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Electrically Assisted Extraction of Soluble Matter from Chardonnay Grape Skins for Polyphenol Recovery

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The objective of this study was to investigate the effects of pulsed electric field (PEF) and high-voltage electrical discharges (HVED) application on the efficiency of aqueous extraction of total soluble matter and polyphenols from grape skins (*Vitis vinifera* L.) at different temperatures within 20−60 °C. The highest level of polyphenol concentration *C* was reached after about 60 min of extraction for HVED treatment: $C_{HVED} = 21.4 \pm 0.8$ μmol of gallic acid equivalent (GAE)/g of dry matter (DM). Almost the same level of *C* was reached after 180 min of extraction for the PEF-treated skins. These levels exceeded the value $C = 19.1 \pm 0.5$ μmol of GAE/g of DM for the untreated samples. The difference between °Brix values for HVED-treated and untreated systems decreased with temperature increase (from 40 to 60 °C), but a large difference in the total amount of polyphenols was observed for HVED-treated and untreated systems. The activation energies were $W_u = 31.3 \pm 3.7$ kJ/mol and $W_{PEF} = 28.9 \pm 5.5$ kJ/mol for untreated and PEF-treated systems, respectively.

KEYWORDS: Pulsed electric field (PEF); high-voltage electrical discharges (HVED); grape skins; polyphenols; extraction

INTRODUCTION

Grape skins are a rich source of polyphenols, which can determine the sensory and health-useful properties of juices and wines (*1*). Polyphenols contained in grapes and wines can be divided into three main groups: phenolic acids (mainly benzoic and hydroxycinnamic acids), simple flavonoids (catechins, flavonols, and anthocyanins), and tannins and proanthocyanidins. The phenolic content of grape skin ranges from 285 to 550 mg of polyphenols/kg of grape skin, depending on the grape variety and type of pretreatment (*2*). The major part of polyphenols remains after pressing of grapes in press residues, or pomace. The grape pomace reaches 20% of the weight of processed grapes, and its utilization is an important environmental problem (*3*). Upgrading of this typically low-value food byproduct can be useful for the production of polyphenol extracts, functional food components, useful health ingredients, and antioxidant additives (*4, 5*).

Different solvent extraction and enzymatic catalysis-based techniques have been proposed for the extraction of polyphenols (*6*−*9*). However, nowadays safe extraction protocols without the use of organic solvents and chemical additives have become more important. On the other hand, methods involving the use of enzymes may be time-consuming. The economical advantages of electrical treatments versus enzymatic methods were studied (*10*). Extraction efficiency can be increased by ultrasonics, high hydrostatic pressure (HHP), and pulsed electric fields (PEF) as was recently discussed (*11*). In particular, PEF application (electric field strength of 3 kV/cm) increased extraction of total polyphenols by approximately 2-fold as compared with the control extraction from ethanol/water mixture (50:50, v/v) during 1 h at $T = 70$ °C. Application of PEF was shown to be useful also for the extraction of phenolic compounds at moderate temperature ($T = 25$ °C) during vinification and fermentation (*12*). In addition to their use for degradation of organic compounds contained in water (*13*) or for inactivation of micro-organisms (*14*), high-voltage electrical discharges (HVED) were also applied for enhanced aqueous extraction of soluble material. This technology was applied to vegetative raw

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material (*15*), linseed (*16*), green tea, and roots of *Datura innoxia* (*17*).

The possible industrial applications of PEF and HVED techniques for solute extraction were recently discussed (*18*−*20*). Semi-industrial systems for the continuous PEF treatment are available now for sugar beets, apples, and grape mash. The development of HVED for solute extraction is limited by the laboratory- and small-scale batch pilots. However, this technique has attracted growing industrial and research interest in several other applications (microbial inactivation of liquid wastes, surface cleaning, drilling, etc.) (*21*). Additional research efforts should be undertaken for the development of large-scale generators and chambers for continuous HVED treatment. Some information about the new developments of larger scale generators is available (*22*).

At present, effective electric treatment protocols for acceleration of extraction of polyphenols from grape skins in aqueous media are still unavailable. The polyphenols bind in the grape skins in different ways and may be classified as cell-wall polyphenols (bound to polysaccharides) and non-cell-wall polyphenols (confined in the vacuoles and cell nucleus) (*1*). Electrical treatment can provoke membrane electroporation (*23*) and biological tissue damage (*24*), but the impact of electrical treatment on the release of polyphenols may be quite different for these two types of polyphenols. PEF is based on the electroporation phenomenon, which was intensively discussed in the biophysical literature (*25*) and in previous works concerning food applications (*26*). The pores created in cell membranes by the PEF treatment can be reversible or irreversible depending on the intensity and duration of PEF application (*27*). Electroporation is considered to be a treatment that permeates the cell membranes but keeps the cell walls relatively intact. It has been shown that the electroporation phenomenon may, in principle, be used for the selective extraction of soluble matter from the interior of cells (*24*). HVED is a more "violent" treatment, which is based on the phenomenon of electrical breakdown of water (*16*). The application of high voltage across the electrodes leads to acceleration of the electrons that achieve enough energy to excite water molecules. Then an avalanche of electrons is created. Air bubbles that are initially presented in water or formed thanks to local heating participate and accelerate this phenomenon. If the electrical field is intense enough, the avalanche of electrons becomes a starting point of streamer propagation from the positive electrode to the negative one. When one of the streamers reaches the negative electrode, electrical breakdown occurs and a discharge channel is created. The electrical breakdown is accompanied by the number of secondary phenomena (high-amplitude pressure shock waves, bubbles cavitation, creation of liquid turbulence, etc.). These secondary phenomena cause particle fragmentation and cell structure damage (*16*). They can also accelerate biomolecule extraction from the interior of the cell tissue.

This work discusses the effects of pulsed electric fields and high-voltage electrical discharges on the acceleration of extraction of total soluble matter and polyphenols from grape skins (white grapes *Vitis vinifera* L. cv. 'Chardonnay') in distilled water at different temperatures from 20 to 60 °C. The level of PEF-induced disintegration at different electrical field strengths (E = 300−1300 V/cm) was estimated from the electrical conductivity measurements. The extraction kinetics at different temperatures were analyzed, and the activation energy was estimated. Both PEF and HVED influence first the total soluble matter extraction. The mechanism of total soluble matter extraction may not be the same as the mechanism of polyphenol extraction because of the hypothetical selectivity of extraction. Therefore, the results of extraction of polyphenols and total soluble matter are compared.

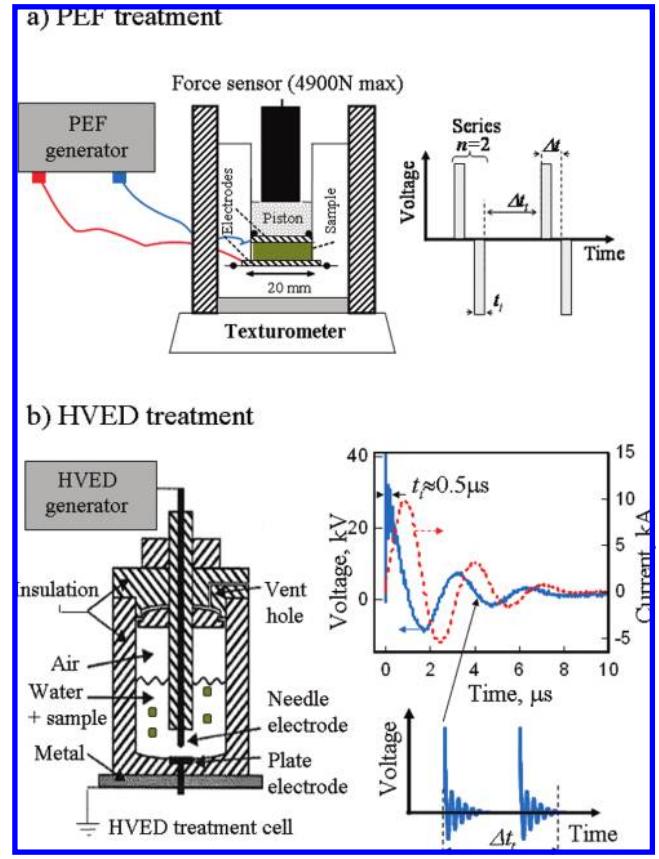

Figure 1. Experimental setups for PEF (**a**) and HVED (**b**) treatments with corresponding pulse protocols.

MATERIALS AND METHODS

Preparation of Grape Skins. White grapes (*V. vinifera* L. cv. 'Chardonnay', vintage 2008) were purchased from the experimental vineyard of Chile. We assume that the effects of electrical treatments obtained for these white grape skins would also be observed for red grape skins. Indeed, with regard to the composition of polyphenols, no principal differences between red and white grape varieties were observed, except for the absence of anthocyanins in white grape pomaces (*7*). Finally, red and white grape pomaces resulting from white winemaking are both characterized by high total polyphenolic content (*28*). Grapes were pressed using a vertical diaphragm wine press (Parapress, Sofralab, France), so there was no soaking of pomace in the juice. The dry matter content in the grape pomace was 25 wt %. Grape skins from the grape pomace were manually separated and washed with distilled water at 25 °C during 30 s to remove external juice.

PEF Treatment Experiments. The experiments were carried out using a PEF treatment cell integrated inside a texture analyzer (model TA-XT Plus, Stable Microsystems) (**Figure 1a**). The PEF treatment cell consisted of a polypropylene cylinder with an inner diameter of 20 mm and a bottom electrode. A stainless steel piston was used as the second electrode. The initial sample weight was 4.0 ± 0.1 g. Skins were placed between the two electrodes, and the final distance between electrodes was fixed at 3 mm. The external electric field was applied to the sample.

Electric field treatment was applied using the PEF generator, 400V-38A (Service Electronique, University of Technology of Compiègne). It provided bipolar pulses of near-rectangular shape. Trains of pulses were used for PEF treatment. An individual train consisted of n pulses with pulse duration t_i and pulse repetition time Δt. There was a pause of Δt_t after each train. The total time of electrical treatment during the

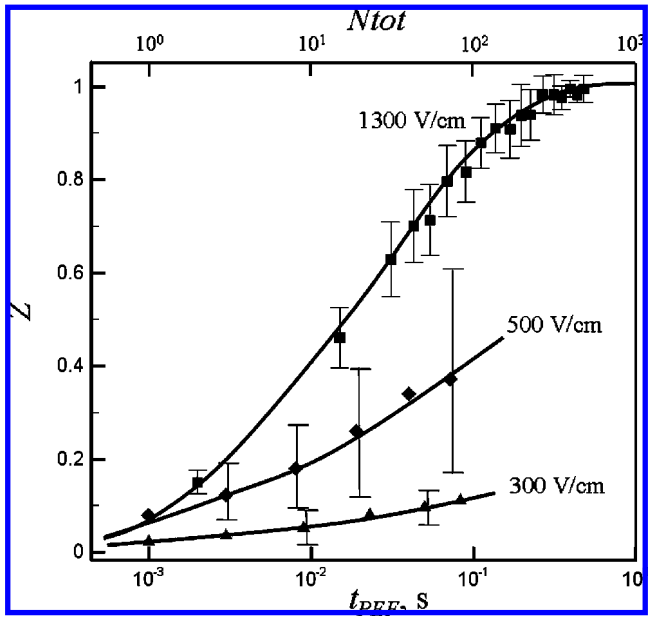

Figure 2. Damage degree Z versus treatment time t_t and total number of pulses N_{tot} at different electric field strengths E in PEF treatment experiments.

PEF experiments was calculated as $t_t = N_{tot}t_i$, where N_{tot} is the total number of pulses and $N_{tot} = Nn$, where N is the number of trains.

The electrodes were connected to the PEF generator, and the electrical conductivity of samples was measured in the intertrain period at a frequency of 0.5 kHz, selected as optimal for the purposes of removing the polarizing effects on the electrodes and tissue sample. All of the output data (current, voltage, electrical conductivity, and temperature) were collected using a data logger and special software, adapted by Service Electronique, University of Technology of Compiègne.

The degree of tissue damage was estimated from the electrical conductivity disintegration index Z (*29*)

$$Z = (\sigma - \sigma_u)/(\sigma_d - \sigma_u) \quad (1)$$

where σ is the measured electrical conductivity value and the subscripts u and d refer to the conductivities of untreated (intact) and maximally damaged skin tissue, respectively. The skin tissue damaged by freezing/thawing is considered here as a maximally damaged tissue. This consideration is usual in the literature to estimate the degree of damage for PEF-treated tissue . In both cases (PEF and freezing/thawing) tissue is not fragmentized and the damage is mainly limited by membrane rupture.

The application of the above equation gives $Z = 0$ for the intact material and $Z = 1$ for the maximally disintegrated material. The electrical conductivity grows under the PEF treatment and gets some saturation level at long times of treatment. This saturation level at the electric field strength $E = 1300$ V/cm was observed for the investigated grape skins at the total time of the PEF treatment $t_t \approx 1$ s, and the corresponding conductivity value was put as σ_d in eq 1.

The temperature inside the sample under PEF treatment was checked in control experiments carried out in online mode in the intertrain period by a Teflon-coated K-type thermocouple (±0.1 K) connected to the data logger thermometer (Center 305/306, IDC Electronic SA).

Examples of damage degree Z versus treatment time t_t at different electric field strengths E are presented in **Figure 2**. The PEF protocol parameters were as follows: pulse duration t_i=1000 μs, number of pulses $n = 2$, pulse repetition time $\Delta t = 1000$ μs, intertrain pause $\Delta t_t = 5$ s, and number of trains N within 1−500. This protocol allowed fine regulation of the grape tissue disintegration index Z without any noticeable temperature elevation ($\Delta T \leq 1$ °C) so the PEF treatment can be considered to be isothermal ($T \approx 20$ °C). The total electric energy W_{PEF} required for high level of Z ($Z > 0.9$) was about 30 kJ/kg of grape skins. These results are in correspondence with the previously estimated electric energy consumption of 20 kJ/kg for PEF treatment of white grapes with a high degree of tissue damage ($Z \approx 0.8$) and electric field strength $E = 750$ V/cm (*30*).

HVED Treatment Experiments. The experimental apparatus (Tomsk Polytechnic University) consisted of a pulsed high-voltage power supply and a laboratory treatment chamber with a needle-plate geometry electrode (**Figure 1b**). A stainless steel needle 10 mm in diameter was used. The grounded plate electrode was a stainless disk of 35 mm in diameter. A positive pulse voltage was applied to the needle electrode. The high-voltage pulse generator provided 40 kV−10 kA discharges during a few microseconds in a 1 L chamber. The inner diameter was 110 mm, and the height of the cell was 315 mm. The distance between the electrodes $d_{electrodes}$ was 10 mm, and the 40 kV peak pulse voltage U was used, which resulted in the electric field strength of 40 kV/cm. The electrical discharges were generated by electrical breakdown in water. Energy was stored in a set of low-inductance capacitors, which were charged by a high-voltage power supply. The effective pulse duration t_i was approximately 0.5 μs (*16*). The total duration of damped oscillations was about 10 μs (**Figure 1b**). The voltage (Ross VD45-8.3-A-K-A, Ross Engineering Corp.) and current (Pearson 3972, Pearson Electronics Inc.) measurement units were connected with a 10^8 Hz sampling system via an oscilloscope (Tektronix TDS1002). The software HPVEE 4.01 (Hewlett-Packard) was used for acquisition of data.

The treatment chamber was initially filled with grape skins (40.0 ± 0.1 g), which were further mixed with distilled water (the liquid−solid ratio, w/w, was fixed at the level of 6) at the diffusion temperature (20, 40, or 60 °C). HVED treatment consisted of applying 60 successive pulses. During the treatment, an electric discharge induced turbulence and cavitation phenomena. After application of 60 pulses, the product inside the cell was assumed to be well mixed. On the other hand, the liquid-to-solid ratio of 6 was highly sufficient to immerge all of the product in water. Therefore, the treatment applied was rather homogeneous. Electrical discharges were applied with a pulse repetition rate of 0.5 Hz ($\Delta t_t = 2$ s, **Figure 1b**), which was imposed by the generator. The total treatment time was then 120 s. The consumed total electric energy W_{HVED} was approximately 120 kJ/kg of grape skins, and the temperature increase after the HVED treatment was between 3 and 5 °C.

The °Brix and content of total polyphenols C were measured before and after treatment.

Extraction/Diffusion Experiments. The cylindrical diffusion cell with distilled water was preheated during 30 min at the desired temperature (20, 40, or 60 °C). The skins (untreated or PEF pretreated) were placed into the diffusion cell. The liquid/solid ratio, w/w, was fixed at the level of 6. The PEF-pretreated samples with high disintegration index ($Z \approx 1$) were used ($E = 1300$ V/cm, $t_t = 1$ s). The HVED treatment was applied to the suspension of grape skins in distilled water preheated at the desired temperature (20, 40, or 60 °C), and then diffusion was studied. A careful agitation at 160 rpm was provided using a round incubator shaker (Infors HT Aerotron). To avoid any evaporation and degradation of polyphenols under the impact of air or light, the diffusion cell was closed and covered by aluminum foil during the extraction process. The concentration of soluble matter content (°Brix) was controlled by a digital refractometer PR-101 (Atago) every 5−10 min at room temperature during the diffusion process.

Quantification of Total Polyphenols. For the determination of the extraction kinetics of total polyphenols, the absorbance of the extracts was measured at 280 nm (*31*) by using a spectrophotometer UV−vis (Libra S32, Biochrom). This is a fast procedure based on absorbance of the aromatic ring. Most catechins have maximum absorption around 280 nm. Catechin is reported to be the major catechin monomer in all grape skins, and the total content of phenols was reported to be highly correlated with absorbance at 280 nm (*31*). For optimization purposes, direct reading of absorbance at 280 nm rather than the Folin−Ciocalteu method may be preferable for total polyphenol evaluation (*8*). Gallic acid (Sigma-Aldrich) was used as standard for the calibration curve. Content of total polyphenols C was expressed as micromoles of gallic acid equivalent (GAE) per gram of dry matter (DM).

HPLC-MS Analyses. Polyphenol analyses of the final extracts were performed by high-performance liquid chromatography (HPLC). Prior

to analytical chromatography, samples were purified to remove interferences of sugars and organic acids from the crude sample. Solid phase extraction (SPE) was conducted on C18 cartridges (500 mg, Isolute, Biotage) preconditioned at pH 7 (*32*). First, the cartridge was preconditioned by sequentially passing 5 mL of absolute methanol and 10 mL of deionized water. Second, a 5 mL portion of crude extract was loaded onto the cartridge. Adding 30 mL of water washed the cartridge. Then the cartridge was dried under vacuum and, finally, 7 mL of methanol was added to elute polyphenols; the fraction was collected and the solvent evaporated to dryness. The purified sample was dissolved in methanol and centrifuged, and the supernatant was injected into the HPLC system.

The HPLC equipment included an autosampler (Famos, Dionex) and a capillary HPLC (Ultimate 3000 LC Packing, Dionex) with UV detection (280 nm) combined with a triple-quadruple mass spectrometer equipped with an electrospray source (Quattro Micro, Waters). The analytical column was a C18 capillary column 100 × 0.5 mm i.d., 3 μm particle diameter and 175 Å pore size (Hypersil Gold, Thermofisher) and maintained at 35 °C. The elution gradient was performed according to the method given in ref *22* with some modifications; the column was initially equilibrated with acetic acid/water (2:98, v/v) as solvent A for *x* min. Polyphenols were eluted with a three-stage linear gradient: from 92 to 76% of A in 20 min, from 76 to 60% of A in 10 min, and from 60 to 0% of A in 15 min with a flow rate of 8 μL/min. A mixture of acetonitrile/acetic acid/water (75:2:23, v/v) was used as solvent B. A wavelength of $\lambda = 280$ nm was used for the absorbance detector. All solvents used were purchased from Sigma-Aldrich (HPLC grade). The injected sample volume was 1 μL. Mass spectrometry analyses were used for the identification of polyphenols. Analyses were performed in the positive SIR mode. Collision energies (20 or 40 eV) and cone voltage (20 V) were set using the following standards: catechin, epicatechin, resveratrol, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, all purchased from Extrasynthese.

Estimation of the Effective Soluble Matter Diffusion Time. The extraction kinetics data were treated using the diffusion theory formalism. For purposes of simplicity, it was assumed that the grape skins were thin slabs of uniform thickness, and solution of the Fick's second law (*33*) was used for estimation of the effective soluble matter diffusion time τ in skins

$$\frac{°\mathrm{Brix} - °\mathrm{Brix_i}}{°\mathrm{Brix_f} - °\mathrm{Brix_i}} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{\exp(-(2n+1)^2 t/\tau)}{(2n+1)^2} \qquad (2)$$

Here, $\tau = 4h^2/\pi D_{\mathrm{eff}}$, h is the skin thickness, D_{eff} is the effective diffusion coefficient, and °Brix$_i$ and °Brix$_f$ are the initial and final values of the soluble matter content, respectively. The value of °Brix$_i$ was determined immediately after the skins had been placed into diffusion cell, and °Brix$_f$ corresponds to the final value of saturation after the infinite time of extraction. This summation series converges rapidly for large values of time *t*. Therefore, only the first five leading terms were taken into account for τ estimation.

Statistical Analysis. Each experiment was repeated, at least, three times, and means and standard deviations of data were calculated. The error bars in all figures correspond to the standard errors.

RESULTS AND DISCUSSION

Effects of PEF and HVED Treatment on Kinetics of Extraction of Polyphenols. Effects of PEF and HVED treatment on acceleration of extraction kinetics at 20 °C are shown in **Figure 3**. The concentration of soluble matter increased with the time and reached the maximum level after 180 min of extraction. The evolution of °Brix was noticeable for both untreated and PEF-treated samples. In contrast, HVED treatment resulted in an increase of the °Brix level immediately after treatment, but then °Brix reached maximum after about 20 min of extraction. An interesting observation was that pretreatment of skins influenced the initial soluble matter content value °Brix$_i$ (inset in **Figure 3**).

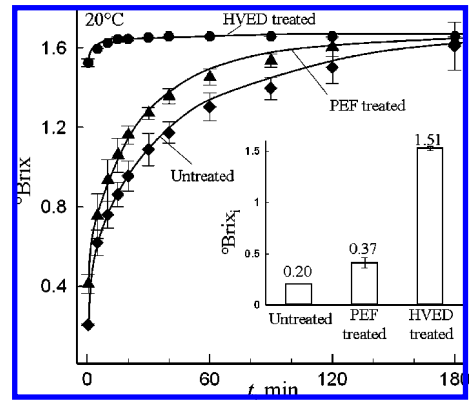

Figure 3. Brix versus extraction time *t* for untreated, PEF-treated, and HVED-treated grape skins at $T = 20$ °C. (Inset) Effect of treatment on initial concentration of the soluble matter °Brix$_i$. (PEF treatment: $E = 1300$ V/cm, $t_t = 1$ s. HVED treatment: $d_{\mathrm{electrodes}} = 10$ mm, $U = 40$ kV, $t_t = 120$ s.)

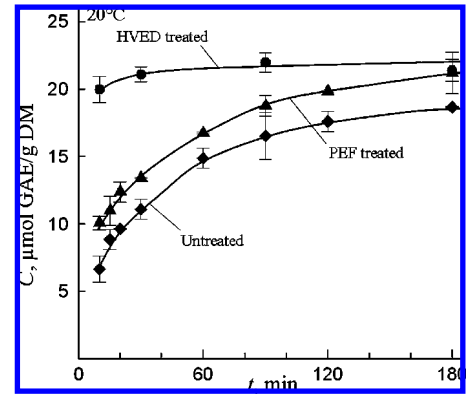

Figure 4. Content of total polyphenols *C* versus extraction time *t* for untreated and PEF-treated grape skins at $T = 20$ °C. (PEF treatment: $E = 1300$ V/cm, $t_t = 1$ s. HVED treatment: $d_{\mathrm{electrodes}} = 10$ mm, $U = 40$ kV, $t_t = 120$ s.)

Note that the composition of grape skin is rather complex and includes superimposed layers with different structures. Most of the polyphenols and soluble matter are located in the inner cell layers (hypodermis), which are closest to the pulp (*34*). The initial jump of °Brix from zero (distilled water) to °Brix$_i$ for untreated samples may reflect the fast release of unbound soluble matter from the damaged cell at the inner surface of grape skins. At room temperature ($T = 20$ °C), the PEF and HVED applications increased the initial value of °Brix$_i$ by approximately 2- and 7.5-fold, respectively, as compared with the untreated control samples.

Kinetics of extraction of the total polyphenols (**Figure 4**) show similar tendencies as observed in the kinetics of °Brix (**Figure 3**). The initial jump of *C* from zero (distilled water) to initial polyphenol concentration C_i was also observed, and it was evidently of the same nature as the initial jump of °Brix. The highest level of *C* was reached after about 60 min of extraction for HVED treatment ($C_{\mathrm{HVED}} = 21.4 \pm 0.8$ μmol of GAE/g of DM). Almost the same level of *C* was reached also after 180 min of extraction for the PEF-treated skins. These levels exceeded the value $C = 19.1 \pm 0.5$ μmol of GAE/g of DM for untreated samples. Note that the concentration of polyphenols was rather small as compared with the concentration of soluble matter in extracts: for example, the ratio of masses for polyphenols and soluble matter was, approximately, 0.5% for extracts obtained at 20 °C during 60 min for untreated grape skins. The differences between extraction kinetics for PEF- and

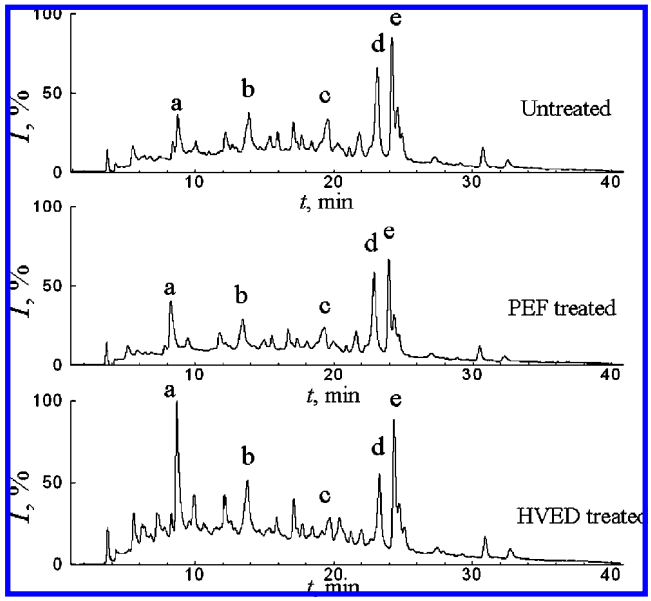

Figure 5. HPLC profiles from the extracts obtained at 20 °C after 60 min of extraction for untreated, PEF-treated, and HVED-treated grape skins. Identified compounds are catechin (a), epicatechin (b), quercetin-3-*O*-glucoside (c), and kaempferol-3-*O*-glucoside (d). (PEF treatment: E = 1300 V/cm, t_t = 1 s. HVED treatment: $d_{electrodes}$ = 10 mm, U = 40 kV, t_t = 120 s.)

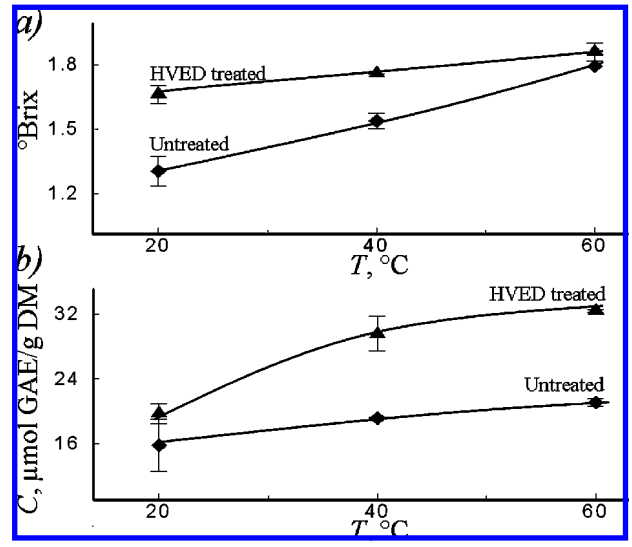

Figure 6. Brix and content of total polyphenols C versus temperature T for untreated and HVED-treated grape skins after 60 min of extraction. The HVED treatment was done for grape skins in distilled water at the same temperature as the temperature of extraction. (HVED treatment: $d_{electrodes}$ = 10 mm, U = 40 kV, t_t = 120 s.)

HVED-treated tissues may reflect the different modes used for both treatments: the PEF treatment was applied to grape skins, whereas the HVED treatment was applied to a skin suspension turbulized by electrical discharges. Therefore, a rather important quantity of soluble matter was extracted from skins directly during the treatment by HVED. Moreover, the HVED treatment leads to tissue fragmentation and consequent increase of transfer surface.

The obtained data evidenced that PEF- and HVED-assisted processes at room temperature (T = 20 °C) allow the acceleration of extraction. After a rather long time of extraction (here, 180 min), the final values of °Brix were approximately the same irrespective of the treatment (**Figure 3**), but the final values of C were slightly higher for treated samples (**Figure 4**). An increase of the concentration of polyphenols after PEF treatment was observed also in the extraction experiments during the fermentation of grape musts (*12*).

The HPLC profiles from extracts obtained at 20 °C after 60 min of extraction evidenced the similar polyphenol compositions of extracts for untreated and PEF-treated grape skins (**Figure 5**). Some anthoxanthins (catechin (peak a), epicatechin (peak b)) and flavonoïds (quercetin-3-*O*-glucoside (peak c), kaempferol-3-*O*-glucoside (peak d)) were identified by comparison of their UV spectra, retention times, and characteristic mass spectra with reference compounds. At the retention time of about 25 min, the mixture of at least three different polyphenols (quercetin glucoside, kaempferol glucoside, or resveratrol) is not well separated, which makes difficult the identification of peak e. All of these compounds are typical for the extracts of polyphenols isolated from Chardonnay grape pomace (*35*). In our experiments, the PEF-assisted extraction by distilled water did not result in any selectivity of extraction of the polyphenols. This result is in contrast with extraction data for the ethanol/water mixture, with which remarkably enhanced extraction of anthocyanin monoglucosides compared to the amount of acylated glucosides was observed (*11*). It is reasonable to assume that the selectivity of PEF-assisted extraction depends on the type of solvent, temperature, time of extraction, and other details of the extraction protocol, but further studies are necessary to elucidate these effects.

Some selectivity was observed for the HVED-assisted extraction, for example; the concentration of catechin (peak a in **Figure 5**) was noticeably higher for HVED-treated samples as compared to untreated or PEF-treated samples. The enhanced selectivity for HVED-assisted extraction of polyphenols can be explained by the effects of shock waves present in the discharge phenomena, which can result in supplementary mechanical damage of skins, disintegration of cell walls, homogenization effects, etc. (*16*).

Effect of Temperature on Extraction Efficiency. The electrically induced damage efficiency can be more pronounced at elevated temperatures as was previously demonstrated for soft cellular tissues (*36*). **Figure 6** compares the effect of temperature on the efficiency of extraction of the soluble matter (**a**) and polyphenols (**b**) for HVED-treated and untreated systems. The HVED treatment was applied to the heated suspension of grape skins, and then extraction was done at the same temperature. The time of extraction was fixed (t = 60 min), and the highest levels of °Brix and C were reached for HVED-treated systems. The data show that the difference between °Brix values for HVED-treated and untreated systems decreases with temperature increase (**Figure 6a**), but it is not so for the content of total polyphenols (**Figure 6b**). The large difference in content of the total polyphenols at elevated temperatures can be explained by the HVED-assisted release of the fraction of polyphenols from the grape skins with relatively low water solubility. Moreover, the hydrophobic substances may become less soluble as the temperature increases (*37*) due to strengthening of the surrounding hydrogen bonds around hydrophobic aromatics (*38*). Therefore, acceleration of extraction of the polyphenols may be also limited by the decrease of their aqueous solubility at elevated temperatures.

Figure 7A shows °Brix versus time t at different temperatures (T = 20−60 °C) for untreated (**a**) and PEF-treated (**b**) grape skins. In all cases, the PEF treatment was done at room temperature. The results of the least-squares fitting of the experimental data to eq 3 are shown in **Figure 7A** by solid

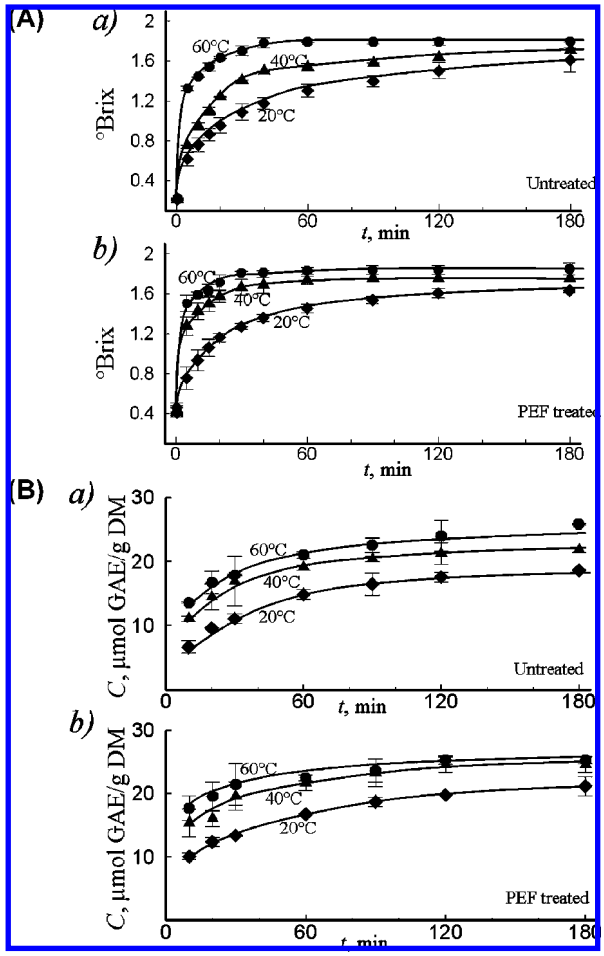

Figure 7. (**A**) Evolution of °Brix during extraction at different temperatures for untreated (**a**) and PEF-treated (**b**) grape skins. Solid lines correspond to the least-squares fitting of the experimental data (symbols) to eq 2. (PEF treatment: $E = 1300$ V/cm, $t_i = 1$ s.) (**B**) Evolution of total polyphenols during extraction at different temperatures for untreated (**a**) and PEF-treated (**b**) grape skins. (PEF treatment: $E = 1300$ V/cm, $t_i = 1$ s.)

lines and the relevant correlation coefficients are rather high, $R^2 = 0.987-0.990$. **Figure 7B** represents total polyphenols versus time t at different temperatures ($T = 20-60$ °C) for untreated (**a**) and PEF-treated (**b**) grape skins. Similar tendencies of the extraction kinetics of total soluble matter and total polyphenols for 20, 40, and 60 °C are observed (**Figure 7**).

The temperature dependencies of the effective diffusion time τ in the grape skins (**Figure 8**) can be satisfactorily described by Arrhenius law, $\tau \propto \exp(W/RT)$, where W is the activation energy and R is the universal gas constant. Both temperature increase and PEF pretreatment accelerated the extraction kinetics.

The activation energies estimated from Arrhenius slopes for effective diffusion time were $W_u = 31.3 \pm 3.7$ kJ/mol and $W_{PEF} = 28.9 \pm 5.5$ kJ/mol for untreated and PEF-treated samples, respectively. The difference between W_u and W_{PEF} was insignificant within the limits of estimation errors, and it reflects the similar mechanisms for the thermally activated diffusion of polyphenols in the untreated and PEF-treated grape skins.

To conclude, both the PEF and HVED treatments allow acceleration of the extraction kinetics of the soluble matter and polyphenols in distilled water at 20 °C. The pretreatment of skins noticeably influences the initial °Brix value and concentration of polyphenols C, and this effect may reflect enhancement

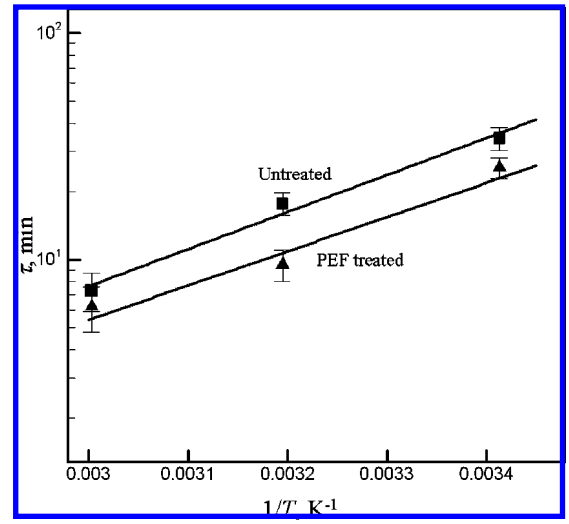

Figure 8. Arrhenius plots of the effective diffusion time τ for the untreated and PEF-treated grape skins. (PEF treatment: $E = 1300$ V/cm, $t_i = 1$ s.)

of diffusion between the cells at the inner surface of grape skins during the PEF or HVED treatment. After a rather long time of extraction (≈180 min), the final values of °Brix were approximately the same irrespective of the treatment, but the final values of concentration of the polyphenols C were higher for treated samples. In our experiments, the noticeable selectivity of extraction of the polyphenols was observed only for the HVED-treated grape skins, and it possibly reflects the effect of the shock waves present in discharge phenomena, which can result in supplementary mechanical damage of skins, disintegration of the cell walls, and mixture homogenization. Electrically assisted extraction at elevated temperatures influenced both the quantity of extracted polyphenols and the rate of extraction kinetics. The efficiency of extraction of the polyphenols may be limited by a decrease of their aqueous solubility at elevated temperatures. The data show that application of HVED treatment may be especially useful for releasing the fraction of polyphenols with low water solubility from the grape skins. Further studies are necessary for elucidation of the effects of extraction protocol (type of solvent, temperature, time of extraction, etc.) on electrically assisted selectivity of polyphenols extraction. To justify application of this technology in the industry, other studies on seeds, stems, and whole grape pomaces should be conducted to confirm the positive effect of these electrical treatments for polyphenol extraction. The energy consumptions of electrical and thermal treatments should also be compared.

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