# Cell Membrane Permeabilization by Pulsed Electric Fields for Efficient Extraction of Intercellular Components from Foods


**E. Vorobiev and N. I. Lebovka**

## Abbreviations

| | |
|---|---|
| AA | antioxidant activity |
| AC | antioxidant capacity |
| AFM | atomic force microscopy |
| CI | color intensity |
| DM | dry matter |
| EE | ethanol extraction |
| FM | fresh material |
| GAE | gallic acid equivalent |
| HVED | high voltage electrical discharges |
| ICUMSA | International Commission for Uniform Methods of Sugar Analysis |
| MW | microwave |
| OD | osmotic dehydration |
| OH | ohmic heating |
| PEF | pulsed electric fields |
| SAE | supplementary aqueous extraction |
| SEM | scanning electron microscopy |
| SG | solid gains |
| TAC | total anthocyanin content |
| TEM | transmission electron microscopy |
| TPC | total phenolic compounds/content |
| TPI | total polyphenol index |

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J. Raso et al. (eds.), *Pulsed Electric Fields Technology for the Food Industry*, Food Engineering Series, https://doi.org/10.1007/978-3-030-70586-2_6

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|---|---|
| US | ultrasound |
| WE | aqueous extraction |
| WL | water losses |

## Symbols

| | |
|---|---|
| °Brix | Brix value |
| $A$ | area of cell |
| $A_P$ | area of a single pore |
| $a$ | modulus of elasticity |
| $b$ | consolidation coefficient |
| $C$ | solute concentration in the solvent |
| $C_m$ | specific capacitance of membrane |
| $D$ | diffusivity |
| $d$ | Diameter of a sample |
| $d_m$ | thickness of membrane |
| $E$ | electric field strength |
| $Fi$ | Fick number |
| $f_e$ | electroporation factor |
| $f_i$ | relative fraction |
| $f_p$ | density of pores in a membrane |
| $G$ | compressibility modulus |
| $h$ | height or thickness of a sample |
| $k$ | electrical conductivity contrast, $k = \sigma_d/\sigma_u$ |
| $k_e$ | hydraulic permeability of the extracellular space |
| $k_i$ | hydraulic permeability of the intracellular space |
| $k_p$ | hydraulic permeability of the pore |
| $k_BT$ | thermal energy |
| $m$ | mass |
| $n$ | number of pulses |
| $N_p$ | number of pores per cell |
| $P$ | pressure |
| $P_{max}$ | fracture pressure |
| $R$ | radius of a cell |
| $r_c$ | critical radius of a pore in a membrane |
| $r_p$ | radius of a pore in a membrane |
| $S$ | firmness (stiffness) coefficient |
| $S_c$ | specific surface of the cell |
| $T$ | temperature |
| $T$ | pressing time |
| $t_c$ | time of a cell charging |
| $t_{c,m}$ | time of a membrane charging |
| $t_p$ | pulse duration |
| $t_{PEF}$ | total time of PEF treatment, $t_{\mathrm{PEF}} = nt_{\mathrm{p}}$ |

| | |
|---|---|
| $U$ | voltage |
| $u_m$ | transmembrane potential (voltage) |
| $W$ | specific energy consumption |
| $W_a$ | activation energy |
| $W_c$ | maximum energy of pore formation in membrane |
| $Y$ | diffusion (juice) yield |
| $Y_c$ | consolidation ratio |
| $Z_e$ | fraction of electroporated cell |
| $Z$ | disintegration index |
| $Z_c$ | electrical conductivity disintegration index |
| $Z_d$ | diffusivity disintegration index |
| $\alpha$ | transmembrane flow coefficient |
| $\alpha_r$ | aspect ratio |
| $\Delta t$ | distance between pulses ( |
| $\varepsilon$ | relative deformation |
| $\gamma$ | surface tension |
| $\eta$ | viscosity |
| $\theta$ | angle |
| $\rho$ | density |
| $\sigma$ | electrical conductivity |
| $\tau$ | characteristic time |
| $\tau_{\mathrm{B}}$ | consolidation time |
| $\tau_{\mathrm{d}}$ | diffusion time |
| $\tau_{\mathrm{r}}$ | retardation time |
| $\omega$ | line tension |

## Introduction

During the last few decades, there has been a growing interest in application of pulsed electric fields (PEF) for processing of food and agricultural materials. The recent efforts have been aimed at applying PEF for extraction of bioactive molecules, osmotic dehydration, expressing of juices, drying, freezing, frying, cooking of foods, fermentation, inactivation of microorganisms, etc. (Vorobiev and Lebovka 2020).

The early interest on effects of electric field on plant growth, seed germination, and living organisms arose in the middle of the eighteenth century, starting from the works of Abbot Nollet (Heilbron 1979). The bactericidal and sterilization effects of electricity in food products were revealed at the end of the nineteenth century (Cohn and Mendelsohn 1879). The "electropure process" for heating the milk was accepted as a safe milk pasteurization technology in Europe and the USA in the 1930s (Moses 1938). In the middle of the twentieth century, the DC and AC electrical treatments were applied for assistance of sugar beet processing (Zagorul'ko 1958), juice extraction from fruits and vegetables (grapes, apples, carrots) (Flaumenbaum 1949),

and processing of vegetable raw materials, meat, and fish (Kogan 1968; Matov and Reshetko 1968; Lazarenko et al. 1977; Rogov 1988). The advantages of DC and AC techniques were the short time of treatment and their relatively easy implementation to assist different processes, like diffusion, pressing, drying, etc. However, the applications of DC and AC electrical treatments were limited by significant elevation of temperature (due to the ohmic heating), thermal degradation of food and biomaterials, significant electrode erosion, and high energy costs. Thus DC and AC methods have found only limited usages.

In this line, the PEF treatment presents a good nonthermal alternative for processing of food and agricultural products without significant elevation temperature and food quality deterioration and with moderate power consumption. Commonly, the PEF causes selective damage of cell membranes in cells without significant effects on cell walls. In plant tissues, an important damage of cell membranes can be observed at the electric field strengths of $E = 100–1000$ V/cm, using pulses with duration between 1 and $10^3$μs and total PEF treatment time within $t_{PEF} = 10^{-4}–10^{-1}$ s (Vorobiev and Lebovka 2008). For microbial killing larger fields ($E = 20–50$ kV/cm) and smaller treatment times ($t_{PEF} = 10^{-5}–10^{-4}$ s) are required (Barbosa-Cánovas et al. 1998). In pioneering works, the application of PEF treatment (with electric field strength up to $E = 20$ kV/cm and exponential pulses with duration of $t_p = 20$μs) was shown to be effective for the selective damage of membranes of sugar beet tissue without significant heating of surrounding media (Zagorul'ko 1958). This phenomenon was called electroplasmolysis. The PEF treatment was also used for inactivation of *Salmonella* in egg powder suspensions and processing of fish products by Doevenspeck in the early 1960s (Doevenspeck 1961) (for more details see the reviews (Sitzmann et al. 2016a, b)). More active research efforts on PEF treatments of food and agricultural materials started about two decades ago (Gulyi et al. 1994; Knorr et al. 1994a, b; Barbosa-Cánovas et al. 1998), and they are basically grounded on the electroporation concept (Pakhomov et al. 2010; Akiyama and Heller 2017; Miklavčič 2017).

This chapter presents the short review on PEF applications for the extraction of intercellular components from biological tissues. The concept of electroporation, main mechanisms of PEF actions, protocols of PEF treatments, techniques to detect PEF-induced changes, recent applications of PEF to assist solid/liquid expression, and solute extraction from different food and biomaterials are presented.

## Electroporation, Its Detection, and PEF Protocols

### *Electroporation of Membranes*

The academic interest on mechanism of PEF actions on biological cell has started in the middle of the twentieth century. The experimentally observed efficiency of high-frequency electric currents for inactivation of bacteria and viruses (Nyrop

1946) was explained by the effects of local overheating (Ingram and Page 1953). The effects of PEF-induced damage of cell membrane (reversible and irreversible) were experimentally observed (Stämpfli and Willi 1957; Stämpfli 1958), and these effects were explained by losses of the membrane's function as semipermeable barriers between the bacterial cell and its environment (Hamilton and Sale 1967; Sale and Hamilton 1967, 1968). The further works performed in the period between 1960 and 1990 revealed detailed mechanisms of PEF action on breakdown of cell membranes (Neumann and Rosenheck 1972; Zimmermann et al. 1974; Riemann et al. 1975; Zimmermann et al. 1976a, b, c, 1980) and formation of pores in membranes (Kinosita Jr. and Tsong 1977a, b; Kinosita and Tsong 1977). The knowledge on the sublethal and lethal effects of PEF in function of pulse intensity, duration, frequency, temperature, and other parameters has been collected in a series of papers (Hülsheger and Niemann 1980; Hülsheger et al. 1981, 1983).

Based on these early works, the electroporation concept was theoretically grounded (Weaver and Chizmadzhev 1996; Miklavčič 2017). Electric field selectively modifies structure of cell membranes. The lipid membranes have significantly smaller electrical conductivity ($\sigma_m$) as compared to internal cytoplasmic ($\sigma_i$) and external extracellular media ($\sigma_e$). Therefore, electric field is predominantly concentrated on the biological membrane, which leads to its alteration and damage.

The transmembrane potential required for the breakdown of biological membrane was experimentally estimated to be in the order of $u_m \approx 1.0$ V (Weaver and Chizmadzhev 1996). Electric field strength applied for the breakdown of membrane with thickness of $d_m \approx 5$ nm can be estimated as $E = u_m/d_m \approx 2 \times 10^{10}$ V/cm, which is a typical value required for the breakdown of insulating materials (Rumble 2019).

For the description of cell membrane breakdown, different physical models have been proposed. These models are based on the considerations of electrically induced thermal, osmotic, mechanical, hydrodynamic, and viscous-elastic instabilities (Chen et al. 2006). Historically, the first speculation on selective breaking of cell membranes was based on electroosmotic effect during the PEF application (Zagorul'ko 1958). Later on it was speculated that the fast molecular exchange induced by electroosmosis could cause chemical imbalances and provoke cell lysis (Dimitrov and Sowers 1990). The ohmic heating could cause selective heating of the membrane as the most resistive part of the cell. However, simulations show that heat diffusion effects effectively prevent the Joule overheating of cell membrane owing to its small thickness ($d_m \approx 5$ nm) (Lebovka et al. 2000).

Electroporation model, based on the formation of electropores inside cell membrane, is actually popular to explain PEF effects (Fig. 1). In this model cell membrane is treated as a condenser (a homogeneous slab of lipids) with a specific capacitance, which can be calculated as $C_m = \varepsilon_m \varepsilon_0 / d_m$ ($\approx 3.5 \times 10^{-3}$ F/m$^2$), where $\varepsilon_m$ ($\approx 2$) is the dielectric constant of a lipid membrane and $\varepsilon_0 = 8.85 \times 10^{-12}$ F/m is the permittivity o free space. The external electric field stimulates creation and growth of pores in a membrane. The energy of aqueous pore formation represents parabolic function of $r$ for the balance between line tension $(2\pi r\omega)$ and surface tension $(\pi r^2 \gamma)$ contributions (Weaver and Chizmadzhev 1996):

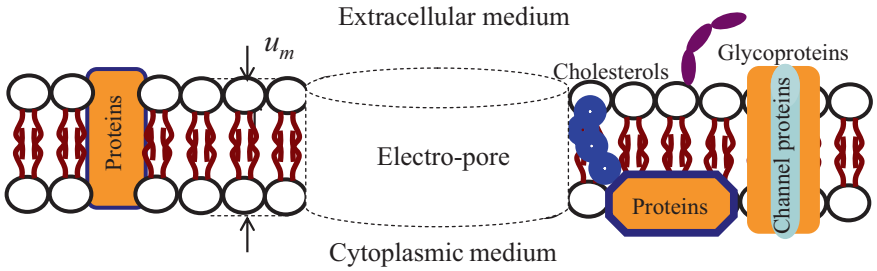


**Fig. 1** Pore induced by external electric field in cell membrane. The real membranes contain a variety of molecules like proteins, glycoproteins, glycolipids, sphingolipids, cholesterol, etc. (Tien and Ottova 2003)

$$W = 2\pi r\omega - \pi r^2\gamma \tag{1}$$

Thus the formation of pores is supported by the surface tension forces and is suppressed by the line tension forces. Inside the pore the lipid part of membrane with low dielectric constant $\varepsilon_m$ (≈2) is replaced by water or ionic solution with high dielectric constant $\varepsilon_w$ (≈80).

The induced transmembrane voltage ($u_m$) increases the effective surface tension and promotes the opening of pores

$$\gamma^* = \gamma\left(1+\left(u_m / u_o\right)^2\right), \tag{2}$$

where is the voltage parameter ($u_o \approx 0.17$ V for the typical values $\gamma \approx 2 \times 10^{-3}$ N/m and $\omega \approx 2 \times 10^{-11}$ N for lipid membranes (Winterhalter and Helfrich 1987).

The critical radius of the pore, $r_c$, and maximum energy of pore formation $W(r_c)$ can be estimated from the condition $\partial W/\partial r = 0$. It gives for the critical radius of the pore $r_c = \omega/\gamma^*$ and for the activation energy $W_c(r_c) = \pi\omega^2/\gamma^* = \pi r_c\omega$. The equilibrium density of pores induced in cell membrane (or pore surface fraction) can be estimated as

$$f_p \approx \exp\left(-W_c / k_BT\right). \tag{3}$$

In absence of the external electric field ($u_m = 0$), the estimations give the values $r_c \approx 10$ nm, where $W_c(r_c) \approx 109kT$ ($k_BT \approx 4.11 \times 10^{-21}$ J at $T = 298$ K) is the thermal energy and $f_p \approx 4.6 \times 10^{-48}$. Therefore formation of pores is a highly improbable event.

However, in presence of the external electric field, the value of $r_c$ decreases and $W_c$ increases with increasing of $u_m$. For example, for the transmembrane potential of $u_m = 1$ V we get

$$r_c = \omega / \gamma* = \left(\omega / \gamma\right) / \left(1+\left(u_m / u_o\right)^2\right) \approx 0.28\,\text{nm}, \tag{4a}$$

$$W_c\left(r_c\right) = \pi r_c \omega \approx 4.28kT, \tag{4b}$$

and $f_p \approx 0.014$; therefore the pore formation can be initiated by thermal fluctuations.

The transient aqueous pore model gives the following relation for estimation of the lifetime $\tau$ of a single membrane (Weaver and Chizmadzhev 1996).

$$\tau\left(u_m\right) = \tau_\infty \exp\left(W_c / kT\right) = \tau_\infty \exp\left(\frac{\pi\omega^2 / \left(kT\gamma\right)}{1 + \left(u_m / u_o\right)^2}\right), \tag{5}$$

where experimental estimation gives $\tau_\infty \approx 3.7\times10^{-7}$ s (at $T$ = 298 K) for the lipid membrane (Lebedeva 1987).

The electropore model presented in Fig. 1 corresponds to the formation of simple pore with hydrophilic internal coating. The electroporation effects for pores with complicated structures were also discussed (Neu and Krassowska 1999). In general, within the temperature range of 20–55 °C, the complex phase behavior and melting transitions were observed for the lipid bilayers (Heimburg 1998; Holl 2008). It can affect the changes in transmembrane potential with temperature elevation (Zimmermann 1986). In general, electroporation evolves changes in the size and number of pores induced by PEF, interactions between pores, aggregation of pores, and transport processes of biomolecules (dyes, vitamins, disaccharides, anticancer drugs, peptides, and genes) through the membrane (Rols 2006). Moreover, electroporation behavior can be influenced by complex composition of real membranes containing a variety of molecules like proteins, glycoproteins, glycolipids, sphingolipids, cholesterol, etc. (Tien and Ottova 2003) (Fig. 1).

In dependence of PEF treatment intensity, sublethal, intermediate, and overlethal electroporation effects can occur. At moderate PEF treatment, the sublethal injury with recovery or resealing effects can be observed (Saulis 1997). Typically, the duration of resealing is of order of 1–100μs, and it significantly depends on the temperature and composition of lipid membrane (Sugar et al. 1987). However, in some cases very prolonged resealing duration (seconds, minutes, and even hours) was also observed (Rols and Teissié 1990; Pakhomov et al. 2009) that can be explained by the lipid peroxidation (oxidative degradation of lipids) mechanism (Rems et al. 2019).

During the last decades, the formation of electropores was intensively studied by computer simulations (Rems and Miklavčič 2016). Particularly, at high electric fields, formation of the bilayer patches containing hydroperoxide lipid derivatives was demonstrated (Rems et al. 2019). Detailed reviews on this subject can be found in the literature (Apollonio et al. 2012; Delemotte and Tarek 2012; Casciola and Tarek 2016).

## *Electroporation of Single Cell and Ensembles of Cells and Tissues*

### Spherical Cell

The electroporation of single cell is complicated by the distribution of transmembrane potential $u_m$ over cell surface. For the idealized spherical cell (Fig. 2a), the distribution of transmembrane potential $u_m$ can be evaluated using Schwan's equation (Grosse and Schwan 1992):

$$u_m = 1.5 f_e E R \cos\theta \left[1 - \exp\left(-t / t_c\right)\right], \tag{6}$$

where $f_e$ is the electroporation factor, $\theta$ is the angle between the external electric field $E$ and the radius-vector on the membrane surface, $R$ is the radius of the cell, and $t_c$ is the time of a cell charging.

The highest drops of transmembrane potential occur at the cell poles, and it decreases up to a zero at $\theta = \pm\pi/2$. The factor $[1\text{-}\exp(-t/t_c)]$ accounts for the membrane charging, and $f_e(\leq 1)$ is an electroporation factor (Kotnik et al. 1997, 1998):

$$f_e = \frac{\sigma_e \left[3 d_m R^2 \sigma_i + \left(3 d_m^2 R - d_m^3\right)\left(\sigma_m - \sigma_i\right)\right]}{R^3 \left(\sigma_m + 2\sigma_e\right)\left(\sigma_m + 0.5\sigma_i\right) - \left(R - d_m\right)^3 \left(\sigma_e - \sigma_m\right)\left(\sigma_i - \sigma_m\right)}, \tag{7}$$

where $d_m$ is the thickness of the membrane and $\sigma_m$, $\sigma_e$, $\sigma_i$ are the electrical conductivities of the membrane, extracellular, and intracellular media, respectively.

The time of a cell charging can be evaluated using the equation

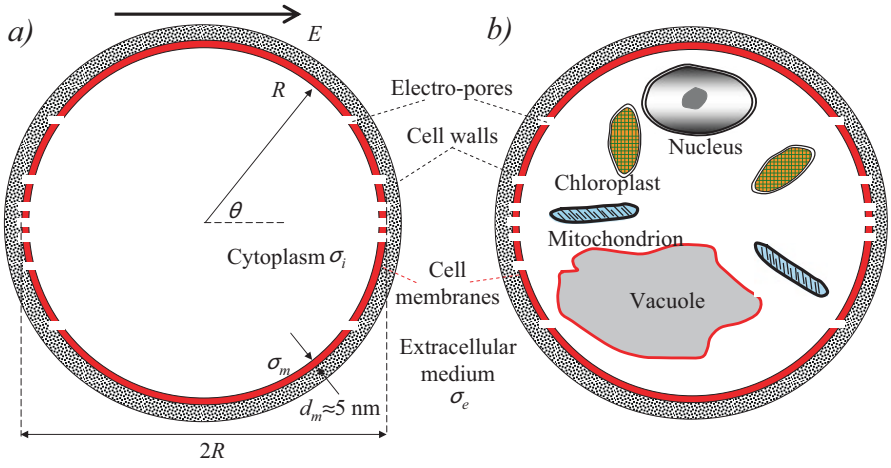


**Fig. 2** A biological cell in the external electric field. Schematic presentations of the idealized spherical cell covered by cell membrane (**a**) and a structure of the eukaryotic plant cell (**b**)

$$t_c = \frac{RC_m}{2\sigma_e\sigma_i/(2\sigma_e+\sigma_i)+\sigma_m R - d_m)} = \frac{t_{c,m}}{\frac{2\sigma_i d_m}{\sigma_m R(2+\sigma_i/\sigma_e)}+1}, \tag{8}$$

where $t_{c,m} = d_m C_m/\sigma_m$ is the time of a membrane charging.

In the limit of nonconducting membrane ($\sigma_m = 0$), we have $f_e = 1$. In general case, the value of $f_e$ depends significantly on the conductivity ratio $\sigma_e/\sigma_i$ and radius of the cell, $R$. For biological cells the typical values of parameters are $\sigma_m \approx 5 \times 10^{-7}$ S/m, $\sigma_i \approx 2 \times 10^{-1}$ S/m, $\varepsilon_m \approx 2$, $d_m \approx 5$ nm (Kotnik et al. 1997), $R \leq 10$μm for yeasts and microbial cells, $R = 10$–$30$μm for animal cells, and $R = 10$–$100$μm for plant cells. Using these values we can estimate $t_{c,m} \approx 35.4$μs, $C_m \approx 3.5 \times 10^{-3}$ F/m$^2$. In vivo conditions (i.e., at $\sigma_e/\sigma_i \approx 1$), $f_e \approx 1$ and $t_c << t_{c,m}$. For external medium with low electrical conductivity (i.e., at $\sigma_e/\sigma_i << 1$), the value of can be $f_e$ is significantly smaller than 1 and $t_c \approx t_{c,m}$.

The real biological cells have more complicated internal structure (e.g., see Fig. 2b). The effect of electroporation is dependent on the size of membrane envelope. Therefore, with moderate electric fields (<1–5 kV/cm), only electroporation of the external cell membrane is expected. The pronounced electroporation of the membranes of intracellular formations can be achieved at more strong electric fields (≈10–100 kV/cm) applied with nanosecond durations (Tekle et al. 2005; Buchmann and Mathys 2019).

### Cells with Different Shapes and Sizes

The real cells show a vast variety of shapes and sizes. For the elongated cell, $u_m$ has its maximum or minimum value when the longest axis of the cell is parallel or perpendicular to the electric field, respectively.

For the prolate spheroid oriented with its long axis along the external field, the stationary value of $u_m$ can be evaluated using the following equation (Kotnik and Miklavčič 2000):

$$u_m = ER\frac{e^3}{e-(1-e^2)\ln(a_r(1+e))}\frac{\cos\theta}{\sqrt{a_r^2\sin^2\theta+\cos^2\theta}}, e = \sqrt{1-a_r^{-2}}, \tag{9a}$$

where $\alpha_r$ (≥1) is an aspect ratio of spheroid (major/minor axis ratio).

In the limiting case of $\alpha_r = 1$ (i.e., for the sphere), this equation reduces to the $u_m = 1.5ER$ that is equivalent to Eq. (6). In the limiting case of large aspect ratio ($a \geq 10$), it reduces to

$$u_m(\theta = 0)/ER = 1-(1-\ln 2+\ln a_r)/a_r^2. \tag{9b}$$

For the arbitrary-oriented spheroidal cells, the values of $u_m$ can be found in the literature (Kotnik and Pucihar 2010). The more complicated electroporation problems

account for the irregular geometry of cells, local heterogeneities in the membrane structure (Gowrishankar and Weaver 2003), nonuniform membrane conductance (Zudans et al. 2007), and changes in the membrane permeabilization and electrical conductivity during the pulsing (Mercadal et al. 2016).

### Ensembles of Cells

In the ensembles of cells (e.g., bio-suspension), electroporation phenomena are complicated by different cell size distributions and different orientations of cells. For example, for the ensemble of spherical cells of different size, simulation predicts for the fraction ($Z$) of electroporated (damaged) cells, the Weibull's kinetics $Z_e = 1\text{-exp-}[(t/\tau_c)^n]$, where $\tau_c$ is the empirical parameter and $n$ is the shape parameter dependent upon the width of the cell size distribution (Lebovka and Vorobiev 2004).

For the elongated cells, the nonexponential kinetics can also reflect the disorder in cell orientations (Lebovka and Vorobiev 2007). The simulations predict upward concavity, $n < 1$, for the prolate spheroids and near-exponential kinetics for the oblate spheroids. The most pronounced deviations from the exponential electroporation kinetics were observed for the disordered suspensions of prolate spheroids having large aspect ratios ($a_r$) and treated by the small electric field strength ($E$). For the partially oriented suspensions, efficiency of electroporation increases with increasing of order parameter and field strength.

In the concentrated ensembles of cells (dense suspensions), the effects of deformation of the external electric field by the adjacent cells are also important. These effects can result in significant violation of Schwan's equation (Eq. (6) (Susil et al. 1998; Pavlin et al. 2002; Qin et al. 2005; Ramos et al. 2006). The corrections were evaluated for different cell packings (Susil et al. 1998; Pavlin et al. 2002; Qin et al. 2005; Ramos et al. 2006; Henslee et al. 2014), and the theoretical estimates were used for explanation of the experimental results on electroporation in dense suspensions of Chinese hamster ovary cells (Pucihar et al. 2007).

### Tissues

In the plant tissues, electroporation phenomena are even more complicated due to the different distributions of local electric fields, different solute concentrations, electrical conductivities, etc. (Pucihar et al. 2007). The tissue electroporation can be monitored by changes of some material property, $P$ (such as the electrical conductivity, textural characteristics, diffusivity, acoustic response, etc.) in the course of PEF treatment (Fig. 3).

It is useful to introduce the disintegration index ($Z$) defined as

$$Z = \left(P - P_i\right) / \left(P_d - P_i\right), \tag{10}$$

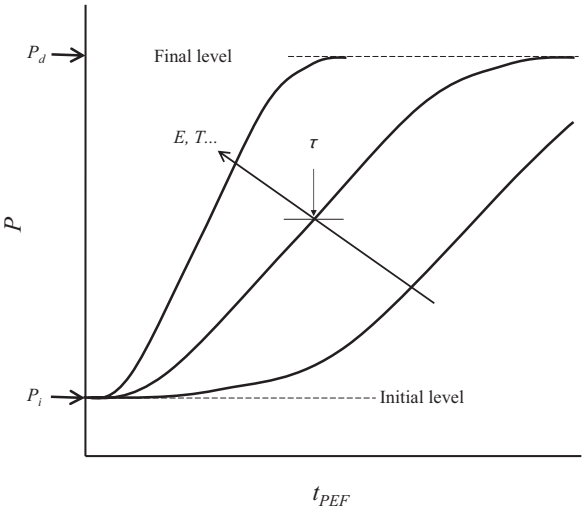


**Fig. 3** Schematical time dependence of some material property ($P$), versus time of PEF treatment ($t_{PEF}$). Here, $P_i$ $P_d$, are the initial (before the PEF treatment) and final (after the long time of PEF treatment) levels of $P$, and $\tau$ is the characteristic damage time. The arrow shows direction of increase of the electric field strength $E$, temperature, $T$, or other processing parameters

where the initial level, $P_i$, corresponds to the intact tissue and the final level, $P_d$, corresponds to the long time of PEF treatment and maximum tissue damage. This equation gives $Z = 0$ for the intact and $Z = 1$ for the completely electroporated material. The characteristic damage time, $\tau$, defined as the PEF duration needed for attaining a half of the material damage ($Z/2$) is also useful for the characterization of electroporation phenomena (Bazhal et al. 2003).

Figure 4a shows typical dependencies of the characteristic damage time $\tau$ versus electric field strength $E$ experimentally estimated for the potato tissue preheated to two different temperatures (Lebovka et al. 2002; Bazhal et al. 2003). The data were obtained by analyzing the changes in low-frequency electrical conductivity during PEF treatment. The value of $\tau$ decreased with increasing of both the temperature $T$ and the electric field strength $E$. The empirical equation similar in shape to Eq. (5) has been proposed to fit the experimental data (Lebovka et al. 2005a)

$$\tau = \tau_\infty \exp \frac{W / kT}{1 + \left(E / E_o\right)^2}. \tag{11}$$

Here, $\tau_\infty$, $W$ and $E_o$ are adjustable parameters.

The observed noticeable decrease of the characteristic electrical damage time after preheating of cell tissues can be explained by the structural transitions (melting) inside the cell membranes and the more pronounced electroporation efficiency at high temperatures (Zimmermann 1986). For the studied case of potato tissue, the direct thermal damage (thermal plasmolysis) was insignificant at temperatures below 50 °C, and the observed effects can only reflect electroporation effects. Note that even at small electric fields ($E < 100$ V/cm), the noticeable electroporation of

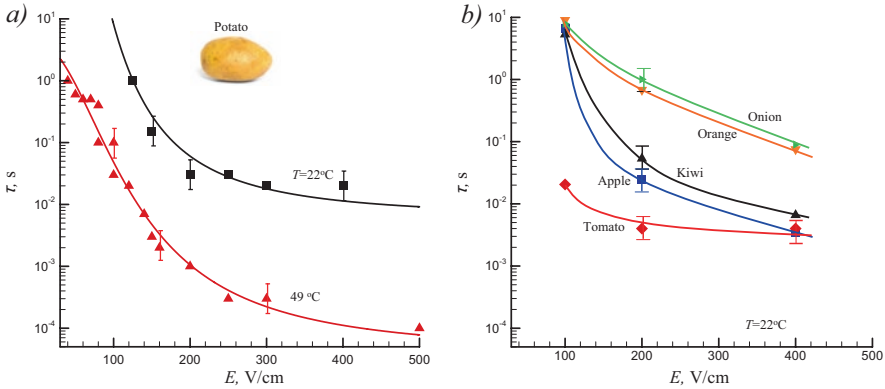


**Fig. 4** Characteristic damage time ($\tau$) versus the electric field strength ($E$) for the potato tissue at two different temperatures (**a**) (Lebovka et al. 2005a) and for the different tissues at room temperature (**b**) (Grimi 2009). The values of $\tau$ were estimated from the measurements of tissue electrical conductivity (**a**) or tissue acoustic properties (**b**)

cell tissues is still possible at the room temperature after prolonged treatment duration, $t_{PEF} \approx$ 10–1000 s.

The $\tau(E)$ dependencies for different plant tissues may be rather different (Fig. 4b), and they can be greatly influenced by the presence of spatial variations in the density, porosity, electrical conductivity, sizes, and shapes of cells. For example, the extracellular porosity of apple tissue is high (14–25%), but the extracellular porosity of carrot (4%), potato (2%), and many other tissues is considerably smaller (Karathanos et al. 1996). Usually, the outer parenchyma porosity is higher than that of the inner parenchyma (Mavroudis et al. 1998).

PEF-treated cells of the plant tissue can be intact (with vital metabolic activity), partially disintegrated (with incomplete metabolic activity), or completely disintegrated (with lost metabolic activity) (Jaeger et al. 2009). In the vicinity of electroporated cells, the local electrical conductivity is high, whereas it is low near the intact cells. This inhomogeneous distribution of electrical conductivity has an impact on the distributions of local transmembrane potentials. The computer simulations (Lebovka et al. 2001) based on the distribution of electrical conductivities of electroporated ($\sigma_d$) and intact ($\sigma_u$) cells ($\sigma_u << \sigma_d$) revealed formation of the conductive paths of electroporated cells between electrodes and correlations between extent of electroporation and changes in electrical conductivity. The data of simulation were in qualitative correspondence with electroporation experiments for the PEF-treated apples (Lebovka et al. 2001). Experimentally observed effects of the pulse frequency were explained accounting for the presence of resealing and mass transfer phenomena.

Simulations have been also used to explain the correlation between electroporation efficiency and electrical conductivity contrast ($k = \sigma_d/\sigma_u$) experimentally observed for different fruit and vegetable plants (apple, potato, carrot, courgette,

orange, and banana) (Ben Ammar et al. 2011a). The simulated data were in good correspondence with existing experimental data (Lebovka et al. 2002). For example, the estimated values of the optimal electric field strength were $E_o \approx 360\ \mathrm{V} \times \mathrm{cm}^{-1}$ ($\sigma_d/\sigma_u \approx 14.3$) for the potato and $E_o \approx 976\ \mathrm{V} \times \mathrm{cm}^{-1}$ ($\sigma_d/\sigma_u \approx 5.6$) for the banana (Ben Ammar et al. 2011a).

## *Techniques to Detect Electroporation*

Disintegration index $Z$ defined by Eq. (10) (sometimes called as electroporation degree or electropermeabilization index) is a very useful characteristic to estimate the degree of electroporation. However, determination of $Z$ requires direct analysis of the microscopic structure of cells or examination of modifications in material properties induced by PEF, e.g., changes of electrical and mass transport characteristics and textural, acoustic, and other properties.

The estimation of extent of electroporation is very important for the optimization of different processing operations assisted by PEF, e.g., pressing and extraction. Unfortunately, it is not a simple or definite task. In principle, disintegration induced by PEF reflects selective damage of cell membranes without direct impact on the cell walls. However, electroporation can also affect the cell walls owing to changes in their moisture content and other secondary effects. Moreover, the electroporation is statistical process, and reversible or irreversible pore formation can develop depending on the PEF protocol. The supplementary effects related to pore resealing, mass transfer processes, and inhomogeneities in tissue structure can also be important.

Nowadays, practically all proposed techniques to detect electroporation are indirect. Moreover, they can be destructive (invasive), affecting the structure of electroporated material. In general, each technique requires a careful adaptation for the studied type of material (Lebovka and Vorobiev 2014, 2016).

### Light and Other Microscopies

Light microscopy is a simple, attractive, demonstrable, and decisive technique. It allows application of modern computerization tools and can be used for the visualization of food material structure and microstructure and quantitative evaluation of changes in size, shape, and other characteristics of biological cell (Chanona-Pérez et al. 2008; Russ and Neal 2016). The effects of disintegration and spatial distributions of different components inside the PEF-treated material may be easily revealed. The light microscopy was used to reveal PEF-induced changes and counting the number of electroporated cells in onion epidermis (Fincan and Dejmek 2002) and in apple parenchyma (Chalermchat et al. 2010). Light microscopy can be used for the visualization of modifications induced by diffusion, osmotic impregnation, drying, and freezing (Ben Ammar 2011; Loginova 2011). Disintegration index $Z$ can be

evaluated from image analysis as the ratio of damaged and total numbers of cells. The disadvantages of this method may be related to problems of tissue preparation, staining strategies, and individual adaptation of pH for selected type of materials.

TEM, SEM, and AFM techniques are also very illustrative and powerful tools for checking the morphological changes in membranes and cell walls (Condello et al. 2013). These methods were tested for the quantification of PEF-induced changes in membrane structure (Chang and Reese 1990), formation of electropores (Chen et al. 2006), and other effects of electroporation (Lee et al. 2012). Particularly, SEM and TEM have been widely applied to study the PEF effects in materials of biological origin such as food materials (Fazaeli et al. 2012). AFM technique has been used for characterization of changes in the membrane and cell walls provoked by electroporation (Suchodolskis et al. 2011; Garcia-Gonzalo and Pagán 2016; Pillet et al. 2016; Napotnik and Miklavčič 2018). In several works magnetic resonance imaging (MRI) techniques have been applied to study the electroporation in vegetable tissues (Hjouj and Rubinsky 2010; Rondeau et al. 2012; Kranjc and Miklavčič 2017; Dellarosa et al. 2018; Suchanek and Olejniczak 2018). However, all these techniques are destructive and require applications of individual methods for specimen preparation (staining, fixation, dehydration, ultrathin sectioning, etc.) or high-vacuum conditions.

### Electrical Impedance Techniques

The electrical impedance techniques are the most popular to detect electroporation. These techniques are straightforward, simple, and non-expensive and can be easily adapted for the continuous monitoring of electroporation during the PEE treatment. In these techniques the electrical conductivity of PEF-treated materials is used as the material property (see Eq. (10)). In a low-frequency (≈1–10 kHz) impedance technique, the electrical conductivity disintegration index $Z_c$ is defined as (Lebovka et al. 2002)

$$Z_c = \left(\sigma - \sigma_i\right) / \left(\sigma_d - \sigma_i\right). \tag{12a}$$

Electrical conductivity ($\sigma$) increases from the value of $\sigma_i$ (intact or untreated tissue) to the value of $\sigma_d$ (completely disintegrated tissue) during the PEF treatment. The value of $\sigma_d$ can be estimated using a long treatment time ($t_{PEF} \approx 0.1$–$1$ s) at high electric field strength ($E > 1000$ V/cm). However, this method can evolve electrochemical reactions and generation of gas bubbles during the prolonged PEF treatment, which can affect the value of $\sigma_d$. Such effects were clearly demonstrated in experiments with sugar beet tissue (Lebovka et al. 2007b). Another method to produce a completely disintegrated tissue is based on the application of freeze-thawing. However, this procedure can violate the structure of cell walls and affect the proper estimation of $\sigma_d$.

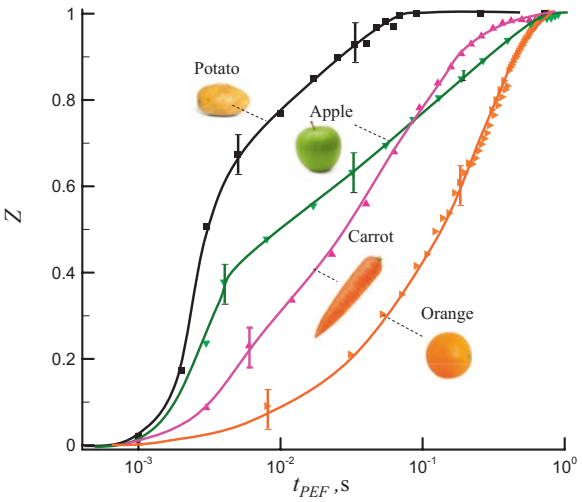


**Fig. 5** Electrical conductivity disintegration index, $Z_c$, versus the time of PEF treatment, $t_{PEF}$, for different food materials obtained using a low-frequency impedance technique. PEF treatment was done at $E = 400$ V/cm using bipolar pulses with duration of $t_p = 1000$μs and pulse repetition time of $\Delta t = 10$ ms. (Adapted from the data presented in (Ben Ammar 2011))

Figure 5 shows temporal behaviors of the electrical conductivity disintegration index $Z_c$ for different food materials (potato, apple, carrot, and orange) obtained on the base of low-frequency impedance technique (Ben Ammar 2011).

In low-high frequency technique, the value of $Z_c$ is defined as (Angersbach et al. 2002)

$$Z_c = \left(\alpha\sigma - \sigma_i\right) / \left(\sigma_d - \sigma_i\right), \tag{12b}$$

using the values of $\sigma$ and $\sigma_i$ (for the partially electroporated and intact materials, respectively) measured at low frequency (≈1–10 kHz); the value of $\sigma_d = \sigma_i^{\infty}$ is measured for the intact material at high frequency (50 MHz). In this equation the measured value of $\sigma$ for PEF-treated material is corrected using the correction factor $\alpha = \sigma_i^{\infty}/\sigma^{\infty}$.

However, electrical impedance techniques are destructive, and they require preliminary cutting of samples to assure good contact with electrodes. Moreover, the electrochemical reactions and generation of gas bubbles inside the treated tissues and near electrodes may affect the measured values of electrical conductivity.

### Diffusivity

These techniques are based on the mass transfer measurements using solid-liquid extraction or drying experiments. They are straightforward, relatively simple, and non-expensive. The diffusivity disintegration index, $Z_d$, can be evaluated by Eq. (10) using solute or moisture diffusivity ($D$) as a material property ($P$) (Barba et al. 2015). However, these techniques are destructive, and estimated values of $D$ may depend on the used experimental procedure.

### Textural Tests

Different textural disintegration indexes, $Z_t$, can be evaluated by Eq. (10) using the cutting force, stress-deformation, or relaxation tests (Lu and Abbott 2004) for the characterization of material property ($P$). These techniques are straightforward, relatively simple, and non-expensive, and they were widely applied for the quantification of PEF-induced changes in different fruit and vegetable tissues (Chalermchat and Dejmek 2005; Grimi et al. 2009a; Liu et al. 2021). However, all these techniques are destructive, the cutting or puncture tests require the penetration of a probe (nail or blade) inside the sample, and in stress deformation or relaxation tests, the sample is compressed.

### Acoustic Test

The test is straightforward, relatively simple, and less destructive and can be used for the detection of electroporation in PEF-treated whole roots or fruits, e.g., sugar beet, potato, tomato, and apple (Grimi et al. 2010a, 2011). The acoustic disintegration index is defined as (Grimi et al. 2010a).

$$Z_a = \left(S - S_i\right) / \left(S_d - S_i\right), \tag{12c}$$

where $S$ is the firmness (stiffness coefficient) $S = f_m^2 m^{2/3} \rho^{1/3}$ (here $f_m$ is the frequency corresponding to the maximum acoustic amplitude, $A$, in the acoustic spectrum, $m$ and $\rho$ are the sample mass and density, respectively).

## *PEF Protocols, Treatment Chambers, and Optimization of the Treatment*

In food processing, the capacitor discharge, square wave, and analog PEF generators can be used (Reberšek and Miklavčič 2011). PEF generators include power source, capacitors, coils of inductance, resistors, and quick switches of different types (Pourzaki and Mirzaee 2008; Toepfl et al. 2014). The electric energy is stored in a bank of capacitors.

### PEF Protocols

Figure 6 demonstrates the most popular exponential decay and square wave shapes for bipolar pulses. The bipolar pulses offer minimum energy consumption with reduced dissolution of the electrodes and reduced electrolysis (Qin et al. 1994; Wouters and Smelt 1997). Note that square wave generators are more expensive and more complex than the exponential decay generators. However, the square wave

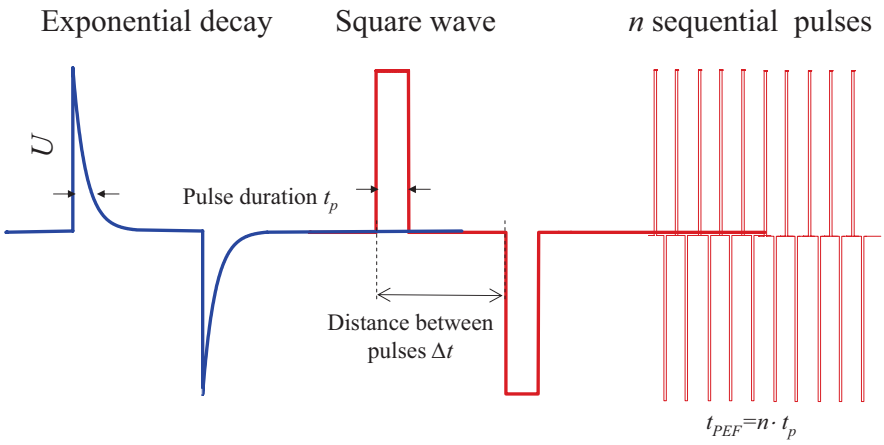


**Fig. 6** The exponential decay and square wave shapes for bipolar pulses and the simple protocol of PEF treatment including $n$ sequential pulses with total duration of $t_{PEF} = nt_p$

generators have better disintegrating and energy efficiencies as compared with the exponential decay ones.

The pulse protocol is defined by the applied amplitude, $U$ (peak voltage); pulse duration, $t_p$; time interval between pulses, $\Delta t$ (or pulse repetition rate $f = 1/\Delta t$); and number of pulses, $n$ (Canatella et al. 2001). The total time of PEF treatment is defined as $t_{PEF} = nt_p$. In more complicated protocols, the trains with long pause between pulses are applied to avoid significant ohmic heating during the PEF treatment.

## Treatment Chambers

Different designs of treatment chambers were used in laboratory and large-scale applications. Nano- and microfluidic systems have been used in electro-transfection experiments for studying electroporation phenomena (Chang et al. 2016), for cell handling and manipulation (Čemažar et al. 2013, 2017), and for in situ observation of electroporation under the optical microscope (Bodénès 2017; Bodénès et al. 2019). Different designs of microscale fluidic chips have been used for analyzing cellular properties or intracellular content of bacteria and yeast (Fox et al. 2006) and studying extraction of cell compounds (Rockenbach et al. 2019). In small-scale laboratory experiments, the cuvettes with parallel configuration of electrodes and 1–4 mm gaps are rather popular (Bio-Rad Laboratories 2019; BTX 2019; Eppendorf 2019).

In food processing and bio-recovery applications, different chambers were proposed for batch and continuous PEF treatment. The batch chambers typically use plate-to-plate electrode geometry. In continuous flow operations, the electrode configurations with parallel plates and coaxial and collinear cylinders were tested

(Reberšek et al. 2014; Toepfl et al. 2014; Raso et al. 2016). Note that for parallel plate or coaxial electrode configurations, the electric field within the interelectrode space can be rather homogeneous. However, the collinear chambers with more inhomogeneous field can be easier inserted in processing lines, and they are more preferable for continuous treatment of solid/liquid suspensions with large particles.

## Optimization of the PEF Treatment

The numerical studies have shown that PEF-induced damage in fruit and vegetable tissues is mainly controlled by the electric field strength $E$ and the treatment time $t_{PEF}$ ($t_{PEF} = nt_p$) (Barba et al. 2015). In general, the electric fields with higher value of $E$ and longer duration of the treatment $t_{PEF}$ lead to the better electroporation efficiency and higher values of disintegration index $Z$.

However, the optimum PEF treatment permitting minimized energy consumption can be achieved using the proper combination of the values of $E$ and $t_{PEF}$. Typically, the values of specific energy consumption, required for the effective PEF damage of fruit and vegetable materials, are situated in the interval of 1–16 kJ/kg, and they are noticeably smaller than the values of specific energy consumption required for the mechanical ($W$ = 20–40 kJ/kg), enzymatic ($W$ = 60–100 kJ/kg), and thermal ($W$ > 100 kJ/kg) disintegration (Toepfl 2006).

For the protocol of PEF treatment including $n$ sequential pulses, the specific energy, $W$ (kJ/kg), can be evaluated using the summing of pulse energy inputs

$$W_i = \int_0 \sigma(t) E^2(t)\, dt / \rho \tag{13a}$$

over the $n$ sequential pulses

$$W = \sum_n^{i=1} W_i. \tag{13b}$$

Here, $\rho$ is the density of the treated material and $\sigma(t)$ is the electrical conductivity of the treated material, which is a growing function of PEF treatment time (Raso et al. 2016).

Therefore, the total specific energy is mainly determined by the behavior of the electrical conductivity ($\sigma(t)$) or the electrical conductivity disintegration index ($Z_c(t)$) of the material during PEF treatment. The total specific energy is approximately proportional to the product of the characteristic damage time ($\tau(E)$) and the square of electric field strength ($E^2$) (Lebovka et al. 2002). The treatment time decreases significantly with $E$ increase, approaching the $\tau_\infty$ value in the limit of high fields ($E \approx 1000$ V/cm) (Fig. 7a), and the product $\tau(E)E^2$ corresponding to the energy consumption goes through a minimum.

This minimum of $\tau(E)E^2$ function determines the optimum value of the electric field strength $E \approx E_0$ (Bazhal et al. 2003). For apple, carrot, and potato, the experimental curves $\tau(E)E^2$ passed through the minimum at $E$ = 200–400 V/cm (Lebovka

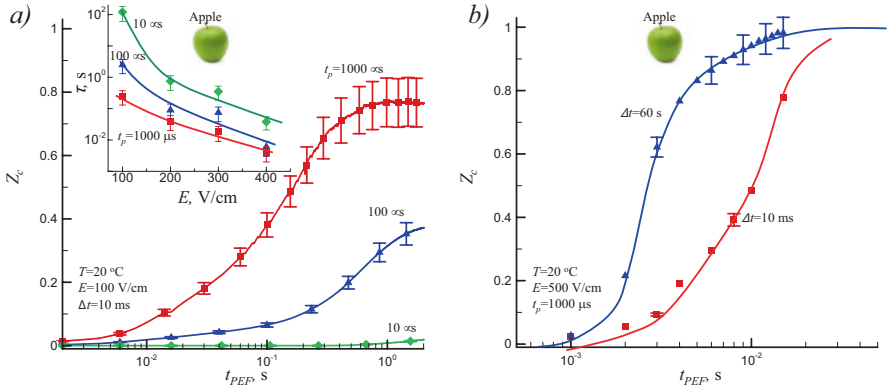


**Fig. 7** Electrical conductivity disintegration index ($Z_c$) for apple tissue (determined by low-frequency impedance technique) versus the time of PEF treatment ($t_{PEF}$): (**a**) at different pulse durations ($t_p$ = 10, 100 and 1000μs), $E$ = 100 V/cm and $\Delta t$ = 10 ms. Inset shows characteristic damage time, τ, versus $E$ at different values of $t_p$ (Adapted from the data presented in (De Vito et al. 2008)); (**b**) at two pulse repetition times $\Delta t$ = 10 ms and 60 s, $E$ = 500 V/cm, and $t_p$ = 1000μs. (Adapted from the data presented in (Lebovka et al. 2001))

et al. 2002). Typically, for many vegetables and fruits, the optimum values of $E$ are situated in the interval between 200 and 1000 V/cm. The PEF treatment at excess values of $E$ above $E_o$ can result in elevated energy consumption without additional electroporation effect. In a general case, the power consumption can be rather complex function of the size and shape of cells, electrical properties of material, and the treatment protocol (Ben Ammar et al. 2010).

The pulse duration ($t_p$) and the distance between pulses ($\Delta t$) may also affect the efficiency of electroporation in plant tissues. For example, the influence of pulse duration (10–1000μs), on the electroporation behavior of grapes, apples, and potatoes, was experimentally demonstrated (De Vito et al. 2008; Vorobiev and Lebovka 2010; Ben Ammar 2011) (Fig. 7a). Longer pulses were more effective, particularly at room temperature and moderate electric fields ($E$ = 100–300 V/cm). The application of PEF protocols with longer distance between pulses ($\Delta t$) at fixed values of $E$ and $t_{PEF}$ also resulted in more accelerated kinetics of disintegration of the apple tissue (Lebovka et al. 2001) (Fig. 7b). Similarly, a cell permeabilization of an onion tissue was significantly higher above the critical distance between pulses, $\Delta t$ = 1 s (Asavasanti et al. 2011b). It was suggested that at low frequencies, convection and cytoplasmic streaming play a significant role in distributing more conductive fluid throughout the tissue (Asavasanti et al. 2012).

During the PEF treatment, the ohmic heating can be also significant (Blahovec et al. 2015). The superposition of the PEF treatment and moderate ohmic heating give synergetic effect for enhancement of electroporation (Lebovka et al. 2005b; Lebovka and Vorobiev 2011). Such synergetic effect can be revealed by analyzing of the behavior of characteristic damage time ($\tau$) at different temperatures (Lebovka et al. 2005a)). The synergy PEF and thermal treatments at moderate temperatures

($T \approx 50$ °C) give a unique opportunity to reach high tissue disintegration at moderate electric fields without noticeable product quality losses.

## Solid/Liquid Expression and Solvent Extraction of Valuable Compounds from Food Plants Enhanced by PEF

### *Solid/Liquid Expression: Principles and Models*

Solid/liquid expression (often named as pressing or pressure extraction) is commonly used for liquid expelling from food and biomass materials. Solid/liquid expression can be produced by movable elements, for example, piston, screw, rolls, etc. (Couper et al. 2012). In hydraulic presses, used to process fruits, oilseeds, and nuts on a small scale, the pressure is applied via an adjustable piston, and the resultant juice is passed through a fine wire screen. In belt presses, the liquid is removed by squeezing the press-cake between compressing rollers (Cheremisinoff 2017). This technique is used for the dewatering of biological sludge, juice production from fruits, etc. In the screw presses, a slowly rotating screw transports and compresses solid-liquid mixtures (Virkutyte 2017; Qingwen et al. 2018). These presses are widely used on the large scale for the oil recovery from oilseeds, dewatering of sugar beet pulp, production of corn fibers and starches, fruit and vegetable juicing, green tea leaf dewatering, winemaking, etc. (Mushtaq 2018). The pressing allows producing fresh-like juices and extracts without undesirable effects of thermal degradation with special interest in applications for temperature or solvent sensible materials (Ashurst 2016).

Solid/liquid expression can be realized using constant pressure or constant rate regimes or their combinations. Constant pressure solid/liquid expression can be performed on industrial pressing equipment like filter press and tube and hydraulic piston presses. In this regime the pressure is suddenly increased up to some given value, which may cause a sudden compression of the material. For biological materials, gentler regime of a constant rate expression with gradually increasing pressure may be preferable. Such regime may be implemented using hydraulic pistons or screw presses. Combined regimes with a constant rate and with a constant pressure for the respectively initial and final pressing periods can be also applied.

Commonly, the juice yield obtained by pressing of coarse (natural) food particles is insufficient. Efficiency of the solid/liquid expression depends greatly on the tissue integrity. It can be significantly improved using preliminary operations like fine grinding of raw material, thermal treatment (heating or freeze-thawing), or enzyme maceration (Monteith and Parker 2016; Sheikh and Kazi 2016; To et al. 2016; Cheremisinoff 2017). However, these operations lead to the passage of cell components like pectins, cellulose, and other impurities into the extracts. As a result, cloudy juices are often produced (Taylor 2016). For further processing of such juices, costly multistage clarification and stabilization procedures are needed

(Albagnac et al. 2002). For example, in sugar production, the fine grinding of sugar beet into the mash was not industrially implemented because of the difficulties for purification of extracted juices (Van der Poel et al. 1998). To overcome the difficulties for juice clarification and purification, coarse fragmentation of raw material (e.g., by slicing) is widely used.

Conventional solid/liquid expression from cellular tissue is accompanied by air expulsion; collapse and different transformations of cellular structure; breakage and separation of cell walls; liquid flowing between intracellular, extracellular, and extraparticle spaces; and many other effects (Das et al. 2018). In rigid materials, the fluid flow is determined by two main characteristics: porosity and permeability. The porosity is a measure of available pore space, and permeability reflects ability of the fluid to flow through the medium under applied pressure.

In compressible porous materials, fluid flow is influenced by the compressibility that is the measure of the mechanical strength (Jönsson and Jönsson 1992). In such materials, the mechanisms of liquid-solid expression are determined by the filtration and consolidation phenomena (Lanoisellé et al. 1996). Moreover, for compressible cellular materials, the cell rupture during liquid expulsing can significantly modify the tissue compressibility. In this case, the simultaneous cell cracking and filtration/consolidation phenomena are manifested. Solid/liquid expression can be also complicated by secondary (creep) consolidation (Lanoisellé et al. 1996). Initial compaction of sliced or grinded particles is needed to start solid/liquid expression. For the description of such phenomena, different mechanistic models have been developed (Lanoisellé et al. 1996; Schwartzberg 1997).

For a constant pressure regime, the model with a spring ($a_1$) and one viscoelastic element ($a_2$, $\eta_2$) (Fig. 8a) predicts three retardation processes related to the liquid flow resistance of the Terzaghi element (time of $\tau_1$), liquid flow resistance of the

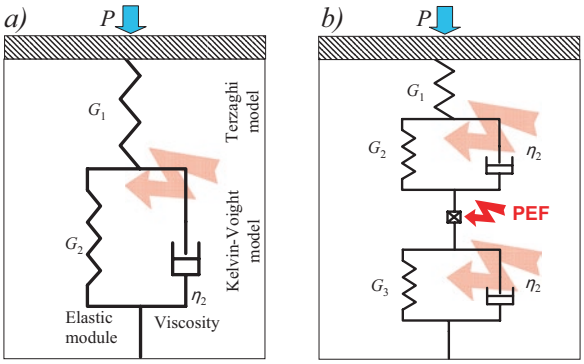


**Fig. 8** Viscoelastic rheological model of cellular tissue with one (**a**) and two (**b**) Kelvin-Voigt elements. Here, Hook's spring $a_1$ is supplemented by viscoelastic elements with the modulus of elasticity ($a_2$, $a_3$) and viscosity ($\eta$). $P$ is the applied pressure. PEF treatment can affect the rheological properties of cellular tissue, and several viscoelastic elements can be used for description of the model

Voigt element (time of $\tau_2$), and creep deformation of the particulate bed (time of $\tau_3$), and it is assumed that $\tau_3 >> \tau_1, \tau_2$ (Shirato et al. 1986).

In rheological models the tissue can be represented by Terzaghi's spring with the compressibility modulus $G_1$ supplemented by one (a) or several (b) Kelvin-Voigt ($G$-$\eta$) elements. The preliminary treatment of tissue (e.g., by application of slicing, fine grinding, heating, or freeze-thawing) causes initial cell damage. The solid/liquid expression starts after compaction of sliced or grinded particles. The initial expression and primary filtration-consolidation behavior are dominated by the compression of the spring $a_1$ (Shirato et al. 1986). The deformation of Kelvin-Voigt elements corresponds to the secondary (creep) consolidation (Lanoisellé et al. 1996).

In general case, biological tissues can include two (Fig. 8b) and even more Kelvin-Voigt elements representing different consolidation periods. Preliminary fragmentation and application of PEF treatment can affect the viscoelastic properties and number of Kelvin-Voigt elements. PEF treatment during the solid/liquid expression can also affect the characteristics of rheological model. For example, PEF application before pressing may influence the behavior of primary consolidation period. PEF application during the pressing may prolong this primary consolidation period or even induce additional consolidation periods (Lanoisellé et al. 1996).

For characterization of compression the consolidation ratio, $Y_c$, defined as

$$Y_c = \frac{h - h_o}{h_\infty - h_0} = \frac{\varepsilon}{\varepsilon_\infty}, \tag{14}$$

is used. Here $h$, $h_0$, and $h_\infty$ are the actual, initial, and final (obtained after the infinitively long time of pressing) thicknesses of the sample, and $\varepsilon = 1\text{-}h/h_o$ is the relative deformation.

Important characteristics of the compression behavior are the infinitive relative deformation ($\varepsilon_\infty$) and the consolidation coefficient, ($b$) expressed in m$^2$/s, which is the analog of diffusion coefficient and represents filtration diffusivity during the press-cake consolidation under applied pressure.

The compression characteristics may significantly depend on the pressure and applied treatment (Grimi et al. 2010b). Figure 9 shows examples of the limiting (infinitive) relative deformation ($\varepsilon_\infty$) (a) and consolidation coefficient ($b$) (b) versus the applied pressure ($P$) for the untreated, PEF-treated (400 V/cm, $t_{PEF}$ = 0.1, $Z_c \approx 0.9$), and freeze-thawed sugar beet tissues (disks with diameter of 25 mm and height of 10 mm) (Grimi et al. 2010b). The pressure behaviors $\varepsilon_\infty$ $(P)$ were rather similar for PEF-treated and freeze-thawed tissues, and the value of $\varepsilon_\infty \approx 0.95$ seems to be maximally attainable for the sugar beet tissue. However, for the untreated samples, the values of $\varepsilon_\infty$ are significantly smaller (e.g., $\varepsilon_\infty$=0.4 even at high applied pressure of 40 bar). The estimated fracture pressure of the untreated sample was of order of $P_{\max} \approx 84$ bar (Grimi et al. 2010b).

The consolidation coefficients ($b$) increase with $P$ for both PEF-treated and freeze-thawed tissues (Fig. 9b). The higher values of $b$ for the freeze-thawed tissue as compared with PEF-treated tissue were observed. This reflects weaker and softer structure of the freeze-thawed tissue.

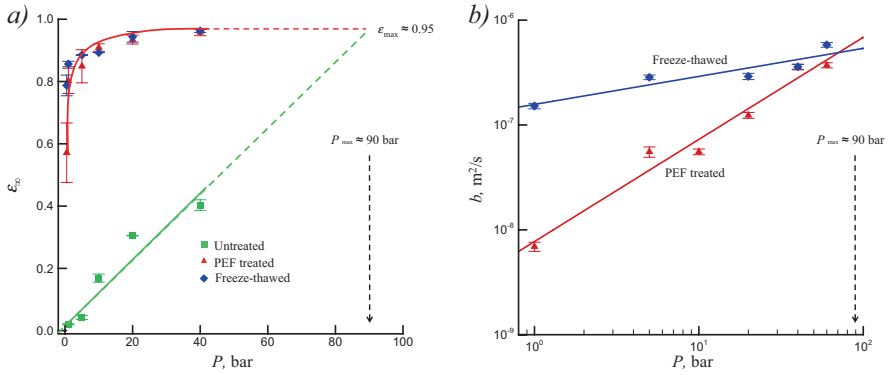


**Fig. 9** Infinitive relative deformation ($\varepsilon_\infty$) $\varepsilon_\infty$ (***a***) and consolidation coefficient (***b***) (***b***) determined at different applied pressures $P$ for the untreated, PEF-treated (400 V/cm, $t_{PEF} = 0.1$, $Z_c \approx 0.9$), and freeze-thawed sugar beet tissues. (Adopted from (Grimi et al. 2010b))

In the generalized model, the primary and creep consolidation periods of the extraparticle volume and the consolidations of both extracellular and intracellular volumes were accounted (Lanoisellé et al. 1996). This model represents the consolidation ratio ($Y_c$) as the sum of different retardation processes:

$$Y_c(t) = f_1 Y_b(t) + \sum_{4}^{i=2} f_i \left(1 - \exp\left(-t/t\right)\right), \tag{15}$$

where

$$Y = 1 - \left(8/\pi^2\right) \sum_{\infty}^{n=0} \frac{\exp\left(-\left(2n+1\right)^2 t/\tau\right)}{2_n + 1)^2}, \tag{16}$$

is the primary consolidation term with a consolidation time of $\tau = \tau_b = 4\ h^2{}_0/(\pi^2 b)$ (here $b$ is the consolidation coefficient), $f_i = (1/G_i)/\Sigma\ (1/G_i)$ ($i$ = 1–4) are the corresponding fractions of the retardation processes related to Hookean springs ($G_i$), and $\tau_i$ are the corresponding retardation times. The compressibility modulus ($G_1$) is attributed to primary consolidation of the tissue, and other terms in Eq. (15) are attributed to the secondary (creep) consolidation of the tissue ($i$ = 2) and to the primary ($i$ = 3) and secondary ($i$ = 4) consolidation of the extraparticle channels.

At large values of $Y$ (when $Y > 0.415$ or $t$ is large), the primary consolidation (Eq.16) can be well fitted (with less than 1% error) by the first term in the series (Schwartzberg 1997):

$$Y(t) = 1 - \frac{8}{\pi^2} \exp\left(-t/\tau\right). \tag{16a}$$

However, the large numbers of terms are needed when $Y$ is small (or $t$ is small). In this case (when $Y < 0.62$), the primary consolidation can be well fitted (with less than 1% error) by the equation (Olson 1986):

$$Y(t) = \left(\frac{16}{\pi^3} t/\tau\right)^{0.5}. \tag{16b}$$

For full diapason, $0 < Y < 1$, the primary consolidation can be well fitted (with less than 0.3% error) by the following equation (Shirato et al. 1980):

$$Y_p(t) \frac{\left(\frac{16}{\pi^3} t/\tau\right)^{0.5}}{\left[1+\left(\frac{16}{\pi^3} t/\tau\right)^{\nu}\right]^{0.5/\nu}}, \tag{16c}$$

where $\nu \approx 2.8$–$2.85$ is an empirical parameter.

Figure 10 illustrates $Y(t/\tau)$ dependencies obtained using the exact theory (Eq. (16)) and different approximations (Eqs. 16a–16c).

For more precise analysis of the deviations of solid/liquid expression from the classical filtration-consolidation behavior, the dual-porosity model was developed

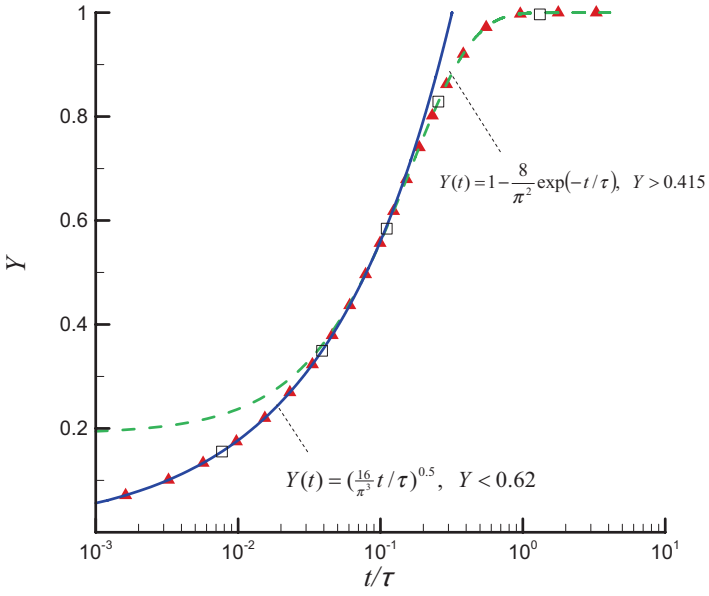


**Fig. 10** Primary consolidation ratio ($Y$) versus the reduced time ($t/\tau$) obtained using the exact theory (solid triangles, Eq. (16)) and different approximations (dashed line, Eq. (16a), solid line, Eq. (16b), and open squares, Eq. (16c), $\nu = 2.8$)

(Mahnič-Kalamiza and Vorobiev 2014; Mahnič-Kalamiza et al. 2015; Mahnič-Kalamiza 2016). In this model the biological tissue was represented by the intracellular (symplast) and the extracellular (apoplast) spaces (Fig. 11). The model postulated that liquid flows from the intracellular to the extracellular space under the pressure gradient. The cells were supposed to be spherical with radius of $R$, the specific surface of the cell was calculated as $S_c = 3/R$, and the cell area was calculated as $A = 4\pi R^2$. The cells are covered by semipermeable cell membranes with permeability, which is a function of the size and the quantity of pores appeared during the PEF treatment. The pore surface fraction was evaluated as $f_p = N_p \cdot A_p / A$, where $N_p$ is the number of pores per cell and $A_p$ is the average single pore area.

The transmembrane flow coefficient ($\alpha$) was calculated as $\alpha = k_p S^2 f_p$, where $k_p$ is the permeability of the pore of radius ($r_p$) induced in cell membrane by PEF. The value of $k_p$ can be estimated based on the Hagen-Poiseuille equation as $k_p = r_p^2/8$. The compressibility of the intracellular space was assumed to be considerably higher than that of the extracellular space. Extracellular space with hydraulic permeability ($k_e$) is formed by the cell wall, extracellular liquid, and air (Fig. 11). Liquid flow in this space was supposed to obey the Darcy filtration law. The permeability of the intracellular space (e.g., $k_i \approx 10^{-24}$ for sugar beet tissue) was neglected

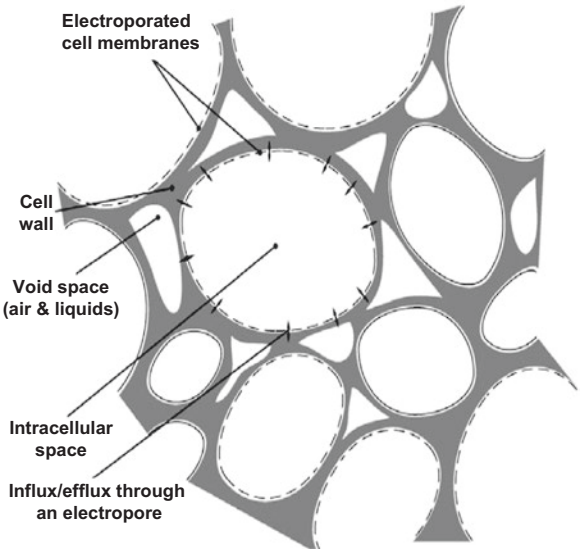


**Fig. 11** A schematic representation of dual-porosity model for description of solid/liquid expression from electroporated cellular tissue. The tissue includes intracellular and extracellular media with semipermeable boundaries (membranes) between them. The membrane has its own hydraulic permeability, which is a function of electroporation parameters. The extracellular space with own hydraulic permeability has intricate structure formed by the cell wall, extracellular liquid, and air. (Mahnič-Kalamiza and Vorobiev 2014)

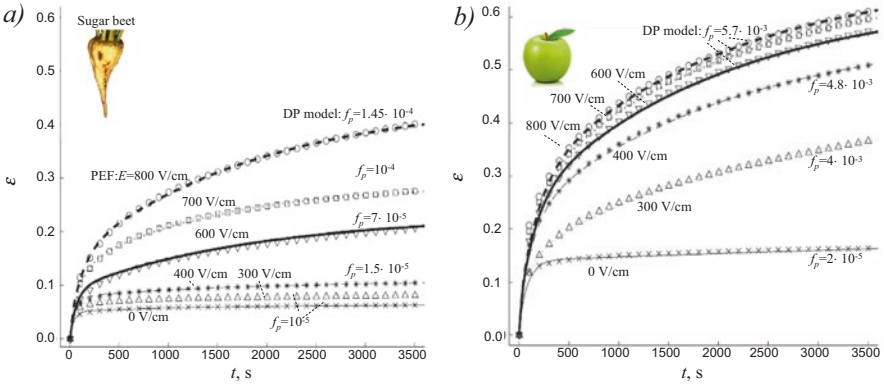


**Fig. 12** Relative deformation, $\varepsilon$, versus the pressing time $t$ for sugar beet (**a**) and apple (**b**) disks. Symbols correspond to the experimental data at different values of electric field strength ($E$), and solid lines correspond to the simulation data obtained using the dual-porosity (DP) model at different values of the pore surface fraction ($f_p$). (Adapted from (Mahnič-Kalamiza et al. 2015))

in comparison to the permeability of the extracellular space ($k_e \approx 10^{-17}$) (Mahnič-Kalamiza and Vorobiev 2014).

The theory based on the dual-porosity model has been tested for checking of the PEF treatment effects on solid/liquid expression (Mahnič-Kalamiza and Vorobiev 2014; Mahnič-Kalamiza et al. 2015; Mahnič-Kalamiza 2016). The disk-like samples of sugar beet and apple (with diameter of 25 mm and height of 5 mm) were PEF treated by bipolar rectangular pulses ($E$ = 300–800 V/cm, $t_p$ = 100μs, $t_{PEF}$ = 1.6 ms) and then pressed at the constant pressures of $P$ = 5.82 bar for sugar beet and $P$ = 2.91 bar for apple.

Figure 12 presents examples of the relative deformation ($\varepsilon$) versus the pressing time ($t$) for sugar beet (a) and apple (b) disks. Symbols correspond to the experimental data at different values of electric field strength ($E$), and solid lines correspond to the simulation data obtained using the dual-porosity (DP) model at different values of the pore surface fraction ($f_p$) (Mahnič-Kalamiza et al. 2015). At the same PEF treatment conditions (the same $E$ and $t_{PEF}$), the estimated values of $f_p$ for sugar beet (Fig. 12a) were an order of magnitude smaller than those for apple (Fig. 12b). It can be explained by the smaller size of cells for the sugar beet.

## *Solvent Extraction of Soluble Substances: Principles and Models*

Transfer of solute from biological solids to solvent is a traditional unit operation in different food applications (extraction of sucrose from sugar beets, of phytochemicals from plants, of lipids from oilseeds, of valuable biomolecules from algae, and

many others) (Aguilera 2003). For example, the conventional hot water extraction at 70–74 °C is widely used in industrial production of sucrose from sugar beet. Such processing permits denaturation of cell membranes (thermal plasmolysis) and accelerates the sucrose extraction from sugar beet slices. Unfortunately, during thermal plasmolysis the cell walls are also subjected to the thermal destruction, and different undesirable substances (like pectin and other colloids) can also be extracted into the juice (Van der Poel et al. 1998). The purification of juice needs numerous operations and is a very costly process.

In recent decades, the solvent extraction from biological solids assisted by different unconventional techniques (high pressure, ultrasound, microwaves, and pulsed electric fields) has been tested (Aguilera 2003; Puri 2017). Application of PEF treatment is of special interest. The early studies demonstrated that tissue treatment even at low gradient electric fields (DC or AC) in the range of 100–200 V/cm could significantly enhance diffusion of soluble substances (Flaumenbaum 1949; Zagorul'ko 1958). However, such treatments can result in undesirable electrolysis and loss of quality of the products (Bazhal and Gulyi 1983), as well as in significant energy consumption and temperature elevation (ohmic heating effects) (Jemai 1997). The PEF treatment is a good alternative to the DC or AC applications. It is a nonthermal cost-effective technique that has shown high potential for extraction purposes. In recent two decades, many studies have demonstrated the PEF effects on solute extraction from plant tissues (Vorobiev and Lebovka 2013; Chan et al. 2014; Vorobiev and Lebovka 2016a, b, 2019).

Solvent extraction goal is the diffusion of biomolecules from a sample into the external solvent. It is a multiphase and unsteady-state mass transfer operation (Aguilera 2003). In the food industry, the commonly used solvents are water, ethanol (or ethanol-water mixtures), and other solvents with preference to the so-called green solvents (Nelson 2003).

The most popular theory for the description of the diffusion processes is based on the analytical solution of Fick's second law (Crank 1979). The kinetics of extraction depends upon the shape of the sample, temperature, type of the solvent, and agitation of the solution. For the infinite slab (plane sheet), the theory gives the following equation for the time evolution of the relative solute concentration in solution (diffusion yield):

$$Y = C(t)/C_\infty = 1 - \left(8/\pi^2\right) \sum_{\infty}^{n=0} \frac{\exp\left(-(2n+1)^2 t/\tau\right)}{(2n+1)^2}, \tag{17}$$

where $C$ is the solute concentration in the solvent, $C_\infty$ is its equilibrium value in the limit of long (infinitive) extraction time, $\tau = \tau_d = h^2/(\pi^2 D)$ is the diffusion time, $h$ is the thickness of a slab, and $D$ is the effective diffusion coefficient (solute diffusivity). It is assumed that at the initial moment of time ($t = 0$), the solute is homogeneously distributed inside the slab, and there is instantaneous equilibrium between the surface of the slab and surrounding solvent. A dimensionless time Fi = $tD/h^2$ is called Fick number (Gekas 1992).

The time dependence of the diffusion yield ($Y$), with time of $\tau_d$ (Eq. 17), is equivalent to the time dependence of the primary consolidation ratio ($Y$), with time of $\tau_b$ (Eq. 16). Therefore, the consolidation coefficient $b$ can be considered as the filtration-consolidation analog of the solute diffusivity ($D$).

The approximate relations represented by Eqs. (16a)–(16c) can be used for estimation of the diffusion time ($\tau_d$) and solute diffusivity ($D$). Fick's solution is frequently used for the estimation of solute diffusivity in different biological tissues (Gekas 1992; Zogzas and Maroulis 1996; Varzakas et al. 2005; Azarpazhooh and Ramaswamy 2009). The values of solute diffusivities in foods have been reviewed extensively in the literature (Schwartzberg and Chao 1982; Aguilera 2003; Varzakas et al. 2005; Saravacos and Krokida 2014).

Single exponential law with coefficient $f = 8/\pi^2$ (and relaxation time $\tau_d$) can be used at the large values of $Y$ (>0.415) for the good fitting of experimental data (see Fig. 10, Eq. 16a). For more precise fitting in whole diapason of $0 < Y < 1$, the relation represented by Eq. (16c) with fitting parameter $\nu$ can be used. Note that for the fitting of the experimental curves of $Y(t)$ other empirical relations were also tested (Varzakas et al. 2005; Chan et al. 2014). We can refer the extended exponential relation (also known as the Weibull or Kohlrausch law (Elton 2018))

$$Y = 1 - fexp\left[\left(-t/\tau\right)^{\beta}\right], \tag{18}$$

or multi-exponential relation

$$Y = \sum_{n}^{i=1} f_i\left(1 - \exp\left(-t/\tau_i\right)\right). \tag{19}$$

Here, $f$, $\beta$ are the fitting parameters, and $f_i$ corresponds to the fraction of the exponential process with relaxation time of $\tau_i$.

Two-exponential law can be used for the fitting of extraction curves, which includes the first step of a fast extraction (washing stage) and the second step related to a slow extraction (solute diffusion) (Chan et al. 2014). Figure 13 presents such situation for the diffusion yield ($Y$) obtained using two-exponential equation (Eq. 19) with fast (washing) contribution ($f_1 = 0.5$, $\tau_1 = 0.1\tau$) and slow bulk diffusion contribution ($f_2 = 0.5$, $\tau_2 = \tau$).

The theory based on the dual-porosity model has been also tested for checking of the PEF treatment effects on diffusion extraction of soluble substances (Mahnič-Kalamiza and Vorobiev 2014; Mahnič-Kalamiza et al. 2015; Mahnič-Kalamiza 2016).

## *Some Examples of PEF-Assisted Processes for Different Foods*

In recent decades PEF-assisted solid/liquid expression and solvent extraction have been tested for different food and biomass materials, like apple, citrus, tomato, sugar crops, potato and carrot crops, grapes and residues of wine industry, other

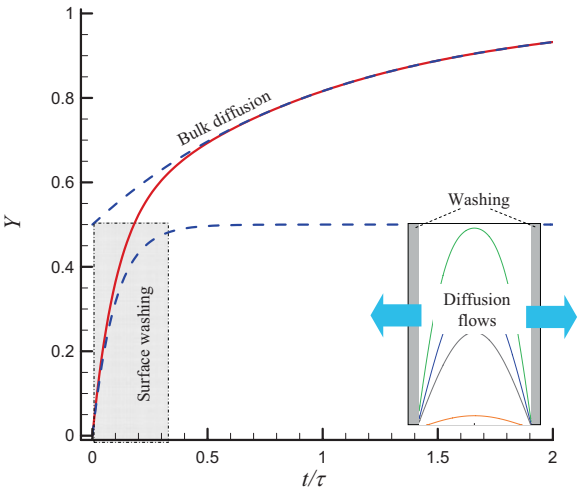


**Fig. 13** Diffusion yield ($Y=C/C_\infty$) versus the normalized time of extraction ($t/\tau$) obtained using two-exponential equation (Eq. (19)) with fast (washing) contribution ($f_1 = 0.5$, $\tau_1 = 0.1\tau$) and slow bulk diffusion contribution ($f_2 = 0.5$, $\tau_2 = \tau$)

fruits and vegetables, microalgae, mushrooms, leaves, etc. Additional recovery of valuable cell compounds, e.g., carbohydrates, polyphenols, proteins, etc., was demonstrated.

## Potatoes and Apples

Potato and apple were frequently used in early studies as model tissues for testing different electroporation effects. For instance, juice expression and solute extraction can be significantly improved when these tissues are electroporated by PEF.

As example, experimental studies revealed complex character of solid/liquid expression from PEF-treated potato slices (Lebovka et al. 2003). Figure 14 presents the juice yield (relative mass losses) $Y = \Delta m/m$ (open squares) versus pressing time $t$ for the untreated potato slices at constant pressure of $P = 5$ bar (symbols). The value of $Y$ is proportional to the consolidation ratio ($Y_c$) defined in Eq. (14). The dashed line represents the least square fit of the consolidation data using the multi-exponential relation

$$Y = \sum_{m}^{i=1} f_i \left[1 - \exp\left(-t/\tau_i\right)\right], \tag{20}$$

where $i = 1$–$3$, $\tau_i$, and $f_i$ are the expression (consolidation) periods and corresponding relative fractions.

The time of the initial expression period was $\tau_1 = 10 \pm 2$ s. During this initial period, about 20% of the juice was expressed ($p_1 = 0.20 \pm 0.05$). The initial period may be attributed to the expression of external juice and air. The value of $\tau_1$ for this initial expression period may depend upon the type of tissue and the degree of tissue fragmentation (Schwartzberg 1997). The parameters for the second expression

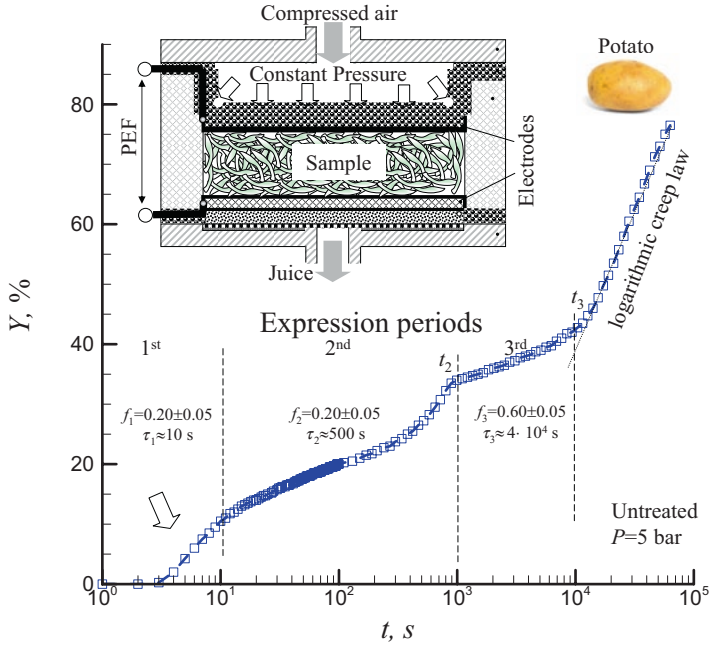


**Fig. 14** Juice yield ($Y$) versus time ($t$) for the constant pressure expression ($P = 5$ bar) from potato slices. Here, $\tau_i$-($i$ = 1–3) are the different expression (consolidation) periods, and $f_i$ are the corresponding relative fractions. Open squares correspond to the experimental data, and the dashed line was obtained using the multi-exponential relation (Eq. (20)). Inset shows experimental device. (Adapted from (Lebovka et al. 2003))

period were $\tau_2 \approx 500 \pm 150$ s and $f_2 = 0.20 \pm 0.05$ (Fig. 14). The third expression period was more prolonged with time of $\tau_3 \approx (4 \pm 1) \times 10^4$ s, and biggest quantity of liquid was expressed during this period ($f_3 = 0.60 \pm 0.05$). After certain transition time, kinetics of the longest expression period may be approximated by Buisman's logarithmic creep law, $Y \propto \ln t$ (Buisman 1936).

Potato was used for checking the effects of PEF protocols on the juice expression behavior. Figure 15a shows typical juice expression curves obtained from the untreated and PEF-treated potato slices at constant pressure ($P = 5$ bar). In these experiments, the electric field strength was fixed at $E = 200$ V/cm, pulse duration was $t_p = 100\mu$s, pulse repetition time was $\Delta t = 10$ ms, number of pulses ($n$) was varied, and total time of the PEF treatment $t_{PEF} = nt_p$ was lasted from 5 ms to 3 s. PEF was applied at $t = 20$ s just after the first consolidation stage when about 20% of juice was expressed (Lebovka et al. 2003). After the PEF application, additional quantity of juice was released from the electroporated cells, and the consolidation process was developed more intensively. Increase of the PEF treatment duration ($t_{PEF}$) leads to a more rapid expression kinetics.

Difference between the behaviors of second consolidation period (lasted from $\approx$20 s to $\approx 10^3$ s) and third consolidation period (lasted from $\approx 10^3$ s to $\approx 10^4$ s) was

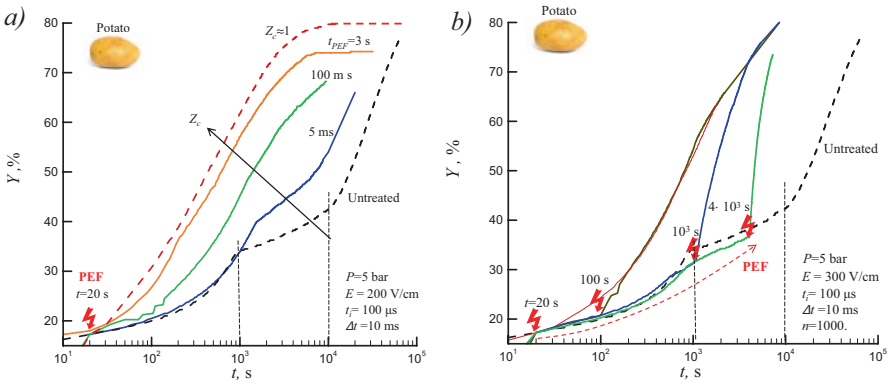


**Fig. 15** Juice yield, $Y$, from PEF-treated potato slices versus time, $t$, for the constant pressure expression ($P$ = 5 bar). PEF treatment was applied at $t$ = 20 s (a) or at the different times $t$ = 20–4000 s (b). PEF treatment parameters were $t_p$ = 100µs, $\Delta t$ = 10 ms, $E$ = 200 V/cm, (**a**), and $E$ = 300 V/cm (**b**), $T$ = 25 °C. (Adapted from (Lebovka et al. 2003))

noticeable for the untreated tissue, and it completely disappeared after the prolonged PEF treatment ($t_{PEF}$ > 100 ms) (Fig. 15a). Figure 15b shows the curves of juice expression $Y(t)$ before and after the PEF treatment of potato slices. PEF treatment was applied at the different moments of time directly during expression. The treatment protocol was $E$ = 300 V/cm, $t_p$ = 100µs, $\Delta t$ = 10 ms, and the fixed number of pulses was $n$ = 1000 ($t_{PEF}$ = 0.1 s) (Lebovka et al. 2003). The delay in PEF application resulted in the retardation of juice release from the electroporated cells and lead to the longer duration of pressing. From an industrial point of view, it is important to reduce the pressing time as much as possible. So, it is desirable to apply the intermediate PEF treatment at the initial period of compression, predominantly before or during the second consolidation period.

Potato was also used for testing the PEF effects on the characteristics of tissue at constant velocity regime of compression. Figure 16 shows the deformation behavior $\varepsilon(t) = 1\text{-}h(t)/h_0$ (a) and the final deformation $\varepsilon_f$ (at $t_f = 1.3 \times 10^4$ s) (b) of the untreated and PEF-treated potato disks (diameter $d$ = 25 mm and initial thickness $h_0$ = 10 mm) when compression is started at the constant velocity ($V$ = 0.1 mm/s) and continued at the constant pressure ($P$ = 10, 20 or 40 bar) (Grimi et al. 2009a). During the constant velocity stage, at $t < t_t$, the deformation increases linearly with time, and pressure also increases up to attain the fixed value of $P$ = 10, 20, or 40 bar (at $t = t_t$) (Fig. 16a).

The difference in the compression behavior for the untreated and PEF-treated potato samples appeared just at $t > t_t$, when pressing is continued at the constant pressure. For the untreated samples, two different effects were observed: (a) the pressure-induced rupture (cracking) of intact cells with release of juice from ruptured cells and (b) the juice expression outside the tissue by filtration-consolidation. At the high level of electroporated cells (conductivity disintegration index $Z_c$ = 0.95), the temporal variation of tissue deformation reflects mainly filtration-consolidation

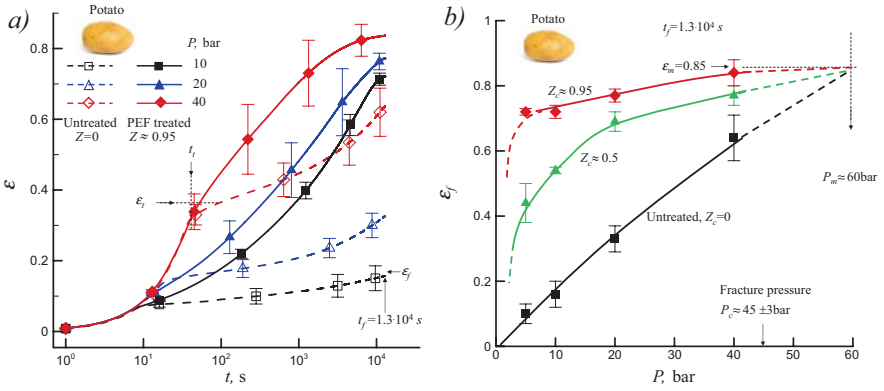


**Fig. 16** Deformation ($\varepsilon$) versus the pressing time ($t$) (**a**) and the final deformation, $\varepsilon_f$, (at $t_f = 1.3 \times 10^4$ s) versus the pressure ($P$) (**b**) for untreated and PEF-treated potato disks. The compression was started at the constant velocity ($V = 0.1$ mm/s) and continued at the constant pressure ($P = 10$, 20 and 40 bar). The conductivity disintegration index ($Z_c$) of PEF-treated specimens was fixed ($Z_c = 0.95$) (*a*) or varied ($Z_c = 0.5$, $Z_c = 0.95$) (*b*). (Adapted from (Grimi et al. 2009a))

behavior (Lebovka et al. 2003). Juice expression from the electroporated cells was much faster than from the mechanically cracked cells for the same values of pressure. For example, 3.5 h and just 70 s of compression at $P = 10$ bar were required to attain the same deformation ($\varepsilon \approx 0.15$) of the untreated and PEF-treated ($Z_c = 0.95$) potato disks, respectively. Final deformation ($\varepsilon_f$) of the untreated and PEF-treated potato disks was estimated after the long compression time ($t_f = 1.3{\cdot}10^4$ s $\approx 3.5$ h). The values of $\varepsilon_f$ obtained at the different pressures are presented in Fig. 16b. It can be seen that extrapolation of the $\varepsilon_f(P)$ curves obtained for the different degrees of cell disintegration ($Z_c = 0$, 0.5, 0.95) gives the same hypothetical pressure value of $P_m \approx 60$ bar, which exceeds the experimentally measured fracture pressure for the potato samples (Grimi et al. 2009a).

PEF-assisted juice expression from apple (Golden Delicious) slices was studied on a laboratory filter-press cell at the constant pressure (Bazhal and Vorobiev 2000). The slices were obtained using a 6 mm grater. For the untreated slices, juice yield was increased from 28% to 61% when pressure, $P$, was increased from 1 to 30 bar. PEF treatment (electric field strength of $E$ = 200–1000 V/cm, pulse duration of $t_p$ = 20–100μs, pulse repetition time of $\Delta t = 10$ ms, number of pulses of $n$ = 3–1000) permitted enhance importantly the juice yield, which attained about 80% at $E = 1000$ V/cm $t_p = 100$μs, $n = 1000$, and $P = 3$ bar.

Correlations between the PEF energy consumption and apple juice yield were established. The maximum juice yield was attained at optimum energy consumption of 3 kJ/kg. The juice obtained without PEF treatment was turbid and required filtration. The juice obtained for PEF-assisted pressing was significantly clearer and did not require additional filtration.

Note that PEF treatment may significantly affect the structure of cell membranes, whereas it seems to retain the cell wall architecture in plant tissues (Fincan and

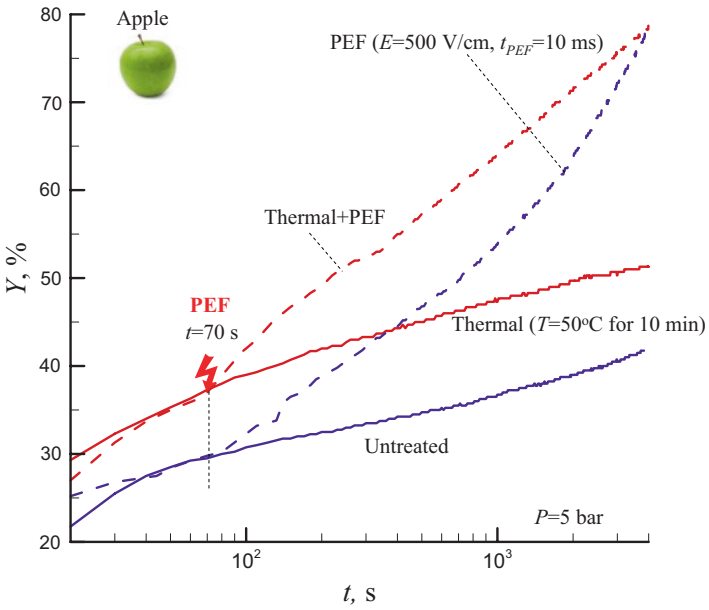


**Fig. 17** Juice yield ($Y$) versus time ($t$) for the constant pressure expression ($P$ = 5 bar) of apple slices. Thermal pretreatment was done at $T$ = 50 °C for 10 min. PEF treatment ($E$ = 500 V/cm, $t_p$ = 10μs, $\Delta t$ = 10 ms, $t_{PEF}$ = 0.01 s) was applied during the pressing at $t$ = 70 s. (Adapted from (Lebovka et al. 2004b))

Dejmek 2003). However, the rigidity of cell walls may hinder the consolidation behavior during solid/liquid expression. The studies have revealed significant effects of mild heating (e.g., at 50 °C) on the textural softening of different food tissues (apple, carrot, potato) (Lebovka et al. 2004a) and juice expression behavior (Lebovka et al. 2004b).

Figure 17 presents the juice yield curves obtained at the constant pressure expression ($P$ = 5 bar) for fine-cut apple slices (1.5 mm in width, 40–50 mm in length). The data for untreated, preheated (thermal), PEF-treated ($E$ = 500 V/cm, $t_p$ = 10μs, $\Delta t$ = 10 ms, $t_{PEF}$ = 0.01 s), and preheated and PEF-treated samples can be compared. Slices were preheated at $T \approx 50$ °C for 10 min, and then they were treated or not by PEF at the ambient temperature. For the preheated sample, the juice yield was significantly higher than for the untreated one. PEF treatment accelerated the juice yield ($Y$) for both untreated and preheated samples. However, the most significant effect of PEF was observed for the thermal + PEF sample.

Ohmic heating (OH) and PEF treatment were applied to enhance the juice yield from potato (1.5 mm in width, 1 mm in thickness, and 30–40 mm in length) and apple (6 mm in width, 1.5 mm in thickness, and 30–40 mm in length) slices (Fig. 18a) (Praporscic et al. 2005). Before OH slices were pre-compacted to ensure the good contact with electrodes. Ohmic heating was realized at $E$ = 30 or $E$ = 50 V/cm for 45 s. The interrupted heating-cooling mode of OH treatment was used when

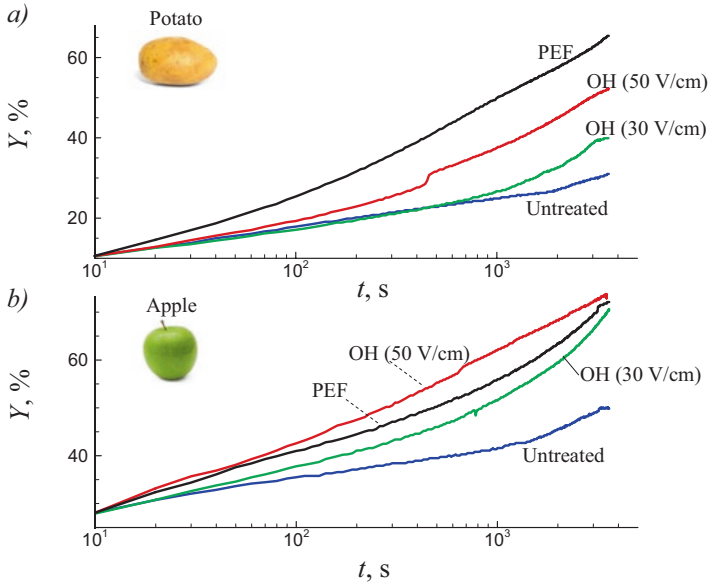


**Fig. 18** Juice yield $Y$ expressed from potato (**a**) and apple (**b**) slices versus pressing time, $t$, at the constant pressure regime ($P = 5$ bar) for the untreated, OH-treated (at $E = 30$ V/cm or $E = 50$ V/cm for 45 s), and PEF-treated ($E = 850$ V/cm and $t_{PEF} = 10$ ms, $t_p = 100$μs, $\Delta t = 10$ ms) slices. OH and PEF treatments were done before pressing. (Adapted from (Praporscic et al. 2005))

the temperature of slices reached 50 °C. Therefore, the final temperature of slices never exceeded 50 °C. Before PEF treatment, slices were also pre-compacted to ensure the good contact with electrodes. PEF treatment was done at $E = 850$ V/cm and $t_{PEF} = 10$ ms ($t_p = 100$μs, $\Delta t = 10$ ms) and ambient temperature. For the potato samples, the highest juice yield was observed after the PEF treatment (Fig. 18a).

However, for apple samples, the highest juice yield was observed after the OH at $E = 50$ V/cm (Fig. 18b). This result can be explained by synergetic effect of thermal and electric field treatments that was more significant for the apple than for the potato (Lebovka et al. 2004a, b).

Aqueous extraction of solutes from apple tissue was also significantly enhanced by PEF (Jemai and Vorobiev 2002, 2003). Different pretreatments were compared to enhance extraction kinetics from apple disks (Golden Delicious): thermal denaturation (plasmolysis) (75 °C, 2 min) followed by diffusion at different temperatures (20–75 °C); (2) PEF treatments ($E = 100$–$650$ V/cm, $t_p = 50$–$200$μs, $n = 1000$) followed by diffusion at 20 °C; and (3) PEF treatments ($E = 500$ V/cm, $t_p = 100$μs, $n = 1000$) followed by diffusion (60 min) at $T = 20$–$75$ °C. Detectable enhancement of diffusion kinetics was observed beginning from the field strengths of $E = 100$–$150$ V/cm. Further increase of $E$ and $t_{PEF}$ resulted in more rapid diffusion kinetics. The temperature dependence of solute diffusivity $D$ followed the Arrhenius law with activation energy $W_a \approx 28$ kJ/mole (for samples without thermal

pretreatment) and $W_a$ ~ 13 kJ/mole (for thermally denaturized samples). For the PEF-treated samples, only one regime with intermediate activation energy, $W_a \approx 20$ kJ/mole, was observed.

PEF treatment ($E$ = 100–1100 V/cm and $t_{PEF}$ = 10–100 ms) was used for accelerating the osmotic dehydration (OD) of apples (*Golden* variety) (Amami et al. 2005). PEF-treated apple disks (2.8 cm in diameter and 0.85 cm in thickness) were placed in stirred hypertonic solution of sucrose. The best results were obtained at $E$ = 900 V/cm, $t_{PEF}$ = 75 ms, and energy input of $W$ = 13.5 kJ/kg. PEF treatment noticeably increased water losses (WL) (on 50%) and solids (sugar) gain (SG) (on 6%) compared to the control samples. Therefore, PEF treatment allowed obtaining the high dehydration effect with minimal sugar uptake by the apple. With increase of sucrose concentration from 44.5 to 55 and 65 w/w, the WL and SG values became higher, especially for the PEF-treated apples (Amami et al. 2006). PEF-assisted OD of apple (*Idared* variety) has been studied (Wiktor et al. 2014). For PEF-treated samples, the increase in WL up to 36–46% was observed.

## Sugar Crops

Sugar crops include sugar beet, sugarcane, chicory, sugar maple, sugar palm, etc. The most intensive studies have been devoted to the PEF-assisted solid/liquid expression and water extraction processes applied to sugar beets. Conventional sugar beet processing employs the hot water extraction followed by very complex multistage juice purification. This is a time- and energy-consuming processing that can be significantly improved by application of PEF treatment.

### Sugar Beet

In the early works, the effect of PEF protocols ($E$ = 215–427 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, $n$ = 250–500) on juice expression from sugar beet cossettes (slices) was studied at the constant pressure regime ($P$ = 5 bar) (Bouzrara and Vorobiev 2000, 2001). An intermediate PEF treatment applied during the pressing enhanced markedly the juice yield, and a correlation between the PEF energy input and juice yield was demonstrated. In addition to the parameters of PEF protocol, other factors, such as pressure and size of sliced particles, have been reported to have significant effects. For the untreated sugar beet slices, pressure increase from 0.5 bar to 10 bar enhanced the juice extraction yield from 1.6% to 34%. PEF treatment ($E$ = 500 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, $n$ = 1000) followed by pressing at $P$ = 10 bar enhanced the juice yield up to 62.3%. The combined process with an intermediate PEF application was most effective and enhanced the juice yield up to 78.5% (at $P$ = 5 bar) and up to 82.4% (at $P$ = 10 bar). Effect of the size of PEF-treated sliced particles (width of 1.5–7 mm, length of 5 mm, and thickness of 1.5 mm) on the kinetics of solid/liquid expression was also evaluated. As expected, the quantity of juice expressed (at $P$ = 5 bar) from small untreated particles was higher (up to 40% for slices with

width of 1.5 mm) than the quantity of juice expressed from large untreated particles (15% for slices with width of 7 mm). Nevertheless, the total juice yield after the PEF application ($E$ = 500 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, $n$ = 1000) was nearly the same for particles with sizes in the range of 1.5–6 mm (76–78%), and it was slightly smaller for the largest particles of 7 mm (73%).

The attractive feature of pressing with an intermediate PEF treatment was a better purity and a paler color of sugar beet juice (Bouzrara and Vorobiev 2001). Moreover, the juice obtained with assistance of PEF had higher sugar concentration and did not contain any pectin substances, which is advantageous for the subsequent filtration and purification processing.

Experiments on PEF-assisted solid/liquid expression from sugar beet cossettes (grated with 6 mm grater) at the constant rate regimes were also performed (Praporscic et al. 2004). During the pressing of sliced particles at the same constant velocity $V$ ($V$ = 0.65 or 5.5 mm/min), the loading pressure increased continuously with the time up to the maximum value of 25 bar, and then pressing was continued at this pressure of $P_m$ = 25 bar. The PEF treatment ($E$ = 0–1000 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, $t_{PEF}$ = 0–0.1 s) was applied before pressing ($P$ = 0) or during pressing at the different moments of time (corresponding to the pressures of $P$ = 1.5–25 bar). It was demonstrated that PEF application to the non-pressurized sugar beet cossettes leads to a higher energy consumption. On the other side, PEF treatment of pressurized cossettes resulted in a delayed kinetics of juice expression. The optimal PEF treatment results minimizing energy consumption were obtained after the pressurization of cossettes at $P$ = 1.5–5 bar.

Figure 19 shows the kinetics of juice yield $Y(t)$ expressed from sugar beet cossettes during the constant rate solid/liquid expression ($V$ = 0.65 mm/min, $P_m$ = 25 bar) for different modes of PEF treatment (Praporscic et al. 2004). In these experiments

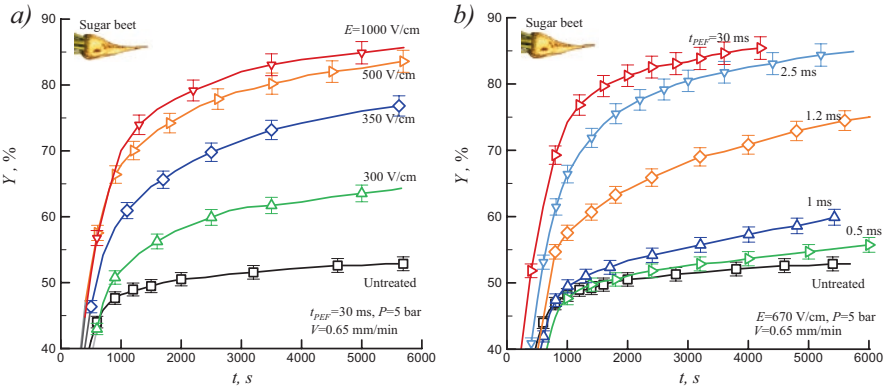


**Fig. 19** Juice yield ($Y$) expressed from sugar beet cossettes versus expression time ($t$) at the constant rate regime ($V$ = 0.65 mm/min, $P_m$ = 25 bar). The PEF treatment ($t_{PEF}$ = 30 ms and different values of electric field strength, ($E$)) (**a**) or ($E$ = 670 V/cm and different values of $t_{PEF}$) (**b**) were applied when pressure attained 5 bar. (Adapted from (Praporscic et al. 2004))

the PEF treatment was applied when pressure attained the value of 5 bar. However, the PEF treatment parameters were different: $t_{PEF}$ = 30 ms and different values of $E$ (Fig. 19a) or $E$ = 670 V/cm and different values of $t_{PEF}$ (Fig. 19b). At the fixed value of $t_{PEF}$ = 30 ms, the noticeable PEF effect on juice yield was only observed for $E$ above 250–300 V/cm (Fig. 19a). However, above 500 V/cm increase in $E$ gave only insignificant supplementary effect. At the fixed value of $E$ = 670 V/cm, the PEF effect was significant for $t_{PEF}$ duration between 1 and 2.5 ms (Fig. 19b).

Enhanced sucrose extraction from PEF-treated sugar beet cossettes has been also revealed (Jemai and Vorobiev 2003). A laboratory-scale solid-liquid extractor was used in these experiments. Soluble matter concentration of the extract and electrical conductivity of the solid-liquid mixture were simultaneously measured during extraction. Significant enhancement of soluble matter release was observed for the PEF treatments above $E$ = 150 V/cm. Later on, PEF-assisted sucrose extraction from sugar beet disks (30 mm in diameter and 8.5 mm in thickness) treated at $E$ = 235–1180 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, and $n$ = 100–1000 were studied in details (El Belghiti and Vorobiev 2004). It was demonstrated that Fick's diffusion law was insufficient to explain the overall mass transfer kinetics from the PEF-treated sugar beet tissue. The extraction kinetics was modeled using a two-exponential model presented by Eq. (19). This model involves two simultaneous processes related to rapid convection of solutes from the tissue surface to the solution (washing process) and with slower transport of solutes from the interior to the exterior of electroporated tissue (diffusion process). During the rapid washing stage of extraction, up to 20–25% of surface sugar was recovered under the optimal PEF treatment conditions. The kinetics of the prolonged diffusion stage of extraction can be accelerated by intensive stirring.

Figure 20 shows kinetics of aqueous sucrose extraction from sugar beet cossettes at different electric field strengths ($E$). Extraction was performed in well-stirred water at 25 °C for 2 h. Just $Y \approx 48\%$ was attained in the absence of PEF in saturated state after 2 h of extraction. This may indicate extraction of sucrose from mechanically broken cells obtained during the slicing of the beet. The yield of solute was significantly increased with PEF treatment attaining $Y \approx 93\%$ for the PEF-treated cossettes at 670–800 V/cm. The influence of PEF treatment time ($t_{PEF}$) and elevated extraction temperatures (30, 40, and 50 °C) were also evaluated. At the fixed electric field strength of $E$ = 670 V/cm, the effective electroporation of tissue was obtained using $t_{PEF}$ = 0.025 s.

The effect of temperature ($T$ = 20–80 °C)) on the aqueous extraction of sucrose from sugar beet cossettes electroporated by PEF ($E$ = 400 V/cm, $t_{PEF}$ = 0.1 s) was studied (Lebovka et al. 2007a, b). The extraction yield was characterized by changes in °Brix measured during extraction:

$$Y_B = \left( {}^{\circ}\mathrm{Brix} - {}^{\circ}\mathrm{Brix}_i \right) / \left( {}^{\circ}\mathrm{Brix}_f - {}^{\circ}\mathrm{Brix}_i \right), \tag{21}$$

where °Brix, °$\mathrm{Brix}_i$, and °$\mathrm{Brix}_f$ are, respectively, the actual, the initial, and the final values of °Brix of the extract.

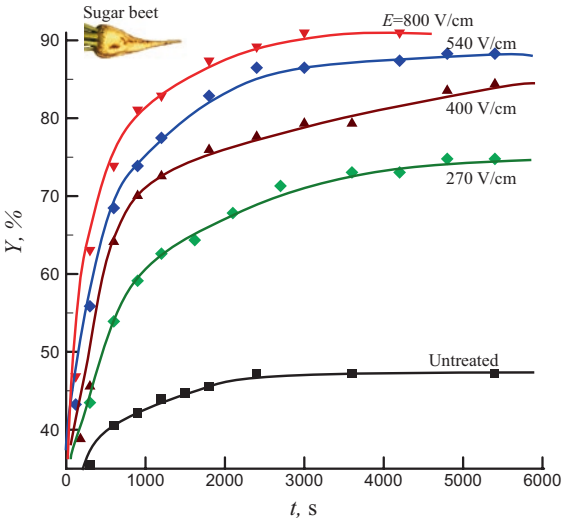


**Fig. 20** Kinetics of aqueous sucrose extraction, $Y(t) = C/C_\infty$, for the PEF-treated sugar beet cossettes at different values of electric field strength, $E$ ($t_p$ = 100μs, $\Delta t$ = 10 ms, $n$ = 1000). Extraction temperature was $T$ = 25 °C. (Adapted from (El-Belghiti et al. 2005a))

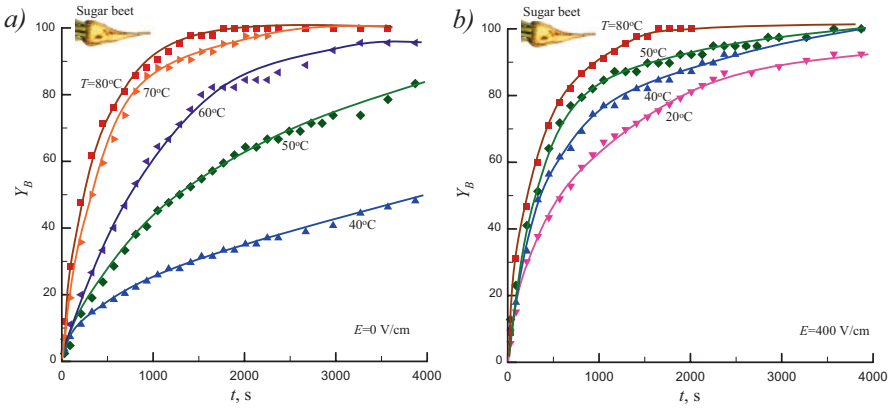


**Fig. 21** Solute extraction yield, $Y_B$ (normalized soluble solid content according to Eq. 21) at different temperatures versus time, $t$, for the untreated (**a**) and electroporated at $E$ = 400 V/cm (**b**) sugar beet cossettes. (Lebovka et al. 2007a, b)

Figure 21 shows the kinetics of soluble solid diffusion from the untreated (*a*) and electroporated (*b*) cossettes at different temperatures (Lebovka et al. 2007a, b). For the untreated cossettes, diffusion duration of 50–60 min was needed at 70–80 °C to attain the maximal value of °Brix, $Y_B$ = 1 (Fig. 21a). At lower temperatures of 40–50 °C, very important time would be needed to extract such quantity of soluble solids. However, for the electroporated cossettes, the maximal value of °Brix was attained more rapidly even at lower extraction temperature of 60 °C. Moreover, effective diffusion can be achieved at lower temperatures of 40–50 °C (Fig. 21b), and even cold diffusion at temperatures of 20–30 °C seemed to be attainable.

Aqueous extraction of sugar from the PEF-treated sugar beet cossettes was studied using the laboratory counter-current extractor with 14 extraction sections (Loginova et al. 2011a, c). Perforated plastic baskets filled with 500 g of cossettes were used for the manual transportation. The most concentrated solution (diffusion juice) was produced in sect. 1 in the contact with fresh cossettes, and the exhausted cossettes (pulp) were obtained in the last section, 14. The total extraction time was 70 min. The draft (juice to cossettes ratio) was varied from 120% to 90%. Exhausted pulp was pressed at 5 bar. The PEF intensity was fixed at 600 V/cm. It was demonstrated that even the cold and warm diffusion at 30–50 °C permitted effective exhausting of electroporated cossettes (Loginova et al. 2011a, c). Cold and warm diffusion led to the lower quantity of colloidal and pectin compounds released from sugar beet tissue to the extract. At such conditions the juice turbidity decreased by 10%, and it was less colored by 27%. The purification of diffusion juice obtained from the electroporated cossettes was evaluated (Loginov et al. 2011, 2012). Filtration properties of the juice of first carbonatation obtained from electroporated cossettes by cold and warm diffusion (at 30 and 50 °C) were considerably better than those of the juice obtained by hot diffusion (at 70 °C). Moreover, the thin juice of the second carbonatation obtained from electroporated cossettes had significantly paler coloration than corresponding thin juice obtained by hot diffusion from the untreated cossettes.

Later on, the characteristics of juices expressed at $P = 5$ bar from untreated, preheated (70 °C), and electroporated ($E = 600$ V/cm, $t_{PEF} = 7$ ms) sugar beet cossettes were compared (Mhemdi et al. 2014). Juice yield expressed from the untreated cossettes was rather low ($Y < 25\%$). Juice yields obtained from the preheated and PEF-treated cossettes were significantly higher (70–75% for the 30 min of pressing). However, the quality of juice expressed from the preheated cossettes was rather poor (purity of 92.5% and color of 7800 ICUMSA units) as compared to the quality of juice expressed from PEF-treated cossettes (purity of 93.5% and color of 5600 ICUMSA units). The worse quality of the juice obtained from preheated cossettes (70 °C) can be explained by the dissolution of hydrosoluble cell pectins and the enhanced enzymatic reactions at this elevated temperature. The better quality of juice obtained by cold pressing of electroporated sugar beet cossettes permits simplification of juice purification with decreased use of lime during carbonatation. Purification of juice obtained by PEF-assisted cold pressing has been studied (Mhemdi et al. 2015). The raw juice was heated to 45 °C and pre-limed with addition of CaO, and then the main liming was heated to 85 °C. The juices of first carbonatation obtained from electroporated cossettes demonstrated better filtration properties than those obtained from thermally treated cossettes (Mhemdi et al. 2015). PEF-assisted procedure permitted to decrease significantly the quantity of lime used for the juice purification and to reduce the quantity of wastes generated by the filtration station of sugar beet factory. Thin juices of second carbonatation obtained from the electroporated cossettes had significantly better quality than those obtained from the preheated to 70 °C cossettes. The purity of juice obtained from PEF-treated cossettes increased from 93.5% (raw juice) to 95.5% (thin juice) (Mhemdi et al. 2015).

The high quality of raw juice obtained by cold pressing of electroporated sugar beet cossettes allowed effective application of membrane purification (Mhemdi et al. 2014). In the proposed method, the juice was clarified by preliminary centrifugation and then filtrated at the laboratory Amicon filter under the pressure of 2 bar using the polyether sulfone (PES) membranes with pore sizes of 10–100 кDa. Ultrafiltration of juices obtained from electroporated sugar beet cossettes using dynamic filter with rotating disk (1000 tr/min) was also investigated (Zhu et al. 2015). The juice purity increased up to 96.4% using PES membrane with pore size of 10 kDa. The juice purity was even higher when lime was added for the cossette pressing (Almohammed et al. 2016a, b).

Combined pressing-diffusion experiments with sugar beet cossettes treated by PEF were conducted in a small pilot scale (Mhemdi et al. 2016). PEF-treated cossettes ($E$ = 600 V/cm, $t_{PEF}$ = 10 ms) were pressed at 5 bar at ambient temperature to obtain 50% of juice from the mass of cossettes. Then pressed pulp was loaded to the laboratory counter-current extractor with 12 extraction sections (Loginova et al. 2011a, c). Different quantities of water were used for the extraction, and the draft (water to cossettes ratio) was varied in the experiments from 80% to 100%. The extraction temperature was fixed at 30 °C or at 70 °C.

Diffusion juice (extract) was mixed with pressing juice to obtain the mixed juice. Cossettes exhausted in the extractor (pulp) were subjected to pressing in the laboratory batch press at 5 bar. Results were compared with those obtained after the conventional aqueous extraction (70 °C, draft of 120%) from the untreated sugar beet cossettes. Mixed juice obtained by pressing-diffusion technology from the electroporated sugar beet cossettes was 30% less colored than the juice obtained by conventional diffusion at 70 °C from the untreated cossettes (approximately 7000 units vs. 10,000 units ICUMSA) (Mhemdi et al. 2016). The purity of mixed juices (92.5–92.8) was higher than the purity of juice obtained by conventional diffusion (91.8%). This was explained by the very high purity of the juice obtained by cold expression from the electroporated cossettes. Decreasing diffusion temperature from 70 °C to 30 °C increased even more the quality of the mixed juice. Such juice had lowest coloration (≈ 5500 units ICUMSA) and highest purity (≈ 93.3%).

Cold pressing of PEF-treated sugar beet tails was studied to produce a fermentable juice (Almohammed et al. 2017a, b). Optimal PEF treatment protocol ($E$ = 450 V/cm, $t_{PEF}$ = 10 ms, and $W$ = 6.84 kJ/kg) allowed increasing the yield of soluble solutes from 16.8% to 79.85%, and the dryness of press-cake was increased from 15% to 24%. Juice expressed from the PEF treated tails was more concentrated (10 vs. 5.2°Brix) and contained more sucrose (8.9 vs. 4.5 °S) than the juice expressed from the untreated tails.

### Sugarcane

The effects of PEF treatment ($E$ = 400–1000 V/cm, $t_{PEF}$ = 4 ms) on sugar extraction from sugarcane stems have been studied (Almohammed et al. 2016a, b). The stems of sugar cane (diameter of 28 mm, thickness of 4 mm) were PEF treated and then

immersed in water for 1 h to extract sugar at different temperatures (20–80 °C). For the untreated samples, solutes were almost completely extracted at 80 °C ($Y_B \approx 0.98$ in Eq. (21)), while just 30% of solutes ($Y_B = 0.3$) were extracted at 20 °C. PEF treatment significantly enhanced extraction yield at lower temperatures. For instance, 60% of solutes were extracted from the electroporated stems at 20 °C (Almohammed et al. 2016a, b).

Samples of sugarcane were PEF treated ($E = 2$ kV/cm and $W = 2.4$ kJ/kg) and then pressed with intermediate water soakings at the temperatures of 45–90 °C (Eshtiaghi and Yoswathana 2012). The yield of sugar extracted from the electroporated samples at the temperature of 45 °C was nearly the same as that extracted from the untreated samples at higher temperatures of 80–90 °C. Moreover, PEF treatment allowed reduction of the extraction time by 20%.

### Chicory

The PEF-assisted ($E = 400$ V/cm, $t_{PEF} = 0.1$ s) extraction of solutes from chicory root slices (2 mm × 10 mm × 20 mm) at different temperatures ($T = 20$–$80$ °C) has been studied (Loginova et al. 2010). Kinetics of solute diffusion from chicory slices was similar to the kinetics of solute diffusion from sugar beet slices. For the untreated chicory slices, the maximum extraction yield ($Y_B \approx 1$) was obtained at $T = 70$–$80$ °C for ≈1 h. The same extraction yield was obtained for the electroporated samples at lower temperature $T = 40$ °C. Pulsed ohmic heating combining both electrical and thermal treatments has been tested to assist inulin extraction from chicory (Zhu et al. 2012). Inulin juice extracted from the electroporated chicory slices had better quality characteristics. Dynamic ultrafiltration with rotating disk was studied for the purification of inulin extracted from PEF-treated and untreated chicory slices (Zhu et al. 2013). A high rotational speed (2000 rpm) and a membrane with large pore size (0.45μm) were selected. Significant differences in the appearance and color were observed for feed juice, permeate, and retentate. Aqueous extraction of inulin from the PEF-treated ($E = 600$ V/cm, $t_{PEF} = 10$–$50$ ms) chicory slices (2 mm × 4 mm × 67 mm) was studied using a laboratory counter-current extractor (Zhu et al. 2012). Extraction temperature was varied from 30 to 80 °C. Inulin content in the juice extracted at 50–60 °C from the PEF-treated chicory slices was comparable with that extracted at 70–80 °C from the untreated slices. The purity of juice extracted from the electroporated chicory slices was higher compared to the purity of juice extracted from the untreated slices.

## Carrots

The effect of PEF treatment ($E = 180$–$360$ V/cm, $t_p = 100$μs, $\Delta t = 10$ ms, $n = 250$–$500$, $t_{PEF} = nt_p = 5$ s) on the solid/liquid expression of carrot slices was studied. Slices were obtained with 6 mm grater and pressed at the constant pressure of $P = 5$ bar (Bouzrara and Vorobiev 2001). Without PEF treatment, the juice yield was 25.6%.

With PEF treatment it was increased up to 38.3% (at $E$ = 180 V/cm) and up to 72.4% (at $E$ = 360 V/cm). Moreover, PEF treatments permitted obtaining a lighter and more concentrated soluble matter juice.

Effects of PEF treatment on the solute extraction kinetics from carrot slices have been investigated by El-Belghiti and Vorobiev (2005). Coarse slices (thickness of 1.5 mm, obtained with 6 mm grater) and fine slices (thickness of 0.5 mm, obtained with 2 mm grater) were treated by PEF ($E$ = 200–650 V/cm, $t_p$ = 100μs, $\Delta t$ = 1 ms, $n$ = 200–1000).

Figure 22a shows kinetics of aqueous solute extraction from coarse and fine carrot slices treated by PEF applied with different energies ($W$). Extraction occurred in well-stirred water at 25 °C for 8 h. In absence of the PEF treatment, only around 45% of solutes were released from the coarse slices after 8 h of extraction. With increase of the energy provided by PEF treatment, the quantity of extracted solutes increased accordingly, until a threshold value of $W \approx 9$ kJ/kg was reached. No further increase of the solute yield was observed above this threshold. Figure 22b compares extraction kinetics for different types of carrot samples (disk-like with diameter of 30 mm and thickness of 8.5 mm, coarse and fine slices). The energy provided by PEF treatment was the same ($W$ = 9 kJ/kg). In these conditions, similar extraction kinetics were observed for both coarse and fine slices, and ≈2 h were needed to attain nearly stabilized solute yield ($Y \approx 93\%$). However, the extraction was considerably slower for the disk-like samples.

Effects of centrifugal force on the extraction from PEF-treated carrot tissue were also studied (El-Belghiti et al. 2005b). Figure 23 presents kinetics of aqueous solute extraction from the untreated and PEF-treated ($E$ = 670 V/cm, $t_p$ = 100μs, $\Delta t$ = 10 ms, $n$ = 300 pulses) carrot slices at different centrifugal accelerations, ($G$) (El-Belghiti et al. 2005b). In the absence of PEF treatment, a yield of around 58% was obtained

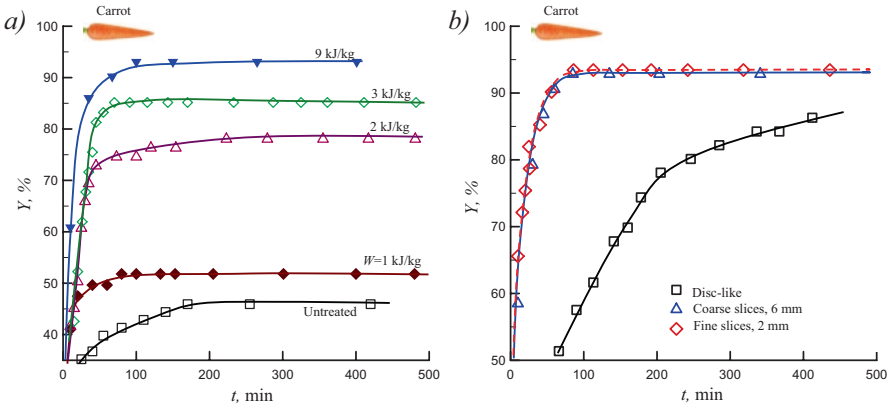


**Fig. 22** Kinetics of aqueous solute extraction ($Y(t) = C/C_\infty$) from PEF-treated carrot slices. (a) Extraction from the coarse carrot slices treated by PEF ($E$ = 300–800 V/cm) with different energies, (b) extraction from the coarse and fine carrot slices treated by PEF ($E$ = 800 V/cm) with fixed energy (9 kJ/kg). Extraction temperature was $T$ = 25 °C. (Adapted from (El-Belghiti and Vorobiev 2005))

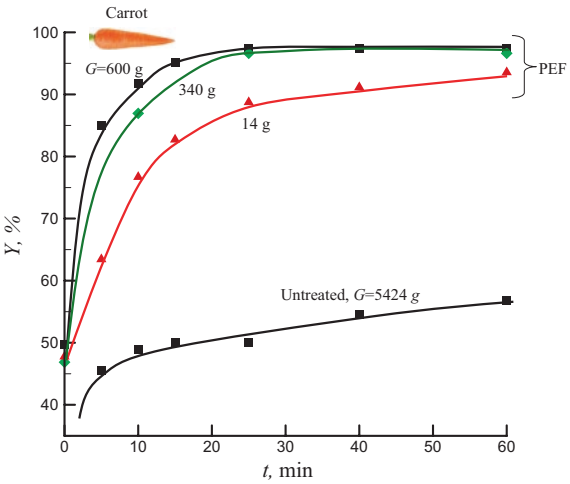


**Fig. 23** Kinetics of aqueous solute extraction ($Y_{(t)} = C/C_{\infty}$), from the untreated and PEF-treated ($E = 670$ V/cm, $n = 300$, $t_p = 100\mu$s, $t_{PEF} = 0.03$ s, $\Delta t = 10$ ms) carrot slices at $T = 20$ °C and different centrifugal accelerations ($G$). (Adapted from (El-Belghiti et al. 2005b))

after 60 min of centrifugal extraction under the high centrifugal acceleration of 5434 g. This yield corresponded to the extracted solutes from cells, which were broken mechanically during slicing. After the PEF treatment, solute yield was significantly higher. Extraction yield increased up to around 92% after 60 min of centrifugal extraction even at low centrifugal acceleration ($G = 14$ g). For the centrifugal accelerations above $G \approx 150$ g, no further enhancement of solute concentration was observed. At this acceleration threshold, the solute concentration of around 97% was reached for 25 min of centrifugation. For the fixed centrifugal acceleration ($G = 150$ g), the final yield of solutes was approximately the same ($Y \approx 97\%$) at various extraction temperatures in the interval between 18 and 35 °C. However, this yield was attained for 15 min at 35 °C and for 40 min at 18 °C.

## Red Beets

PEF-assisted extraction of pigments from red beets has been studied in several works (López et al. 2009; Fincan 2017). Water-soluble betalains (red-violet betacyanins and yellow betaxanthins) are the main red beet pigments (Jackman and Smith 1996; Rodriguez-Amaya 2019). The important fraction of pigments (75–95% mass) is related to betanine. These pigments have application in the food, medical, and pharmaceutical industries, owing to their visual and health-promoting functional properties (Tiwari and Cullen 2012). However, the temperature factor is very important for the extraction of these heat-sensitive pigments (Leong et al. 2018). A PEF treatment of 1 kV/cm allowed releasing ≈ 90% of the total red pigment during 1 h of aqueous extraction (Fincan et al. 2004). The kinetics of PEF-assisted extraction from red beet slices was described using a bimodal Fickian diffusion model (Chalermchat et al. 2004). PEF treatment (1–9 kV/cm) was applied to extract betanine from red beet roots at different temperatures ($T$ = 10–60 °C) and pH values

(3.0–6.5) (López et al. 2009). At optimal operational conditions ($E$ = 7 kV/cm, $t_{PEF}$ = 10μs, $T$ = 30 °C and pH 3.5), ≈90% of total betanine was extracted after 300 min. Effects of PEF treatment ($E$ = 375–1500 V/cm) and temperature ($T$ = 30–80 °C) on the kinetics of extraction and degradation of colorants of red beet have been studied (Loginova et al. 2011b). PEF treatment was found to be effective for the acceleration of colorant extraction and reducing operational time. Increase of the temperature caused acceleration of both extraction and colorant degradation processes. The colorant degradation was rather important at $T \geq 60$ ° C. PEF treatment allowed effective "cold" extraction with a high level of colorant extraction and a small level of colorant degradation. For example, for extraction at $T$ = 30 °C, PEF treatment allowed reaching of the high yield ($Y \approx 0.95$) at the low level of degradation ($D \approx 0.10$).

The efficiencies of PEF-assisted extraction of colorant from red beets have been compared for different protocols (Luengo et al. 2016). For PEF protocols applied at $E$ = 0.6 kV/cm, $t_{PEF}$ = 40 ms, $W$ = 43.2 kJ/kg and at $E$ = 6 kV/cm, $t_{PEF}$ = 150μs, $W$ = 28.8 kJ/kg, the betanine extraction yield increased by 6.7 and 7.2 times, respectively, compared to the untreated samples.

## Onions

In many studies, onions have been used as a model material to investigate effects of PEF on the cell membrane permeabilization (Fincan and Dejmek 2002; Ben Ammar et al. 2011b). For PEF treatments above a threshold level of $E$ = 350 V/cm, damage of onion cells was observed (Fincan and Dejmek 2002). The permeabilization occurred along preferential paths connecting the electrodes. Effects of PEF protocols (electric field strength, pulse width, total pulse duration, and frequency) on the onion damage efficiency was studied in several works (Asavasanti et al. 2010; Ersus et al. 2010; Asavasanti et al. 2011a, 2012). Irreversible cell rupture was observed for PEF treatment of 333 V/cm (Ersus and Barrett 2010).

At constant total PEF treatment time, the degree of tissue disintegration was increased with shorter pulse widths and a larger number of pulses (Ersus et al. 2010). With application of ten pulses of 100μs, two critical values of electric field strength $E$ were established: a lower value of $E$ = 67 V/cm (for cell membrane breakdown) and a higher value of $E$ = 200 V/cm (for tonoplast membrane breakdown) (Asavasanti et al. 2010). The effect of PEF frequency on the integrity of onion tissues was studied. It was established that lower frequencies ($f$ < 1 Hz) can cause more important disintegration effect than higher frequencies ($f$ = 1 to 5000 Hz) at the same value of $E$ = 333 V/cm (Asavasanti et al. 2011a). This effect was explained by the influence of PEF frequency on the cytoplasmic streaming (Asavasanti et al. 2012).

Effects of PEF treatment ($E$ = 0.3, 0.7 and 1.2 kV/cm) on the volatile compounds produced in onion cultivars were studied (Nandakumar et al. 2018). Significant increases in the concentrations of alkanes, aldehyde (2-methyl-2-pentenal), and the sulfur-containing compounds were observed. These changes were explained by

PEF-induced electroporation and facilitation of the enzyme-substrate reactions after the PEF treatment. PEF-assisted (2.5 kV/cm) aqueous extraction of phenolic (PC) and flavonoid (FC) compounds from the onion tissue was also investigated (Liu et al. 2018). Under the optimal treatment conditions, the content of PC and FC in extracts was increased by 2.2 and 2.7 times, respectively, in comparison to the control (untreated) sample. PEF treatment (0.3–1.2 kV/cm) was applied to bunching onion bulbs (Liu et al. 2019). The carbohydrate leakage and fructan leakage from onion bulbs were observed.

### Tomatoes

PEF treatment ($E$ = 3–7 kV/cm, $t_p$ = 3μs, $t_{PEF}$ = 0–300μs) was used to enhance the carotenoid extraction from pulp and peels of tomato (*Canario* variety) (Luengo et al. 2014a). Carotenoid extraction from tomato pulp was not significantly increased by the PEF treatment. However, the PEF treatment ($E$ = 5 kV/cm, $t_{PEF}$ = 90μs) of tomato peels was more successful and permitted improvement of the carotenoid extraction yield by 39% as compared to the control extraction in a mixture of hexane:ethanol:acetone (50:25:25).

Moreover, PEF treatment permitted reduced hexane proportion in the mixture and decreased extraction time without affecting extraction yield. Extracts obtained with PEF treatment had a higher antioxidant capacity than the control ones due to their higher carotenoid concentration. The extractability of carotenoids from tomato peels (*Pachino* variety) treated by PEF ($E$ = 0.25–0.75 kV/cm, 1 kJ/kg) and steam blanched (1 min at mild temperatures 50–70 °C) was also studied (Pataro et al. 2018). Extraction was realized in acetone. PEF treatment alone increased the carotenoid extractability by 44%, 144%, and 189%, respectively, at $E$ = 0.25, 0.50, and 0.75 kV/cm. Steam blanching alone also increased the carotenoid extractability (60–188%). Meanwhile, a synergetic effect of combined PEF and steam blanching was observed (Pataro et al. 2018).

PEF treatment ($E$ = 4–24 kV/cm) applied for the assistance of lycopene extraction from tomato pulp (*Savoura* variety) permitted improvement of the extraction yield by 68.8% using optimal value of $E$ = 16 kV/cm (Gachovska et al. 2013).

### Citruses

The citrus peels resulted from industrial production of different juices (orange, lemon, grapefruit, pomelo, and lime) constitute about 50% of fresh fruit weight, and they represent the reach source of polyphenols, pigments, and essential oils (Putnik et al. 2017). Recovery of these components can be significantly improved with assistance of PEF. The effects of PEF treatment at $E$ = 1, 3, 5, and 7 kV/cm on the extraction by pressing of total polyphenols and flavonoids (naringin and hesperidin) from orange peel have been investigated (Luengo et al. 2013). For treatment at $E$ = 7 kV/cm after pressing for 30 min at $P$ = 5 bar, the total polyphenol extraction

yield increased by 159%, and the antioxidant activity of the extract increased by 192%. PEF treatment at $E$ = 10 kV/cm has been applied for the assistance of polyphenol extraction from orange peels in ethanol-water solutions (0–50% for 1 h) (Kantar et al. 2018). With 50% of ethanol in solution, the extraction yields were 12 and 22 mg GAE/g DM, respectively, for the untreated and PEF-treated samples. PEF treatment at $E$ = 3–9 kV/cm and 0–300μs on the extraction of polyphenol from lemon peel residues by pressing has been also investigated (Peiró et al. 2019). The effects of PEF were independent of lemon residue size. The treatment at $E$ = 7 kV/cm increased the efficiency of polyphenol extraction by 300%.

### Winemaking from Grapes

The red and white grape berries are rich in bioactive molecules such as sugars, carbohydrates, fruit acids (mainly tartaric and malic), mineral elements, and vitamins (Rousserie et al. 2019). During the last decades, different applications of PEF treatment have been tested for the assistance of maceration/extraction processes in red and white winemaking and for the recovery of bioactive molecules from pomace, grape seeds, and vine shoots. Different examples of PEF applications for the improvement of winemaking in batch and continuous flow modes were described (Praporscic et al. 2007; López et al. 2008a, b; Grimi 2009; Noelia et al. 2009; Grimi et al. 2009b; Donsi et al. 2010; Puértolas et al. 2010a; Sack et al. 2010; Puértolas et al. 2010b; Donsi et al. 2011; Delsart et al. 2012; El Darra et al. 2013a; Garde-Cerdán et al. 2013; López-Alfaro et al. 2013; El Darra et al. 2013b; Delsart et al. 2014; Luengo et al. 2014b; López-Giral et al. 2015; El Darra et al. 2016a, b; Saldaña et al. 2017; Vicaş et al. 2017; Comuzzo et al. 2018).

### Mushrooms

Mushrooms are rich in antioxidant, anticancer, and anti-inflammatory components including polysaccharides, antioxidants, polyphenols, proteins, lipids, and vitamins (Muszyńska et al. 2018). They also contain bioactive molecules like β-glucans and triterpenoids that can act as immune-modulators (Rathore et al. 2017). However, the conventional solid/liquid extraction of these bioactive molecules involves organic solvents and requires elevated temperatures. This may result in the noticeable degradation of valuable cell compounds.

PEF-assisted solid/liquid extraction of polysaccharides from mushroom *Inonotus obliquus* (commonly known as chaga) was investigated (Yin et al. 2008). PEF treatment was applied at $E$ = 30 kV/cm at the solid/liquid ratio of 1:25. The data were compared with alkali extraction, microwave-assisted extraction, and ultrasonic-assisted extraction. The PEF-assisted method gave the highest extraction yield and better purity of extracts. The PEF-assisted solid/liquid extraction of exopolysaccharides from Tibetan spiritual mushroom was also studied (Zhang et al. 2011). The

PEF treatment at $E = 40$ kV/cm under the optimal conditions allowed increasing the extraction yield by 84.3% compared to the control sample.

Later on, the efficiency of extraction and stability of extracts obtained from mushroom (*Agaricus bisporus*) by pressure extraction (PE) and by pressure extraction combined with PEF (PE + PEF) was studied (Parniakov et al. 2014). Extraction was conducted at room temperature at a constant pressure of 5 bar. In PE + PEF experiments, the PEF treatment was done in nonstationary conditions, because the thickness of mushroom cake decreased and the applied electric field strength increased during pressing (from 800 V/cm up to 1333 V/cm). The obtained data were compared with hot water extraction (WE) at elevated temperature $T = 70$ °C for 2 h and with ethanol extraction (EE) at $T = 25$ °C for 24 h.

Application of WE or EE methods resulted in high contents of proteins, total polyphenols, and polysaccharides in extracts. However, these extracts were cloudy and unstable. The extracts, produced by PE and PE + PEF methods, were clear and their colloid stability was high. Moreover, application of PE allows selective extraction of bioactive compounds with high purity, fresh-like proteins and polysaccharides. Both PE and PE + PEF methods allowed selective separation of different components. Application PE + PEF method gave highest nucleic acid/proteins ratio and allowed production of mushroom extracts with high contents of fresh-like proteins and polysaccharides. Supplementary EE from solid residues (cakes) allowed additional increase of yield of bioactive compounds.

For example, Fig. 24 compares concentration of total polyphenols obtained by PE, PE + PEF, aqueous (WE), and ethanol extraction (EE) methods. Supplementary ethanol extraction was also evaluated.

PEF-assisted solid/liquid extraction of polysaccharides from mushroom (*Agaricus bisporus*) was studied (Xue and Farid 2015). PEF treatment was applied

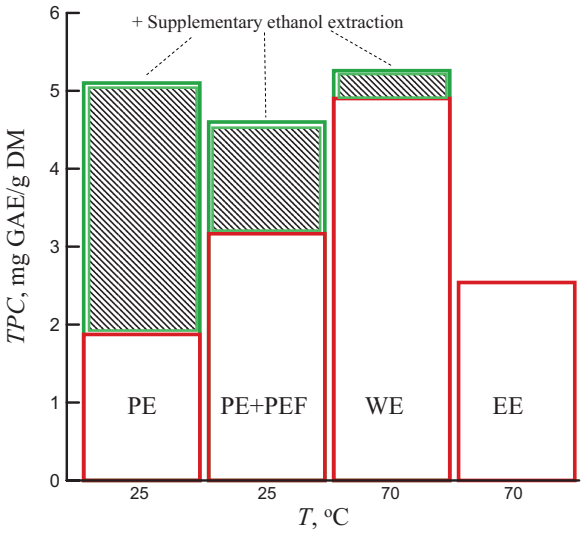


**Fig. 24** Concentration of total polyphenols (TPC), in the mushroom extracts obtained by pressure extraction (PE), PEF-assisted pressure extraction (PE + PEF), aqueous extraction (WE), and ethanol extraction (EE). The effects of supplementary ethanol extraction ($T = 25$ °C, 24 h) from mushroom cakes are shown by dashed areas. (Adapted from (Parniakov et al. 2014))

to a mushroom suspension (9% w/w) in continuous mode in flowing chamber. PEF treatment ($E$ = 38.4 kV/cm, $t_{PEF}$ = 272μs, and a treatment temperature of $T$ = 85 °C) permitted extract ≈98% polysaccharides, ≈51% total polyphenols, and ≈49% proteins, whereas the conventional extraction (at 95 °C for 1 h) resulted in recovery of only ≈56% polysaccharides, ≈25% total polyphenols, and ≈45% proteins.

PEF-assisted solid/liquid extraction of Morchella esculenta polysaccharide (MEP) from morel mushroom (*Morchella esculenta*) was studied (Liu et al. 2016). PEF treatment was applied at $E$ = 15–25 kV/cm at the solid/liquid ratio of S/L = 1:20–1:40. For PEF-assisted procedure, the extracted polysaccharides were less degraded compared to those obtained by heat extraction.

A general review on the nonconventional methods of valuable compound recovery from mushrooms (including enzyme-assisted extraction, PEF, ultrasound, microwaves, subcritical and supercritical fluid extraction) was recently published (Roselló-Soto et al. 2016).

## Conclusions

Pulsed electric fields (PEF) can be used as effective tool for the permeabilization of cell membrane by electroporation mechanism. Efficiency of electroporation depends critically on pulse protocols and properties of food materials. Under properly adapted PEF protocols, the efficient and highly selective extraction of useful molecules from food plants and residues is possible. Moreover, PEF treatment allows the "pure" extraction with preservation of quality, color, flavor, vitamins, and important nutrients of products. PEF-assisted extraction is in a full correspondence with the green extraction concept, i.e., it allows excluding the toxic organic solvents. Many promising examples of the PEF-assisted extraction of juices, sugars, proteins, polyphenolic substances, and oils from different foods were already demonstrated. However, more investigations at pilot and preindustrial scales are still required to evaluate the advantages of PEF treatment taking into account quality of products, economic efficiency, and environmental aspects.

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