Application of Pulsed Electric Energy for Grape Waste Biorefinery

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Eugène Vorobiev and Nikolai Lebovka

Abstract

Grape is the most usable and claimed fruit that is rich in bioactive compounds and especially in phenolic compounds. Facilitation of extraction of these compounds is important problem in modern processes of bioconversion and biorefinery of winery waste (pomace, skins, stalks, seeds). Different constituents of grape are rich in phenolic compounds. These compounds have excellent antioxidant, antiallergen, antibacterial, antifungal, anticancer, cardioprotective, vasodilatory, antimicrobial, and antiviral properties. For example, polyphenols have known health promoting effects and prevent or delay lipid oxidation in diverse food systems. Commonly, the existing techniques for extraction of these compounds are time consuming, can require large quantities of relatively costly or nongreen solvents, introducing of chemicals and enzymes, processing at high temperatures and pressures, and can cause large losses of nutrient resources and polyphenols recovery. This chapter concentrates on application of two modern electroporation-based extraction techniques: pulsed electric fields (PEF) and high voltage electrical discharges (HVED). Different applications of PEF and HVED treatment for improvement of juice and wine quality are discussed. The

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different examples of pulsed electric energy applications in pressure extraction systems are discussed. The efficiency of extraction of phenolic compounds and effects of pulsed electric energy on elimination of residual fungicides and microbial stabilization are considered. An introduction to the main aspects PEF and HVED treatments in relation with grape waste biorefinery is also provided.

Keywords

Pulsed electric fields (PEF) • High voltage electrical discharges (HVED) • Grape waste • Wine production • Biorefinery

Contents

Introduction

The biorefinery is aimed on the valorization of by-products, effluents, waste, and surplus with minimal or zero residual waste as well on extraction or separation of bioactive and other useful substances in the most economical and ecological manner. The grape berries are widely used for making wine, juice, vinegar, jam, jelly, grape seed extract, and grape seed oil. The direct industrial residue of grapes (grape pomace) typically contains skins and pulp, stalks, and seeds. The other residues of grapes include also wine shoots, leafs, and debris.

Work presented in this chapter concentrates on the application of pulsed electric energy (without discharges: pulsed electric fields (PEF) and with discharges: high voltage electrical discharges (HVED)) in relation with grape waste biorefinery. The both techniques assume effective electroporation of treated material. The PEF treatment is typically applied under the nonthermal condition without significant elevation of temperature. The HVED treatment is typically accompanied with electrical breakdown, propagation of streamer, shock waves, significant elevation of temperature, and mechanical fragmentation of treated materials. Application of PEF and HVED can positively affect juice and wine quality and improve biorefinery of grape waste. The objective here is to provide readers with a short overview of the biorefinery of grape waste and to outline some recent developments on bioconversion and traditional methods of biorefinery of grape waste as well as on pulsed electric energy applications to grape products and waste. The present chapter does not cover the details of PEF or HVED processing systems and basic of breakage/

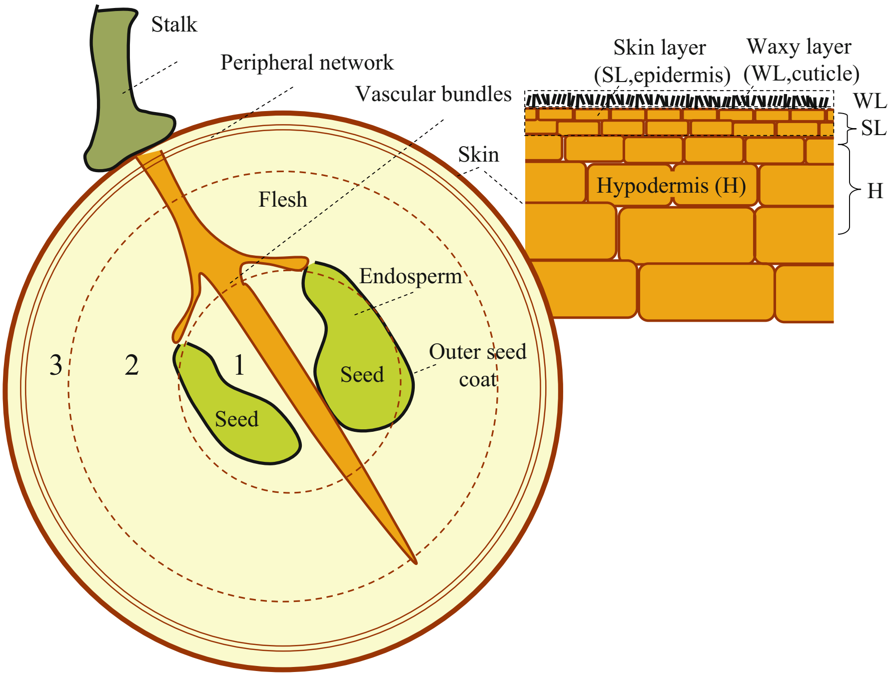


Fig. 1 Schematic structure of a grape berry. The soft part of the grape can be divided into: (*1*) central zone (malic acid, sugars); (*2*) intermediate zone (tartaric acid, sugars); and (*3*) peripheral zone (potassium, aromas, astringene substances, oxidases (group of enzymes), sugars). The details of cross-section through the cuticle, epidermis, and hypodermis are shown

electroporation of membranes and disintegration of cells. The main focus is on the efficiency of different PEF and HVED protocols and possibility of extraction of valuable polyphenolic substances, antioxidants, and other high-valued substances.

Winery Waste

The annual world grape production foots up to 67,067,128 metric tons (2012) and the major quantity of grapes (≈71%) is used for making wine. Moreover, more than 60% of wine worldwide is produced in European Union (mostly in France, Spain, Italy, and Germany). The cellular juice in wine grapes can be rather sweet and contains ≈24% sugar by weight. Winery waste are the lingocellulosic biomass that contains cellulose (≈50%), hemicellulose (≈35%), and lignin (≈15%).

Figure 1 presents the structure of grape berry. The soft grape flesh is noticeably inhomogeneous and can be divided onto three different zones with different composition of constituents. The grape berry is covered by skin and included seeds. The skin (epidermis) of the grape berry is covered by a waxy layer (cuticle). The outer layers are rich in tannins. Under the outer layer of skin a tightly packed layer of

Table 1 The quantity of residues as the result of the production of 100 L of white and red wine pomace (Compiled from the data presented in Prozil et al. (2012))

	Skins (kg)	Stalks (kg)	Others
White wine	17	4	10.2
Red wine	13.2	4	7.8

flattened cells (hypodermis) is located. This layer is rich in phenolic compounds and tannins that contribute to aroma, flavor, color, and astringency of wine and define its antioxidant properties. Grape seeds consist of the outer seed coat and the endosperm (Jackson 2008).

The direct industrial residue of grapes (grape pomace) typically contains skin and pulp (≈40–50%), stalks (≈25–30%), and seeds (≈25–30%). The mixture of grape skin and pulp is called also as marc. The other residues of grapes include wine shoots, leafs, and debris.

Table 1 represents the quantity of residues that accompanies the production of 100 L of white or red wine.

In general, different constituents of grape berry are rich in phenolic compounds. These compounds present the most valued component and have excellent antioxidant, antiallergen, antibacterial, antifungal, anticancer, cardioprotective, vasodilatory, antimicrobial, and antiviral properties. Polyphenols have known health promoting effects and prevent or delay lipid oxidation in diverse food systems.

The grape *pomace* is produced after pressing the crushing grapes (white wine) after fermentation and maceration (red wine). The grape pomace can represent up to 20% of the grape weight. It is very rich in bioactive compounds (cellulose, hemicellulose, tannins, resveratrol, anthocyanins, catechins, flavonol glycosides, phenolic acids, etc.).

The data of physicochemical analysis of grape pomace (*Vitis vinifera* L.) flour are presented in Table 2 (Sousa et al. 2014). The grape pomace is also rich in minerals (iron, potassium, zinc, calcium, and manganese) and the big concentration of total dietary fiber allows including the residue in the daily diet as a source of fiber and nutritional supplement.

Grape *skins* include cellulose (≈50%), hemicellulose, pectin, and lignin and have a large concentration of different phenolic constituents.

Wineries also produce a large quantity of the residues of grape *stalks* (the grape skeleton obtained from destemmed grape vines). Grape stalks contain a high degree of fibers (lignin and cellulose) and a high percentage of nutritive mineral elements, especially nitrogen and potassium (Table 3) (Prozil et al. 2012; Bertran et al. 2004). The rich composition of these residues allows them to be reapplied to the soil as compost.

The grape *seeds* contain fiber (≈40%), proteins (≈11%), phenols (≈7%) as well as oils (≈8–22%), nonphenolic antioxidants (tocopherols and β-carotene), sugars, and mineral salts. The oil content includes linoleic, oleic, palmitic, and stearic acids. Grape seed extracts are widely used in pharmacological applications.

Table 2 Chemical composition of grape pomace (*Vitis vinifera* L.) (Compiled from the data presented in Sousa et al. (2014))

Bioactive compounds			
Vitamin C (mg ascorbic acid/100 g)	26.25 ± 0.01	Soluble dietary fibers (g/100 g)	9.76 ± 0.03
Total anthocyanins (mg/100 g)	131 ± 0.4	Insoluble dietary fibers (g/100 g)	36.40 ± 0.84
General content (% dry basis)			
Moisture	3.33 ± 0.04	Pectin	3.92 ± 0.02
Ash	4.65 ± 0.05	Fructose	8.91 ± 0.08
Total lipids	8.16 ± 0.01	Glucose	7.95 ± 0.07
Proteins	8.49 ± 0.02	Total dietary fibers	46.17 ± 0.80
Carbohydrates	29.20		
Minerals (mg/100 g)			
Calcium	0.44 ± 0.715	Manganese	0.817 ± 0.550
Magnesium	0.13 ± 0.255	Phosphorus	0.183 ± 0.255
Sodium	0.044 ± 0.056	Sulfur	0.089 ± 0.336
Potassium	1.40 ± 0.313	Zinc	0.98 ± 0.702
Iron	18.08 ± 0.03		

Table 3 Chemical composition of grape stalks (*Vitis vinifera* L.) from red grape pomaces (Compiled from the data presented in Prozil et al. (2012))

Fibers (%, w/w)			
Cellulose (Kürschner–Höffer)	30.3	Hemicellulose	21.0
Klason lignin	17.4		
General content (%, w/w)			
Ash	7	Tannin	15.9
Proteins	6.1		
Monosaccharides (% wt)			
Rhamnose	1.7	Mannose	4.8
Fucose	<0.2	Galactose	4.9
Arabinose	5.5	Glucose	62.7
Xylose	20.4		
Minerals (mg/100 g)			
Calcium	0.15	Zinc	0.01
Potassium	0.9	Magnesium	0.02
Sodium	<0.01		

The wine *shoots* consist of ≈41% of cellulose, ≈26% of hemicellulose, ≈20% of lignin, and ≈9% of extractables (polyphenols, proteins, sugars (sucrose, glucose, fructose)).

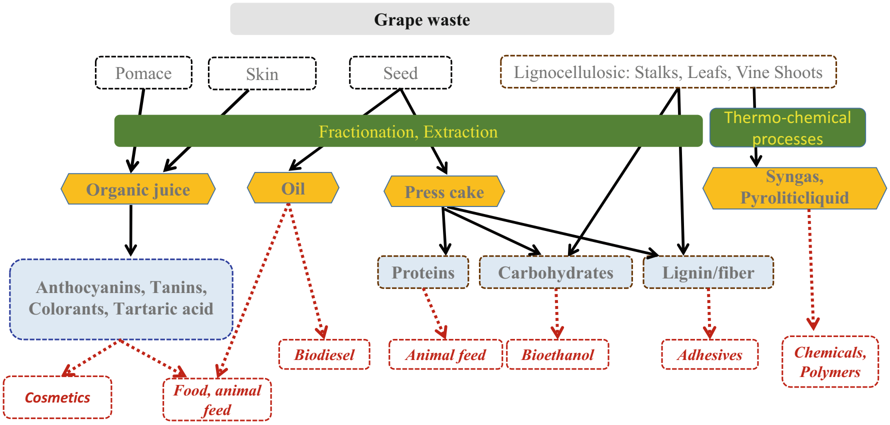


Fig. 2 Grape waste biorefinery

Concept of Biorefinery

Nowadays the concepts of biorefinery of bio-based waste are becoming more and more popular. First of all the biorefinery is aimed on the valorization of agro food by-products, effluents, waste, and surplus with minimal or zero residual waste. The very important application of biorefinery is aimed on extraction or separation of bioactive and other useful substances from the biowaste products in the most economical and ecological manner. Then these substances can be converted into value-added chemicals and nutraceutical products useful in food, medicine, cosmetic, and pharmaceutical industries. The biorefinery is also aimed on development of effective methods for production of bioenergy such as biofuels for transport (biodiesel or bioethanol), generation of electricity, and production of heat (Kamm and Kamm 2004).

Figure 2 presents the schema of grape waste biorefinery, including pomace, skin, seeds, stalks, leafs, and wine shoots.

Traditional Methods of Grape Waste Biorefinery

The important aspect of biorefinery includes the extraction and isolation of bioactive compounds and antioxidants from grape waste. Note that during vinification the extraction of phenolic compounds (anthocyanins, catechins, procyanidins, flavonol glycoside, phenolic acids, and stilbenes) is not very efficient and ≈60–70% of their content are lost in a grape pomace. The phenolic compounds are presumably located in skins, seeds, and short stems. The red grape pomace is more rich in anthocyanins, whereas the white grape pomace is rich in flavonols. Grape pomace also contains low-extractable polyphenols (polymerized tannins and complexes of polyphenols with fiber).

The *conventional* methods of obtaining phenolic-rich extracts from grape pomace include diffusion controlled by solid–liquid extraction, supercritical fluid extraction, hot-compressed water or subcritical water extraction, and different other methods. However, commonly the conventional methods are time consuming and they require large quantities of relatively costly solvents (ethanol, methanol, acetone, etc.), introducing of chemicals and enzymes, and processing at high temperatures and pressures. Moreover, the existing conventional methods can cause large losses of nutrient resources and polyphenols recovery.

Application of *nonconventional* methods of phenolic extraction from *Vitis vinifera* waste (grape seeds and skins) such as ultrasound (UAE), microwaves (MAE), high pressure, and temperature extraction (HPTE) have been recently discussed (Casazza et al. 2010). The methanol and ethanol were used as the solvents. The both UAE and MAE techniques cause cell disruption that result in enhancing mass transfer processes. In general, UAE technique allows facilitation of the rate and efficiency of extraction of polyphenols with high antioxidant activity. MAE technique is also well suited for the extraction of high level phenolics without altering the antioxidant potential of the extracts. Comparison between the efficiency of classic solid–liquid extraction (SLE) technique and UAE, MAE, and HPTE techniques in term of extraction yield and antioxidant power of the extract was done. The highest content in total polyphenols, o-diphenols, and flavonoids, both for seeds and skins, was obtained with application of HPTE technique. From other hand, the highest antiradical power was determined in seeds extract from MAE. It was noted that prolonged extraction times (>30 min) result in increase in the content of total polyphenols and decrease in the amount of flavonoids and the antiradical power.

Grape seeds contain 15–20% high polyunsaturated fat content and are rich on vitamin *E* important for human health. They also have high protein content (Göktürk Baydar and Akkurt 2001). Oil is industrially extracted from grape seeds by pressing, and press cake can be used for animal feed as well for protein, carbohydrate, and lignin extraction (Fig. 2). Grape seeds are rich in polyphenols. The main polyphenols isolated from grape seeds are catechins (catechin, epicatechin, and epigallocatechin) and their polymers (Prieur et al. 1994).

Wine shoots resulting from the pruning activities conducted in the vineyards are often abandoned on the ground or burned and have environmental drawbacks. They can be used in biorefineries for thermochemical transformation producing biogas or pyrolytic liquid (Fig. 2). Polyphenols obtained by extraction of wine shoots have important antioxidant capacities and effective for medical applications. The valorization of wine shoots using polyphenol extraction permits converting them into added-value products.

The general state of the art related with different methods of the extraction of bioactive compounds and useful components from grape waste and by-products was recently presented (Barba et al. 2016).

Pulsed Electric Energy Applications to Grape Products and Waste

Among different nonconventional methods the pulsed electric energy (PEE)–assisted extraction gets a special attention (Mahnič-Kalamiza et al. 2014). There are many successful examples of PEE-assisted extractions from different types of biomass: fruits and vegetables, eggs, tea, mushrooms, yeast, microalgae, etc. Application of PEE allows enhancing the yields of juice, sucrose, inulin, proteins, phenolics, etc. Moreover, the PEE-assisted processing can be applied for high efficient biorefinery of agricultural and forestry residues, energy crops and municipal waste etc. Note that PEE-assisted processing solves 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 (nondenatured and biodegradable) and extracts co-products instead of waste. Note that agro-solvents (bioethanol, esters of fatty acids and derivatives of glycerol etc.) can be derived from agriculture (from farming/breeding, forestry, and aquaculture), and they have a high solvent power. In general, they are biodegradable and nontoxic.

The two different methods of application of PEE are based on treatment by pulsed electric fields (PEF) at relatively low voltages without development of discharges (hereinafter simply referred to as PEF treatment) and on treatment at relatively high voltages with development of high voltage electrical discharges (hereinafter simply referred to as HVED treatment). Impact of PEF on material includes mainly consequence of electroporation of cell membranes. HVED treatment also causes electroporation and can accompany with particle fragmentation and damage of cell walls, etc. Both PEF and HVED treatments result in accelerating the extraction of intracellular compounds.

The different variants of PEF and HVED treatment for improvement of juice or wine quality and biorefinery of grape waste have been tested in recent years.

Improvement of Juice and Wine Quality

Pressure extraction (expression) is widely used for production of juices and wines. The impact of PEF on expression behavior and juice quality has been studied using different varieties of white grapes (Muscadelle, Sauvignon, and Semillon (Praporscic et al. 2007)). PEF treatment in a laboratory vertical filter-press cell operated at electric field strength $E = 750$ V/cm, total time of PEF treatment $t_{PEF} = 0.3$ s, and constant pressure, $P = 5$ bar allows increasing the juice yield from 49% to 54% (untreated grapes) to 76–78% (PEF-treated grapes) at 45 min of pressing (Praporscic et al. 2007).

Figure 3 presents examples of juice yield expression kinetics for untreated and PEF-treated Muscadelle grape. The observed effects can be explained by presence of electroporation of cells that accelerates the expression kinetics. The effects are more pronounced for PEF pretreatment before pressing ($t = 0$ s) than for the intermediate PEF-treatment at $t = 1000$ s. It is interesting that PEF treatment results in noticeable

Fig. 3 Juice yield, *Y*, versus expression time, *t*, in the pressing experiments without PEF treatment, with PEF pretreatment ($t = 0$ s), and intermediate PEF treatment ($t = 900$ s) for Muscadelle grape (Compiled from Praporscic et al. (2007))

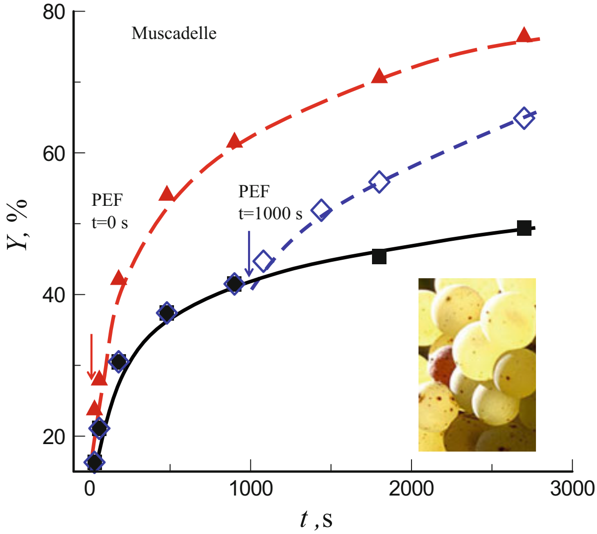
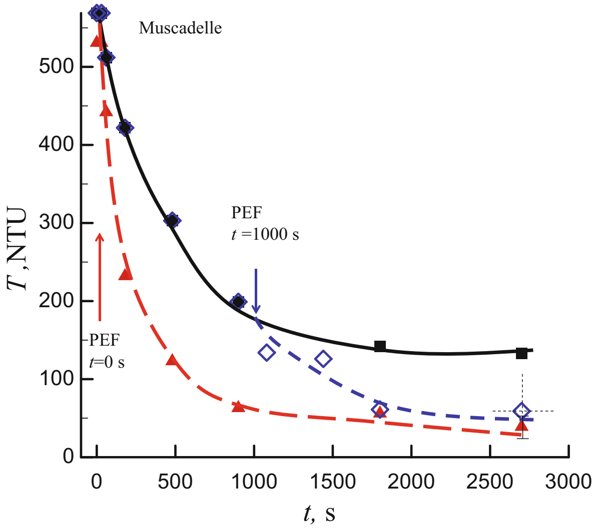


Fig. 4 Juice turbidity, *T*, versus expression time *t* in the pressing experiments without PEF treatment, with PEF pretreatment ($t = 0$ s), and with intermediate PEF treatment ($t = 1000$ s) for Muscadelle grape (Compiled from Praporscic et al. (2007))



decreasing of juice turbidity *T* (Fig. 4), and the effects are more pronounced for PEF pretreatment before pressing ($t = 0$ s). This observation can be explained by diminishing of solid impurities after the PEF treatment as the result of the selective damages on membranes without changes in the structure of cell wall components. The energy input of order $W \approx 20$ kJ/kg at electric field strength $E = 750$ V/cm is shown to be optimal for effective PEF-assisted expression. The results evidence the advantages of PEF-assisted expression for production of higher quality juices (Praporscic et al. 2007).

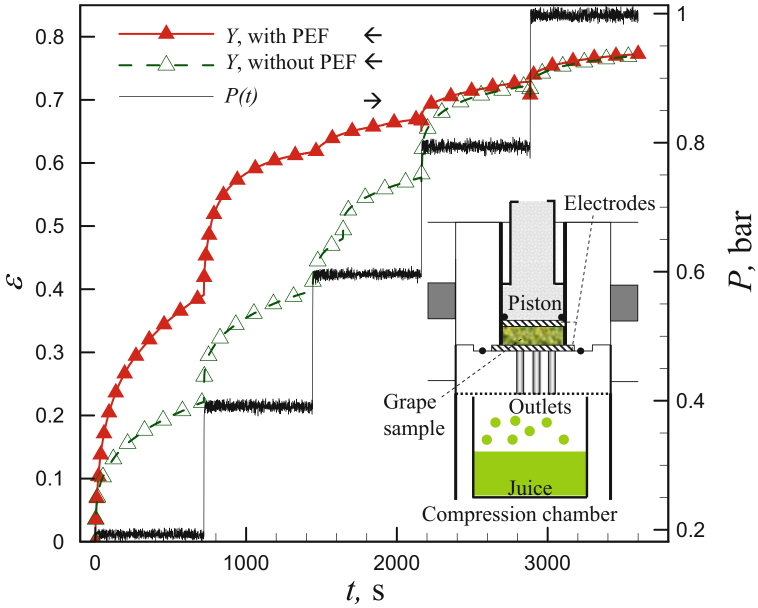


Fig. 5 Deformation of grape sample ε versus expression time for pressing at progressive pressure increase from 0 to 1 bar with and without PEF. The time evolution of pressure P is also shown. *Inset* shows PEF-treatment compression chamber. PEF pretreatment was performed before pressing ($t = 0$ s) (Compiled from Grimi et al. (2009))

The value of pressure and rate of its increase are important factors determining the quality of juice. Rapid rate of pressure increase results in high level of suspended solids, which can induce undesirable enzyme catalyzed oxidation and hydrogen sulfide production. Commonly, winemakers apply gentle pressing with pressure maximum of 1.25–2 bar and pressing duration of about 1.5–2 h. Two regimes of PEF-assisted juice expression from Chardonnay white grapes were compared at: constant pressure (0.5 or 1 bar) and progressive pressure increase (from 0 to 1 bar during 3000 s) (Grimi et al. 2009). The last one was chosen for the scale-down modeling of industrial grape pressing process. The experiments were carried out using a texture analyzer equipped with a PEF-treatment compression chamber operated at moderate electric field strength $E = 400$ V/cm and time of PEF treatment $t_{PEF} \approx 0.1$ s (Fig. 5). Such protocol allows reaching of a high level of cell disintegration, $Z \approx 0.8$, with energy consumption $W \approx 15$ kJ/kg.

The impact of PEF was evaluated by measuring of sample deformation, ε (Fig. 5), juice yield, Y, turbidity, T, and content of total polyphenols, C.

The final juice yield, Y, and turbidity, T, after 1 h of pressing for the constant pressure ($P = 1$ bar) and progressive pressure increase (0–1 bar) for untreated and PEF-treated (shaded) grapes are presented in Fig. 6.

For both pressing regimes PEF treatment results in juice yield increase from ≈67% to ≈75%. The turbidity of juice, T, was noticeably smaller for the regime of

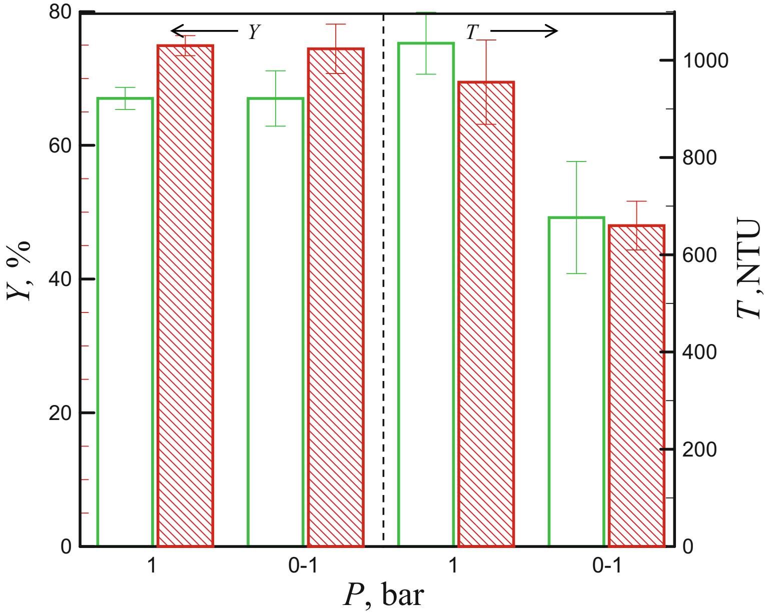


Fig. 6 Final juice yield, *Y*, (*left axis*) and turbidity, *T*, (*right axis*) after 1 h of pressing for the constant pressure ($P = 1$ bar) and progressive pressure increase (0–1 bar) for untreated (*unfilled bars*) and PEF-treated (*shaded bars*) grapes (Compiled from Grimi et al. (2009))

progressive pressure increase (0–1 bar). However, the turbidity of juice was approximately the same (within the limit of errors) for PEF treated and untreated samples for all the pressing conditions, i.e., impact of PEF on the value of turbidity was insignificant.

At constant pressure regime ($P = 1$ bar) PEF treatment did not significantly affect the content of polyphenols ($C \approx 13$ µmol GAE/g DM). For the regime of progressive pressure increase ($P = 0$–1 bar) the content of polyphenols was $C \approx 17$ µmol GAE/g DM and $C \approx 26$ µmol GAE/g DM for untreated and PEF treated grapes, respectively. Thereby, this pressure regime impact of PEF on the content of polyphenols was insignificant. Possibly this phenomena reflects impact of pressure regime on the filtration of juice through the filter cake, the cake-filtering capacity, and retention capacity of polyphenols by cake (Grimi et al. 2009). Note that high level of polyphenols is not desirable in white wine production (it is important for red wine production). However, the high level of polyphenols may be interesting for production of juices and beverages with antibacterial, antiviral, antioxidant, anti-inflammatory, and anti-carcinogenic properties.

For production of red wines the fermentation maceration of the must together with grape skins is used. At this stage the production of ethanol by yeasts and extraction of phenolic compounds from grape skin run simultaneously. Phenolics are important wine components defining organoleptic attributes like color, astringency, and bitterness. Effects of PEF treatment on the improvement of wine quality produced from red grapes (Tempranillo, Garnacha, Mazuelo, Graciano, Cabernet Sauvignon, Syrah, and Merlot) have been widely investigated (see (Luengo et al. 2015; Puértolas et al. 2010) for review). The evolution of color intensity, anthocyanin content, and total polyphenolic index during vinification were tested, and it was demonstrated that these parameters increased in the final wine when the electric field strength is raised from 2 to 10 kV/cm. PEF treatment (5 kV/cm) of the destemmed, crushed, and slightly compressed Cabernet Sauvignon grape pomace (skins, pulp, and seeds) before vinification led to freshly fermented wines that were richer in color intensity, anthocyanins, and tannins and showed better visual characteristics. Moreover, PEF allowed decreasing the maceration time from 268 to 72 h and was shown to be more effective in terms of color intensity and phenolic content than the enzymatic ones. The effects of PEF treatment (5 kV/cm) and macerating enzymes (Lallzyme EX and Lallzyme OE) on phenolic content and color of Cabernet Sauvignon red wine have been compared (Luengo et al. 2015; Puértolas et al. 2010). Both PEF and enzymatic treatments were effective for enhancing polyphenols extraction. However, compared with enzymatic pretreatment, PEF technology was more effective for extraction of phenolic compounds. The combined PEF and enzymatic interactions can be even more powerful technique for extraction of phenolic compounds. It is important accounting for the relatively high cost of the enzymes applied in wine production (Yang et al. 2016).

PEF protocol can affect the properties of the red wines (Delsart et al. 2014). PEF treatment of cabernet sauvignon grape berries was performed prior to vinification at $E = 0.7$ kV/cm, $t_{PEF} = 200$ ms, $W = 31$ Wh/kg (PEF_1) and at $E = 4$ kV/cm, $t_{PEF} = 1$ ms, $W = 4$ Wh/kg (PEF_2). PEF_1 treatment results in changes in the structure of the skins and in high concentration of tannins (34%). PEF_2 treatment results in alternation of the visual appearance of phenolic compounds in the skins and in better extraction of the anthocyanins (19%). Moreover, PEF1 treatment has more impact on the parietal tannins and the cell walls of the skins while PEF_2 modifies more the vacuolar tannins.

Impacts of three techniques, (PEF (E = 5 kV/cm, t_{PEF} = 1 ms), enzymatic treatment (macerating enzyme Lafase HE), and thermovinification (70 °C for 30 min)), on the characteristics (phenolic compounds, color, copigmentation, nondiscolored pigments) of red wine from Cabernet Sauvignon grapes have been compared (Table 4, El Darra et al. 2016). The intensity and composition of the freshly fermented wine color were related to the amount of native polyphenols and other phenomena such as copigmentation.

Phenolic extraction during fermentation of the red grapes was tested with continuous PEF system at the pilot-plant scale. The obtained data have evidenced attractiveness of this PEF-assisted technology at the commercial scale (Puértolas et al. 2010). PEF-assisting winemaking allowed bottling of wines with better characteristics (Folin-Ciocalteu index, color intensity, concentrations of polyphenols). It was also reported that PEF application increased the antioxidant activity of the extracts from grape by-products making it twofold higher than in the control extraction.

Table 4 The increase in color, content of flavonols, and total phenolic of red wine from Cabernet Sauvignon grapes after application of PEF, enzymatic, and thermovinification treatment (Compiled from the data presented in El Darra et al. (2016))

Technique	Color	Content of flavonols	Concentration of total phenolic
Pulsed electric fields	56%	48%	18%
Enzymatic treatment	62%	97%	32%
Thermovinification	22%	4%	3%

It is interesting that pulsed electric energy can have positive effect on elimination residual fungicides and microbial stabilization in wines. The significant reduction of four residual fungicides (pyrimethanil, vinclozolin, cyprodinil, and procymidone) and in concentrations of pesticides after PEF treatment at $E = 5–20$ kV/cm, $t_{PEF} = 0.5–2$ ms, $W = 10–160$ kJ/L in dry white wine has been observed (Delsart et al. 2016a). Application of PEF treatment ($E = 20$ kV/cm) and HVED treatment ($E = 40$ kV/cm) allows the inactivation of alteration yeasts (*B. bruxellensis* CB28) and bacteria (*O. oeni* CRBO 9304, *O. oen*i CRBO 0608, and *P. parvulus* CRBO 2.6) (Delsart et al. 2016b). PEF treatment has no negative impact on the composition of wines compared to the HVED treatment. Contrary to PEF treatment, the phenolics compounds are degraded the physicochemical composition of wine are modified after the HVED treatment.

Biorefinery of Grape Waste

During the last decade application of pulsed electric energy (PEF and HVED) became rather popular in relation to valorization of winery waste and by-products (Barba et al. 2016).

The most efforts were devoted to extraction of high-added substances from grape *skins*. The effects of PEE (PEF and HVED)-assisted recovery of total soluble matter and polyphenols from Chardonnay white grape skins in aqueous medium at different temperatures within 20–60 °C have been investigated (Boussetta et al. 2009). These experiments showed that PEF and especially HVED enhance the extraction kinetics (Fig. 7). The highest yield of polyphenols (≈21.4 μmol of gallic acid equivalent/g of dry matter) was reached after ≈60 min of extraction for HVED treatment and after ≈180 min of extraction for PEF treatment. This level exceeded the value ≈19.1 μmol of gallic acid equivalent/g of dry matter for the untreated samples. The choice of effective HVED treatment time (number of electrical discharges) should be accurately evaluated, as excessively prolonged treatment may deteriorate phenolic compounds (Boussetta et al. 2011). With increasing the energy input up to 80 kJ/kg, both polyphenols content and antioxidant activity were increased. However, above this energy threshold, a negative effect of HVED is observed corresponding to the decrease of polyphenols content, probably due to their degradation (Boussetta et al. 2011).

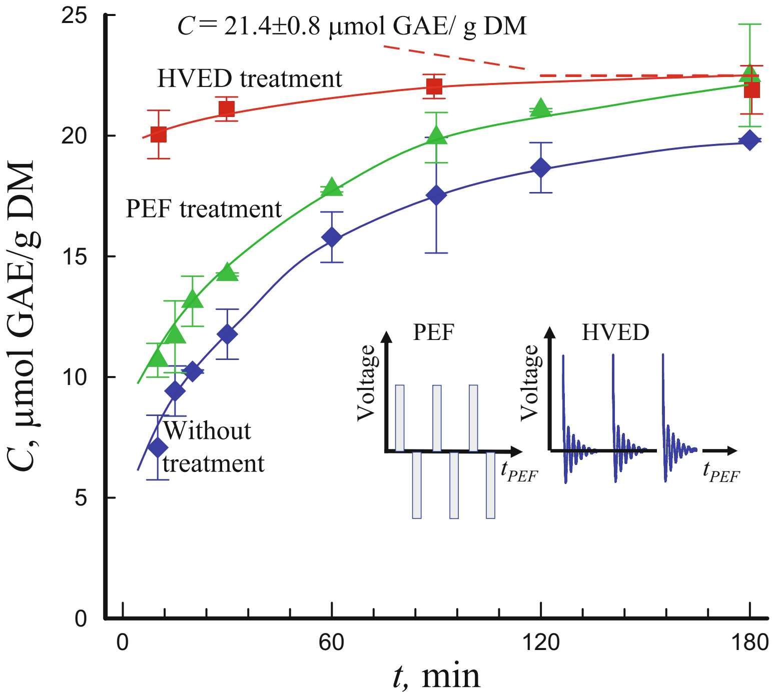


Fig. 7 Kinetics of changes in content of total polyphenols C for aqueous extraction from untreated and PEF-treated (1300 V/cm) and HVED treated (≈40 kV/cm) grape skins at T = 20 °C (Compiled from Boussetta et al. (2009))

HVED treatment (voltage from 30 to 60 kV, needles-to-plane electrode treatment chamber with a distance between electrodes of 1.0 cm) has been applied to suspension of grape skins in distilled water (Takaki et al. 2011). The six-stage Blumlein-line was used to generate 140 ns width pulse voltage. The cell morphological changes after HVED treatment were observed by using a microscopy. The results showed that many anthocyanoplasts in cells were collapsed. The increased yields of the total polyphenols were 16.7% at 30 kV and 84.2% at 60 kV. The obtained results were explained by development of electroporation process.

The extraction of polyphenols from grape *seeds* assisted by PEF (8–20 kV/cm, 0–20 ms), high voltage electrical discharges (HVED) (10 kA/40 kV, 1 ms), and grinding (180 W, 40 s) has been studied (Boussetta et al. 2012). The diffusion was performed after pretreatment with a mixture of water and ethanol. These two pretreatments increased the extraction kinetics, and the final polyphenols content was 9 g GAE/100 g DM after 15 min of extraction with grinding and after 60 min for PEF- or HVED-assisted extraction in ethanol. The subsequent solid–liquid separation was faster with PEF as compared to grinding and HVED treatments. The impact of electrical discharges of low energy on the extraction of polyphenols from grape seeds has been also studied (Boussetta et al. 2013). Three basic phenomena were involved in the discharge process: pulsed electric field (PEF), prebreakdown phase (streamer), and breakdown phase (arc). The polyphenol extraction was much more efficient with arcs, compared to streamers and PEF. The energy consumption required to extract 5000 mg GAE/100 g DM with the arc discharges was 16 kJ/kg, which is 27 times lower compared to streamers alone and 47 times lower compared to PEF. The mechanical effects of arcs (shock waves, expanding cavity, and strong turbulence) were responsible for the fragmentation of grape seeds and strongly promoted the release of polyphenols. The polyphenol extraction was further enhanced when grape seeds were in close proximity with the breakdown arc.

Application of pulsed electric energy is also very promising for valorization of wine *shoots*. The effects of PEF (13 kV/cm), HVED (40 kV), and ultrasonic (US, 400 W) treatments of wine shoots on aqueous extraction (pH 13) of polyphenols and proteins have been studied (Rajha et al. 2013). The yields of extracted components were significantly increased for HVED, PEF, and US-assisted processing as compared to the standard solid–liquid diffusion. The significant extraction required some energetic thresholds: 25.4 kJ/kg (HVED), 51 kJ/kg (PEF), and 3428 kJ/kg (US). For polyphenols the following yields were obtained: 35 mg GAE/g DM (HVED), 22 mg GAE/g DM (PEF), and 16 mg GAE/g DM (US). The effects of HVED were also highest in terms of tissue damage. Efficiency of PEE (PEF and HVED)-assisted extraction of polyphenols from viticulture and winemaking by-products (grape pomace and wine shoots) has been compared (Rajha et al. 2015). Grape pomace treatments have shown that particle size reduction (grinding) accelerates extraction. The extraction was more efficient in 70% ethanol/water solvent under high temperature (140 °C) and pressure. PEE treatment of wine shoots prior to the alkaline extraction (0.1 M NaOH) resulted in enhancement of polyphenols diffusion. The most efficient was HVED-assisted extraction that allowed enhancing the extraction yield by 2.3 times. The detailed study of mechanisms of HVED-assisted extraction of polyphenols from wine shoots has been also presented (Rajha et al. 2015). The effect of (1) the fragmentation process and (2) the initial particle size on the efficiency of HVED in terms of polyphenols extraction from wine shoots was studied. The increasing of HVED energy inputs ($W = 100$–600 kJ/kg) provoked wine shoot fragmentation and particle size reduction. For HVED treatment with $W = 300$ kJ/kg the small fragmentation was observed, whereas the polyphenol concentration significantly increased up to 110 mg/L. It was concluded that the efficiency of the HVED treatment is related with mechanical and nonmechanical action mechanisms.

The earlier studies were focused on the HVED-assisted extraction of total phenolic compounds. Recently, results on HVED-assisted extraction of flavan-3-ols, flavonols, and stilbenes grape *stems* have been reported (Brianceau et al. 2015). Main extraction parameters affecting the extractability of these compounds such as treatment time, pH, and ethanol concentration were optimized through response surface methodology. Optimized extraction parameters (pH =2.5; treatment time = 4.0 ms; ethanol addition = 50%) were compared to a conventional hydro-alcoholic extraction. HVED improved significantly the extraction of flavan-3-ols and flavonols but was less efficient on stilbenes. Optimized extraction conditions allowed the release of almost 35% of additional phenolic compounds and led to a better extractability of flavan-3-ols (+21%) and of flavonols (+12%), compared to the conventional hydro-alcoholic extraction.

A new process has been recently investigated for the valorization of fermented grape *pomace* (Brianceau et al. 2016). This process combines preliminary densification of pomace to decrease electrical conductivity of mixture and following PEF treatment. Different pressures in the range of 0–10 bars were applied to the grape pomace in order to study the effect of the density, ranging from 0.6 to 1.3 $g \cdot cm^{-3}$, on PEF efficiency. Hydro-alcoholic extraction was then used at different temperatures for polyphenols recovery. Optimal process parameters (pomace density $\rho = 1.0\ g \cdot cm^{-3}$, field strength $E = 1.2\ kV \cdot cm^{-1}$, energy input $W = 18\ kJ \cdot kg^{-1}$) permitted to attain highest polyphenols content in the extract (increase of 15%). Densification allowed the air evacuation and better electrical contact between pomace particles, thus increasing electrical conductivity of pomace. When PEF was applied to a more compacted grape pomace (ρ =1.3 $g \cdot cm^{-3}$), the effect of the electric treatment on the recovery of polyphenols was less pronounced (increase of 7.5%). The ratio of total anthocyanins to total flavan-3-ols at 20 °C was equal to 7.1 and 9.0 for control and PEF treated samples, respectively. These results demonstrate the selectivity of PEF treatment for anthocyanin extraction and thus present new possibilities to produce extracts with different biochemical compositions (Brianceau et al. 2016). Moreover, this study has demonstrated that a relatively dry pomace (relative humidity $\approx$ 55%) can be effectively treated by PEF after previous densification. The PEF treatment of densified fermented grape pomace required lower output current and lower specific energy input than PEF treatment applied without pomace densification.

Conclusions

Pulsed electric energy (PEE) has found application in many branches of food, agricultural, biotechnological, and medical sciences. Modern developments in the field provide unique possibilities of gentle regulation of membrane permeability via phenomenon of electroporation without essential impact on the other constituents of biological systems. The two different methods based on application of pulsed electric fields (PEF) at relatively low voltages and high voltage electrical discharges (HVED) can be effectively integrated in different processing related with raw bioproducts. In many cases, PEE-assisted processing allows preservation of flavor, color, vitamins, and important nutrients of the products. Recent laboratory efforts from different groups have already demonstrated promising examples of the PEF-assisted processing for improvement of juice or wine quality and biorefinery of grape waste. Such processing gives unique possibilities for extraction of valuable proteins, polyphenolic substances, enzymes, and polysaccharides. From other hand, HVED-assisted processing allows increasing the yield of valuable components, and it is very promising for extraction of polyphenols from the grape pomace. It can be expected that with the growing availability of high efficient and low-cost PEE systems electroporation-assisted processing became more and more popular in different grape product biorefinery applications.

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Cross-References

- ▶ Application of Pulsed Electric Energy for Lignocellulosic Biorefinery
- ▶ Application of Pulsed Electric Fields for Root and Tuber Crops Biorefinery
- ▶ Application of Pulsed Electric Field Treatment for Food Waste Recovery Operations
- ▶ Impact of Pulsed Electric Field Treatment on Must and Wine Quality
- ▶ Polyphenol and Protein Extraction from Rapeseed Stems and Leaves Assisted by Pulsed Electric Fields
- ▶ Pulsed Electric Fields and High-Voltage Electrical Discharge-Assisted Extraction of Biocompounds from Vine Shoots
- ▶ Pulsed Electric Fields-Assisted Extraction from Exotic Fruit Residues
- ▶ Pulsed Electric Fields in Wineries: Potential Applications
- ▶ Selective Extraction of Molecules from Biomaterials by Pulsed Electric Field Treatment

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