). These biomolecules can be used for development of new food products, food additives, and nutraceuticals.

To the best of our knowledge, the studies reporting the extraction of polyphenols and proteins from the solid waste and by-products of olive oil production are rather limited, and indeed, the application of emerging non-thermal technologies for this purpose is scarce. Thereby, the objectives of the present study were as follows: (i) to evaluate the potential of HVED, PEF, and US application for recovery of the above compounds from olive kernels; (ii) to select the most appropriate technology; and (iii) to optimize the selected methodology in order to improve the extraction of polyphenols and proteins, and antioxidant capacity of the recovered extracts from olive kernels of different varieties.

Material and Methods

Samples

Samples were collected from two three-phase production units, placed in Valencia (Spain) and Chania (Greece). Olive varieties in the first and second cases were Cornicabra (dry matter 34.33 %) and Koroneiki (dry matter 40.51 %), respectively. The samples, of each variety, were placed in a cold storage room at 4±2 °C. The storage duration never exceed 1 week.

Treatments

HVED and PEF electrical treatments were done using the same high voltage pulsed power 40 kV-10 kA generator (Tomsk Polytechnic University, Tomsk, Russia), 1-l cylindrical batch treatment chamber, and different types of electrodes (Fig. 1).

The HVED treatment chamber was equipped with needle-plate geometry electrodes. The diameters of stainless steel needle and the grounded disk electrodes were 10 and 35 mm, respectively. The distance between the electrodes was 5 mm. Energy was stored in a set of low-inductance capacitors, which were charged by the high-voltage power supply. The electrical discharges were generated by electrical breakdown in water at the peak pulse voltage (U) of 40 kV. Damped oscillations were thus obtained with the total duration t_i of ≈10 μs. The total treatment duration t_t was calculated from Eq (1):

$$t_{\mathrm{HVED}} = n \times t_i \tag{1}$$

The specific energy input W (kJ/kg) was obtained from Eq. (2):

$$W = \frac{\sum_{i=1}^{n} W_{\mathrm{HVED}}}{m} \tag{2}$$

where W_{HVED} is the pulse energy (kJ/pulse), n is the number of pulses, and m is the product mass (kg). W_{HVED} is determined from Eq. (3).

$$W_{\mathrm{HVED}} = \int_{o}^{t} UIdt \tag{3}$$

where U is the voltage (V) and I is the current strength (A).

Two parallel stainless disks were used as electrodes in PEF experiments. The electrode area was 95 cm^2. The distance between the electrodes was 3 cm, which corresponds to the

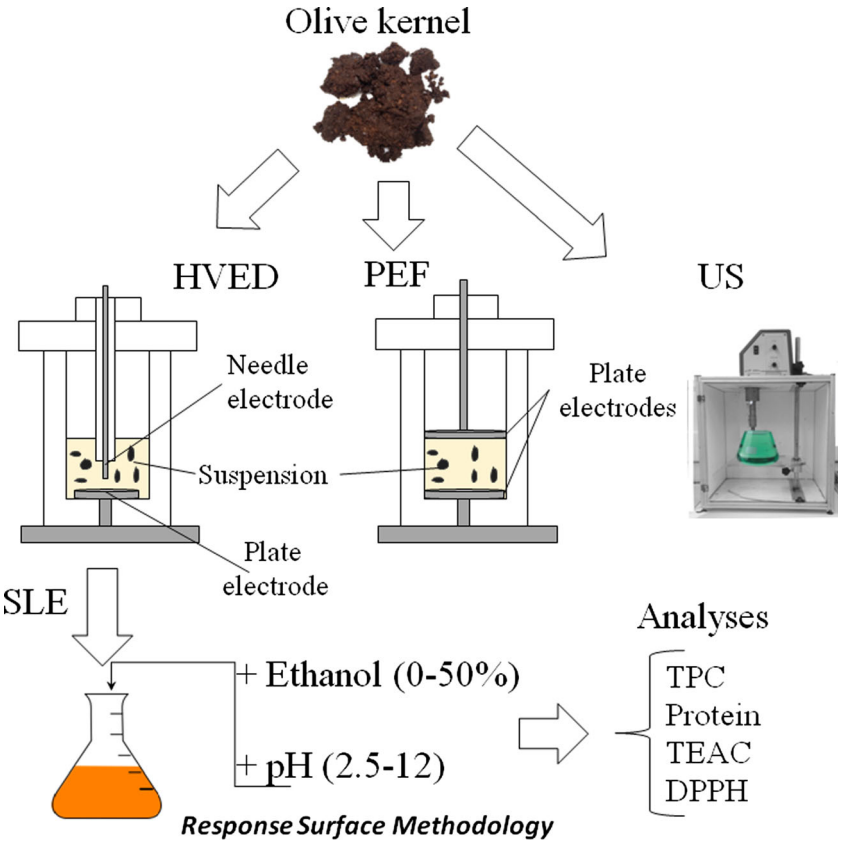

Fig. 1 Experimental setups for high voltage electrical discharges (HVED), pulsed electric fields (PEF), and ultrasounds (US) treatments followed by solid–liquid extraction in hydro-ethanolic solutions and analysis procedures

electric field strength E=13.3 kV/cm. The circuit configuration generated exponential decay pulses. The total treatment duration t_t was varied by increasing the number of pulses n from 0 to 300. The PEF pulse length was about t_i=10 μs and the pulse repetition rate was f=0.5 Hz. The total treatment duration and the specific energy input were determined using Eqs. (1)–(3), in which t_{HVED} and W_{HVED} were replaced by t_{PEF} and W_{PEF} (kJ/pulse). The energy input of PEF and HVED treatments varied from 0 kJ/kg (for t_{PEF}=t_{HVED}=0 ms) to 141 kJ/kg (for t_{PEF}=t_{HVED}=3 ms).

The voltage (Ross VD45-8.3-A-K-A, Ross Engineering Corp., Campbell, California, USA) and current (Pearson 3972, Pearson Electronics Inc., Campbell, California, USA) measurement units were connected with a 108-Hz sampling system via an oscilloscope (Tektronix TDS1002, Beaverton, Oregon, USA). The software HPVEE 4.01 (Hewlett-Packard, Palo Alto, USA) was used for data acquisition.

For ultrasound (US) assisted extraction, an ultrasonic processor UP 400S (Hielscher GmbH, Germany), which operates at 400 W and 24 kHz, was used. The amplitude was adjustable from 20 to 100 %. For the experiments of the present study, the amplitude was fixed at 100 %.

The titanium sonotrode H14 having the diameter of 14 mm and the length of 100 mm was used for transmission of ultrasound inside the sample. The sample was immerged into a cooling bath to avoid the heating induced by US irradiation. The energy input of US treatment was calculated as follows:

$$W_{US} = \frac{P_g t_{US}}{m} \quad (4)$$

where t_{US} is the total treatment duration (s), m is the product mass (kg), and P_g is the generator power (400 J/s). The solid/liquid ratio was 1/5 for all the treatments.

Solid–Liquid Extraction

A response surface methodology (RSM) analysis was designed for the evaluation of the effects of solid–liquid extraction (Fig. 1). After the HVED treatment, a supplementary amount (200 g) of distilled water or a mixture of ethanol and water was added. The suitable liquid-to-solid ratio (w/w), allowing to maintain a homogeneous solid–liquid extraction, was 10. The extraction was studied in a cylindrical cell of 10 cm in diameter. A gentle 2-min agitation at 150 rpm was provided by a round incubator shaker (Infors HT Aerotron, Bottmingen, Switzerland). The total extraction time (pretreatment+agitation) was fixed at 20 min, which

corresponded to the effective PEF and HVED treatment time of 4 ms. The extraction temperature was controlled (20±2 °C). The same protocol was used for experiments without pretreatments. After the extraction, the samples were filtered as well as centrifuged, to obtain the extracts, and then stored at −20 °C, until needed for analysis.

The concentration of total polyphenols, proteins, and antioxidant capacity (Trolox equivalent antioxidant capacity (TEAC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) values) of the recovered extracts was measured. Experimental conditions were selected on the basis of previously reported data (Boussetta et al. 2013).

Analysis

The olive kernel extracts obtained after HVED, PEF, or US treatment and solid–liquid extraction were analyzed using different procedures (Fig. 1).

Physicochemical Properties

The pH of the solution was measured using a pH meter (CONSOR C931, Bioblock Scientific, France) at 20 °C. The concentration of total soluble matter (or total solutes) was measured by a digital refractometer (Atago, USA) at room temperature. The results are expressed in °Brix (g of dry matter DM/100 g solution).

Total Protein Content

The concentration of proteins was determined using the Bradford protein assay (Bradford 1976). A volume of 0.2 mL of extract and 1.8 mL of threefold diluted Bradford Reagent (Sigma-Aldrich, St-Quentin Fallavier, France) were mixed. The sample was kept for 5 min at room temperature. The absorbance was measured at 595 nm by the UV/Vis spectrophotometer (LibraS32, Biochrom, Lagny-sur-Marne, France). Bovine serum albumin (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve.

Total Phenolic Compounds

Total polyphenols content (TPC) was determined colorimetrically by means of the Folin–Ciocalteu method based on oxidation/reduction reactions of phenols (Singleton et al. 1999) with some modifications (Barba et al. 2013). A volume of 0.2 mL of diluted extract and 1 mL of tenfold diluted Folin–Ciocalteu reagent (Sigma–Aldrich, St-Quentin Fallavier, France) were mixed. Then, 0.8 mL of Na_2CO_3 (75 g/L) (VWR, Fontenay-sous-Bois, France) was added. The sample was incubated for 2 h at room temperature. The absorbance was measured at 750 nm by the UV/Vis spectrophotometer (Libra S32, Biochrom, Lagny-sur-Marne, France). Gallic acid (Sigma–Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. Results were expressed as gram GAE/100 g dry matter (DM). The analyses were performed in triplicate and standard deviation was calculated.

Determination of Chlorophyll and Carotenoids

The content of chlorophyll *a*, chlorophyll *b*, and carotenoids was estimated spectrophotometrically according to the method of Lichtenthaler (1987). Aliquots of the extracts were diluted 15–300 times by 90 % (*v*/*v*) methanol/water and absorbances were measured at 470, 647, and 663 nm. The content of carotenoids, chlorophyll *a*, and chlorophyll *b* was calculated using the Lichtenthaler equations:

$$\begin{aligned}
\text{Chlorophyll } a &= (12.25 \times \text{Absorbance at 663 nm}) - (2.79 \times \text{Absorbance at 647 nm}) \\
\text{Chlorophyll } b &= (21.50 \times \text{Absorbance at 647nm}) - (5.10 \times \text{Absorbance at 663 nm}) \\
\text{Total carotenoids} &= (1,000 \times \text{Absorbance at 470}) - (1.82 \times \text{chlorophyll } a) - (85.02 \times \text{chlorophyll } b)
\end{aligned}$$

DPPH Assay

DPPH-free radical method is an antioxidant assay based on electron transfer that produces a violet solution in ethanol. The method used was as described by Keceli and Gordon (2001). The reaction was begun by adding a suitable dilution of the methanol beverage extract to the DPPH-colored radical. Absorbance was measured at 515 nm every 15 min for 1 h until equilibrium was reached (Samaniego Sanchez et al. 2007).

TEAC Assay

TEAC measures the antioxidant capacity, as compared to the standard, Trolox. The method used is based on the capacity of the sample to inhibit the ABTS radical (ABTS•+) (Sigma-Aldrich, Steinheim, Germany) compared with the reference standard of antioxidant (Trolox®) (Sigma-Aldrich, Steinheim, Germany) (Re et al. 1999). The radical was generated using 440 μL of potassium persulfate (140 mM). The solution was diluted by ethanol (Baker, Deventer, The Netherlands) until

absorbance of 0.70 was reached at 734 nm. Once the radical was formed, 2 mL of ABTS•+ was mixed with 100 μL of appropriately diluted sample and the absorbance was measured at 734 nm for 20 min in accordance with (Carbonell-Capella et al. 2013). The results, obtained from duplicate analyses, were expressed as millimolar Trolox equivalents.

Experimental Design

A multiple regression analysis was performed to study the influence of different factors on the given parameter. Face-centered central composite design was used with three levels (maximum, minimum, and central) of each independent variable, power input (0–109 kJ/kg), concentration of ethanol (0–50 %), and pH (2.5–12), leading to 16 combinations of these variables. Independent variable levels were selected accounting for the sample and the potential TPC degradation after HVED at higher energy inputs. The combinations included HVED–Ethanol–pH conditions with an intermediate level (central point) of the three variables replicated two times, which was used to check the reproducibility and stability of the results. The experimental design was performed twice giving two blocks of experiments. Accordingly, samples were treated by duplicate and analyzed by triplicate in all the cases. Experiments were randomized to minimize the systematic bias in the observed responses due to extraneous factors and for higher precision. Finally, it was studied whether there were correlations between a pair of variables. All statistical analyses were performed using the software Statgraphics® Centurion XV (Statpoint Technologies, Inc., USA).

Statistical Analysis

Significant differences between the results were calculated by analysis of variance (ANOVA). Differences at $p<0.05$ were considered to be significant. The Least Significant Difference (LSD) test was applied to indicate the samples between which there were differences.

Results and Discussion

Comparison of HVED, PEF, and US-Assisted Extraction Efficiency Using Water as Solvent

The first objective of this study was to evaluate and to compare the potentials of HVED, PEF, and US on the recovery of nutritionally valuable compounds. For this purpose, three

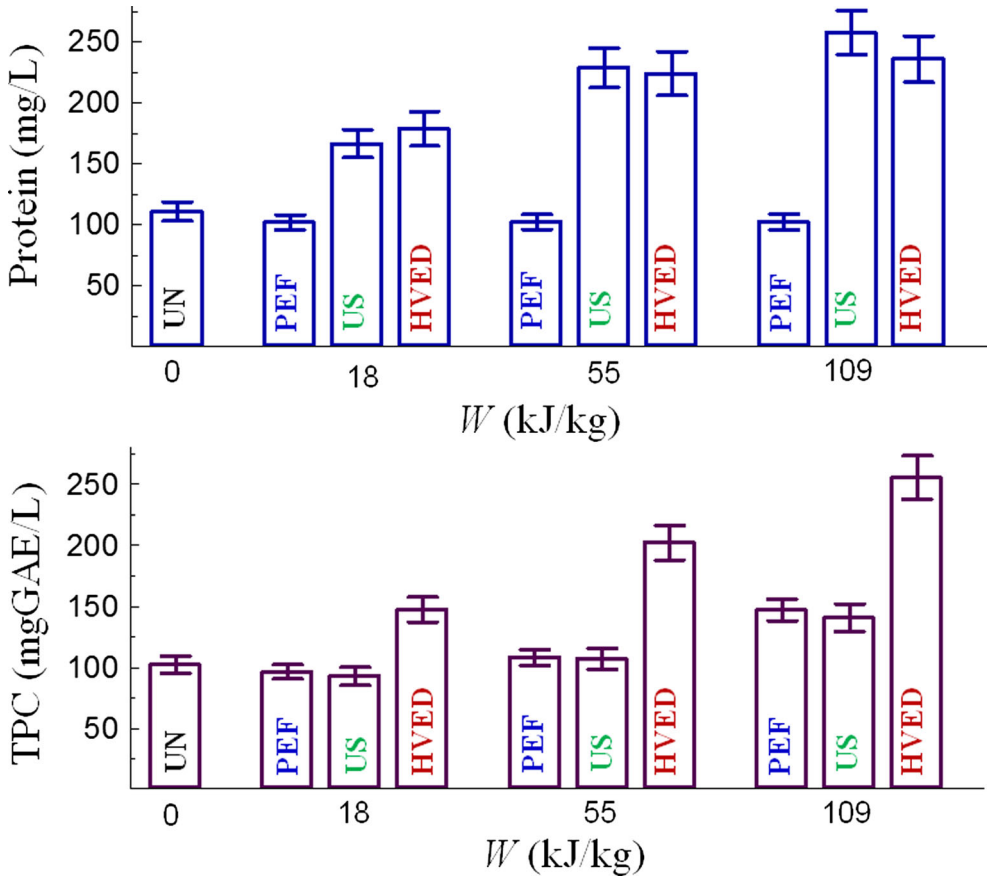

Fig. 2 Content of proteins (**a**) and polyphenols (**b**) versus the input energy for treated (high voltage electrical discharges, HVED; pulsed electric fields, PEF; and ultrasounds, US) and untreated (UN) samples. The Cornicabra olive kernel variety was chosen as an example. The diffusion was done in water (pH 7) and the temperature was maintained at 25 °C

different treatments (HVED, PEF, and US) at equivalent energy inputs (18, 36, 55, 73, 91, 109 kJ/kg) were used for obtaining water extracts. Results were compared with the control sample (Fig. 2).

The levels of total carotenoids, chlorophyll *a*, and chlorophyll *b* were, respectively, 324.05±19.26, 346.22±18.78, and 791.03±62.20 mg/100 g for untreated (control) sample. Non-statistically significant differences were obtained for these compounds compared to the control sample when various (HVED, PEF, and US) treatments were applied.

Two-way ANOVA analysis (treatment and energy input) showed that both treatment and energy input had significant effect ($p<0.05$) on the extraction of total phenolic compounds (TPC), proteins, and antioxidant capacity of the extracts. HVED technology was demonstrated to be more effective than ultrasound and pulsed electric field technology in terms of energy input and effective treatment time required for extraction of TPC (Fig. 2). A possible explanation is that application of electrical discharges to different biological materials results in fragmentation of treated particles of olive kernels due to propagation of the shock waves and explosion of cavitation bubbles, thus facilitating the extraction of phenolic compounds as suggested by Boussetta and Vorobiev (2014). Moreover, phenolic compounds can form complexes with proteins, starch, cellulose, minerals, and other substances. So, application of HVED can affect phenolic binding thus increasing extractability of these compounds. However, it is necessary to optimize HVED processing conditions. As can be seen in Fig. 2b, HVED at high energy inputs (more than 100 kJ/kg) can promote TPC degradation. These results are in close agreement with those found by Rajha et al. (2014) when they evaluated the effects of HVED, PEF, and US on polyphenol and protein recovery from vine shoots. They found that HVED (254 kJ/kg), used as pretreatment, increased significantly ($p<0.05$) the extraction of polyphenols and proteins. They also have found that polyphenol purity was the highest (89 %) after HVED treatment in comparison to polyphenol after PEF (88 %) and US (84 %) treatments.

Response Surface Methodology Design for Optimization of HVED Energy Input, pH, and Ethanol Concentration

HVED was selected at the most appropriate treatment for the recovery of TPC and other antioxidant compounds that can be found in the analyzed extracts (TEAC and DPPH values). The multiple RSM was used for the evaluation of the effect of HVED at different values of energy input (0–109 kJ/kg), pH (2.5–12), and ethanol concentration (0–50 %, *v*/*v*). Some factors, such as pH and ethanol concentration, are known to influence the extractability of proteins, phenolic compounds, and some other antioxidants. Table 1 compares the impact of HVED treatment (energy input), pH and ethanol concentration on TPC, protein content, and antioxidant capacity (TEAC and DPPH values) of the extracts obtained from Spanish olive kernel.

As it is shown in Table 1 and Fig. 3a–c, HVED treatment had a significant effect on TPC, protein, and antioxidant recovery from olive kernel samples. HVED used as pretreatment

Table 1 Impact of high voltage electrical discharges (HVED) treatment (energy input), pH, and ethanol concentration on total phenolic compounds (TPC), content of proteins and antioxidant capacity (Trolox equivalent antioxidant capacity, TEAC, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate, DPPH) of the extracts from olive kernels (Cornicabra)

Energy input (kJ/kg)	pH	Ethanol (%)	TPC (mg GAE/L)	Protein (mg/mL)	ABTS (mM TE)	DPPH (mM TE)
109	12	0	230.23±4.02a	0.16±0.02a	4.00±0.07a	3.01±0.08a
55	12	25	579.66±3.21b	0.48±0.02b	4.71±0.23b	3.69±0.03b
0	2.5	50	520.00±5.62c	0.16±0.02a	7.56±0.04c	5.93±0.28c
109	7.25	25	552.95±4.02d	0.23±0.03c	5.14±0.05d	4.11±0.19d
55	2.5	25	555.80±3.21d	0.12±0.02ad	7.98±0.07e	6.15±0.11ce
0	7.25	25	389.32±4.02e	0.14±0.03ade	3.94±0.20af	3.09±0.06af
0	2.5	0	263.75±3.31f	0.09±0.02df	5.17±0.01dg	4.14±0.07dg
109	12	50	503.52±4.82g	0.19±0.01agh	8.59±0.05h	6.79±0.05h
0	12	0	316.59±10.45h	0.21±0.03cgh	5.47±0.08i	4.30±0.06i
109	2.5	0	209.20±4.82i	0.10±0.03df	4.67±0.11bj	3.60±0.10bj
0	12	50	368.30±4.95j	0.18±0.02acgh	2.46±0.15k	1.90±0.26k
55	7.25	25	477.39±4.82k	0.21±0.03acgh	8.27±0.23ehl	6.28±0.16cel
55	7.25	25	470.00±4.02k	0.30±0.03i	8.29±0.20ehl	6.41±0.03l
55	7.25	0	391.02±3.27e	0.23±0.02cgh	7.64±0.09cm	5.98±0.06cem
109	2.5	50	607.50±4.03l	0.19±0.02acgh	7.83±0.10mn	6.07±0.13cem
55	7.25	50	492.73±16.87km	0.19±0.02acgh	7.86±0.18emn	6.34±0.26celm

Different letters (a–m) in the same column indicate significant statistical differences. The samples were analyzed immediately after HVED treatment

increased significantly the aqueous and hydro-ethanolic extractions of total polyphenols of recovered extracts when energy input was increased, independently of the pH used. These results are in close agreement to those found by Boussetta et al. (2013) who noted a significant increase in TPC after aqueous extraction both in crushed and non-crushed flaxseed cake when HVED was used as pretreatment.

Moreover, pH had also a significant effect on TPC, protein, and antioxidant recovery (Table 1, Fig. 3a–c). Impact of pH on the extraction of TPC can be explained by changes in their solubility depending on polyphenol family and alternation of the interactions with plant material (Meireles 2009). As can be seen in Fig. 3a, the optimum phenolic yield was found when pH 2.5 (555.80 mg GAE/L) and pH 12 (579.66 mg GAE/L) were used at 25 % of ethanol. However, RSM shows that the maximal yield can be obtained when pH 2.5 (607.50±4.03 mg GAE/L) at 50 % ethanol. This increase can be explained by two factors: (1) enhanced solubility of specific phenolic compounds, which are highly soluble in acidic media, and (2) hydrolysis products that are formed when acidic conditions are used. These compounds can react with Folin–Ciocalteu reagent, thus increasing TPC values (Meireles 2009). Moreover, these results are in close agreement with those reported by Rawel et al. (2005), who demonstrated that the non-covalent binding of some phenolic compounds (chlorogenic, ferulic, and gallic acids, quercetin, rutin, and isoquercetin) to different proteins may be influenced by different factors, e.g., temperature, ionic strength of solution, as well as decreasing pH can cause a diminished binding, thus facilitating the extractability of TPC and proteins.

In addition, it should be noted the important TPC yield found when pH 12 was used. These results were in close agreement to those found by other authors who found that the alkaline hydrolysis releases polyphenols linked by ester bonds in vine shoots, thus improving their extractability (Rajha et al. 2014).

Regarding protein recovery, a noticeable increase was observed when pH and HVED energy input were augmented. A

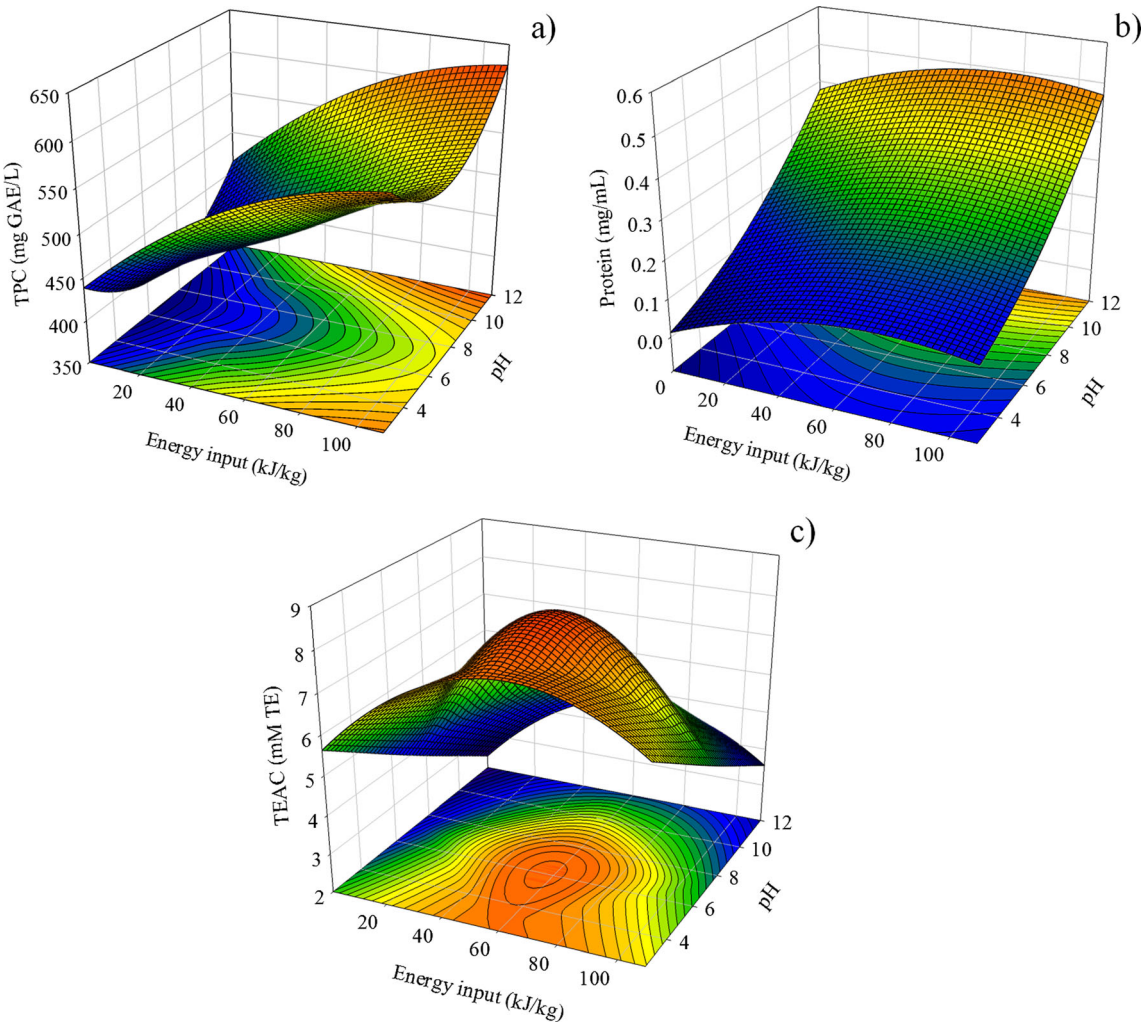

Fig. 3 Response surface plots for **a** total phenolic compounds (TPC); **b** protein; and **c** antioxidant recovery from olive kernel samples (Cornicabra) with 25 % ethanol as affected by high voltage electrical discharges (HVED) at different energy inputs (0–109 kJ/kg) and pH (2.5–12)

possible explanation can be that a large proportion of the proteins (80 to 90 %) contained in olive kernel is linked to the lignocellulose fraction (Nefzaoui et al. 1983). So, the binding can be destroyed by HVED due to its ability to promote turbulation of suspension and fragmentation of pills. Moreover, the interesting observation is that increasing pH resulted in an important increase of the concentration of proteins. This fact can be explained by the ability of sodium hydroxide to increase the accessibility of solid residue by removing the lignin physical barrier (Max et al. 2010), thus facilitating protein recovery.

On the other hand, different trends were observed for antioxidant capacity as a function of the energy input and pH. As it is shown in Fig. 3c, a significant increase of antioxidant capacity was found when energy input was augmented up to 66 kJ/kg. However, it was followed by a significant decrease after this energy. In addition, the interesting observation is that increased pH resulted in a decrease of the antioxidant capacity of the extract, while pH 2.5 was the optimum to recover antioxidant capacity. A possible explanation is that electrical discharges at high energy inputs and certain pH may produce chemical products of electrolysis and free reactive radicals, which can reduce the nutritional quality of some antioxidant compounds that are not determined in the present work.

Moreover, ethanol percentage had also a significant influence in TPC, protein, and antioxidant recovery. As can be seen in Table 1 and Fig. 4a–c, a significant increase in TPC was found when ethanol percentage was augmented.

These results were in close agreement with those obtained by Boussetta et al. (2013) who found that HVED noticeably enhanced the hydro-ethanolic extractions of TPC from both crushed and non-crushed flaxseed cake. They used ethanol at different percentages (0–25 %), obtaining the highest TPC recovery (almost twofold higher) when they used 25 % ethanol.

In another study, Rajha et al. (2014) evaluated the effects of different ethanol contents (25–75 %) on polyphenol recovery

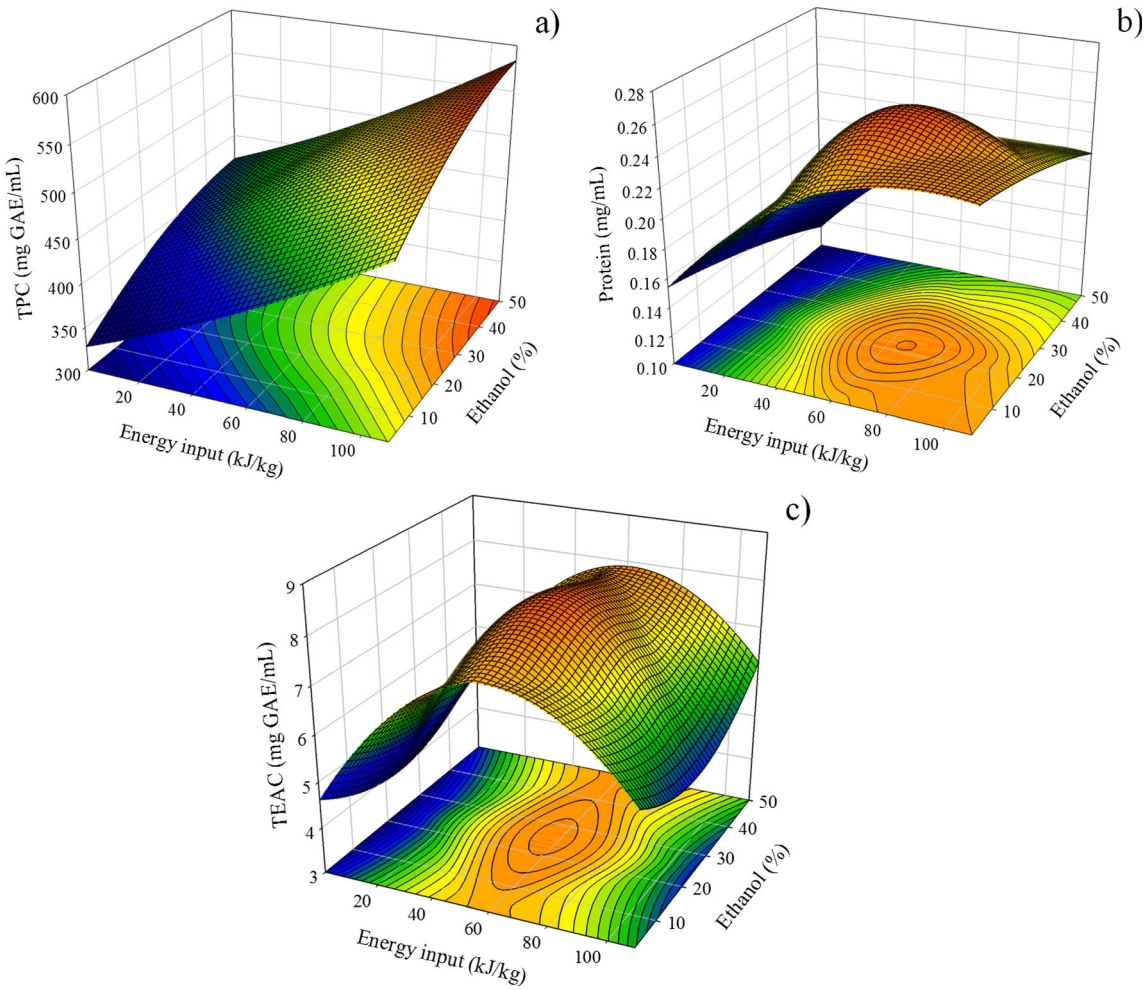

Fig. 4 Response surface plots for **a** total phenolic compounds (TPC); **b** protein; and **c** antioxidant recovery from olive kernel samples (Cornicabra) as affected by high voltage electrical discharges (HVED) at different energy inputs (0–109 kJ/kg) and hydroalcoholic mixtures with different ethanol percentages (0–50 %)

from vine shoots. They found that ethanol content had a significant influence in the recovery of phenolic compounds. However, they obtained the maximum polyphenol yield when ethanol was used at the intermediate concentration (50 %). They attributed this effect to the different phenolic profile of the samples with such a high diversity in water and ethanol solubility.

Protein recovery was significantly affected by ethanol concentration. As it is shown in Fig. 4b, an increase in protein yield up to 25 % ethanol concentration and a decrease when ethanol percentage was higher were observed. This fact can be attributed to coagulation of proteins, thus promoting conformational changes in the structure of the protein, which can damage the integrity of the protein or can decrease their extractability. These results are in the line to those reported by Rajha et al. (2014) who obtained the highest protein yield (≈1.3 mg BSA/g DM) when they used a hydroalcoholic mixture with ethanol content of 25 %.

It can be seen that application of HVED+ethanol extraction allowed a significant enhancement of the yields of antioxidant capacity (TEAC and DPPH values) at pH 7.25 up to 66 kJ/kg. However, TEAC and DPPH values were decreased when higher energy inputs were used. This fact can be explained by the ability of ethanol for extracting fat-soluble compounds with antioxidant capacity (e.g., vitamin E), which can be found in olive-derived samples. For instance, HVED can promote loss of these compounds at high energy inputs, thus reducing antioxidant recovery.

As it was found for phenolic compounds, the optimum antioxidant recovery was obtained when pH 2.5 was used. This fact can be attributed to better stability of the phenolic compounds contained in olive kernel when strongly acidic conditions are used. When the possible correlation (Pearson test) between TPC and antioxidant capacity (TEAC and DPPH values) was studied for the different pH and ethanol concentrations, it was found that there was a significant positive correlation between TPC and TEAC and TPC with DPPH.

The multi-response analysis of RSM using the desirability approach was used to optimize the energy input, pH, and ethanol concentration. The desirability function is an approach for solving the problem of optimization of the several responses and is applied when various responses should be considered simultaneously. The desirability function is constructed first independently for each individual response, and then it is possible to obtain the overall desirability.

Table 2 Response surface methodology (RSM) data for optimum conditions for the olive kernel samples (Cornicabra) that were analyzed immediately after high voltage electrical discharges (HVED) treatment

	Energy input (kJ/kg)	pH	Ethanol
TPC	81	2.5	47
Protein	54	12.0	23
TEAC	67	2.5	50
DPPH	67	2.5	50
Total	66	2.5	49

TEAC Trolox equivalent antioxidant capacity, *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate

Table 3 Comparison of the effects of optimum high voltage electric discharges (HVED) conditions at olive kernels of different varieties (Cornicabra and Koroneiki)

Variety	TPC (mg/L)	Proteins (mg/mL)	TEAC (mM TE)
Cornicabra	618.86±7.23	0.198±0.001	9.30±0.09
Koroneiki	106.36±7.23	0.208±0.001	2.79±0.10

TPC total phenolic compounds, *TEAC* Trolox equivalent antioxidant capacity

Table 2 shows the optimum conditions for enhancing the yields of TPC, proteins, and antioxidant compounds after HVED treatment. Multiple response optimizations indicated that HVED pretreatment with energy input of 66 kJ/kg followed by short solid–liquid extraction in 49 % *v*/*v* ethanol/water solution gave the highest yields of TPC, proteins, and antioxidant capacity in the extracts obtained immediately after the treatment of Spanish olive kernels. Under such conditions, TPC, content of proteins, TEAC, and DPPH were 626.6 mg GAE/L, 0.225 mg/mL, 9.80 mM TE, and 7.61 mM TE, respectively (Table 3).

The mean contents were compared by a *t* test, and the results show that there are no significant differences ($p>0.05$) between TPC, content of proteins, and antioxidant capacity after applying the optimized method and the experimental values.

Finally, TPC, proteins, and TEAC values of the extracts obtained from olive kernels (Koroneiki) at the optimized conditions were evaluated and compared to those obtained from Spanish olive kernels (Table 3). It was found that under these conditions, antioxidant compounds were almost fivefold lower to those obtained for Spanish olive kernels. This observation may be attributed to the initial composition (initial concentration in the raw material) of olive kernels varieties (Cornicabra and Koroneiki).

Conclusions

The results obtained in the present study showed the potential of HVED-assisted technology to improve the recovery of high-added value compounds from olive kernels (polyphenols and proteins). At equivalent energy inputs, HVED technology was demonstrated to be more effective than US and PEF technologies in terms of polyphenol extraction (255 mg GAE/L for HVED versus 140 and 146 mg GAE/L for US and PEF, respectively). The results also showed faster

extraction kinetics when HVED was used as compared to US and PEF treatments. This technology allows recovery of biomolecules with antioxidant capacity that can be used as food additives and/or nutraceuticals. Moreover, it is in accordance of the modern concept of green extraction, which assumes using of renewable plant resources, alternative solvents (water or agro-solvents), reduction of energy consumption, production of high quality and purity extracts (non-denatured and biodegradable) and extracts co-products instead of wastes. The application of HVED treatment is a promising technology to improve the extraction of valuable compounds from olive kernel. The HVED treatment is a green extraction technique and required low energy input (60–80 kJ/kg). However, the feasibility of the application of HVED at pilot or industrial scales is still unknown. The advantages of HVED should encourage the development of new generators with high voltage output, which can help on the scaling up of this process.

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