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Mass Transfer Enhancement by Means of Electroporation

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1. Introduction

PEF treatment involves the application of repetitive ultra-short pulses (from ns to μ s) of a high-strength electric field (0.1-10 kV/cm) through a material located between two electrodes. The application of the external electric field induces the permeabilization of cytoplasmatic membranes. The main advantages of PEF with respect to other treatments addressed to disrupt the cell membranes, such as the application of heat or the addition of pectolytic enzymes, are as follows:

- Cost reduction due to lower energy consumption and unnecessary enzyme addition
- Higher purity of the extracts, since upon the PEF treatment the permeabilized cell membranes maintain their structural integrity and are not disrupted in small fragments
- Lower processing times thanks to the increased mass transfer rates.

The application of PEF as a permeabilization treatment to increase the rates of mass transfer of valuable compounds from biological matrices was demonstrated to be effective in drying, extraction, and diffusion processes.

This chapter reviews the basic mechanisms of PEF-induced permeabilization of plant tissues, discusses the methods of detection of electrically induced cell damages and analyses the influence of PEF process parameters on mass transfer. Furthermore, mathematical models to describe the mass transfer rates from PEF-treated vegetable tissue are discussed and some criteria of energy optimization are given as well as some examples on the recovery of polyphenolic compounds from food matrices and on the integration of PEF treatments in the winemaking industry.

2. Basic considerations and mechanism

The application of pulsed electric fields to biological cells (plant or animal) mainly affects the cell membranes, inducing local changes in their structures and promoting the formation of pores. This phenomenon, named electroporation (or electropermeabilization), causes a drastic increase in the permeability of cell membranes, which lose their semipermeability, either temporarily or permanently (Weaver & Chizmadzhed, 1996). Electroporation is today widely used in biotechnology and medicine to deliver drugs and genes into living cells

(Neumann et al., 1982; Fromm et al., 1985; Mir, 2000; Serša et al., 2003; Miklavčič et al., 2006). Recently, the interest in electroporation has considerably grown, as it offers the possibility to develop different non-thermal alternatives to the traditional processing methods of the food industry requiring the disintegration of cell membrane. For example, the complete damage of the microbial cell membrane induced by the application of intensive PEF process conditions has been intensively studied in the last twenty years as a new non-thermal method of food preservation (Barsotti and Cheftel, 1999; Mosqueda-Melgar et al., 2008; Pataro et al., 2011). More interestingly, it has been also reported by several research teams that the application of a pulsed electric fields pre-treatment of moderate intensity to biological tissue may considerably increase the mass and heat transfer rates between plant cells and the surroundings, making it suitable for enhancing the efficiency of the pressing, extraction, drying and diffusion processes of the food industry (Angersbach, 2000; Vorobiev et al., 2005; Vorobiev and Lebovka, 2006; Donsì et al., 2010b).

The exact mechanism of electroporation is not yet fully understood. Several theories (Chang, 1992; Neumann et al., 1992; Zimmermann, 1986) based on the experiments carried out on model systems such as liposomes, planar bilayers, and phospholipid vesicles were proposed to explain the mechanism of the reversible electroporation and/or the electrical membrane breakdown. All of these theories in their differences are characterized by advantages and disadvantages, but they share a common feature: the cell membrane plays a significant role in amplifying the applied electric field, as the conductivity of intact membrane is several orders of magnitude lower than the conductivities of extra cellular medium and cell cytoplasm (Weaver and Chizmadzhev, 1996). Hence, when the biological cells are exposed to an external electric field E , the trans-membrane potential (u_m) increases as a result of the charging process at the membrane interfaces. In Fig. 1 the simple case of a sphere shaped biological cell is considered. The trans-membrane potential u_m can be derived from the solution of Maxwell's equation in spherical coordinates, assuming several simplifying restrictions (Neumann, 1996), according to Eq. 1, where r_{cell} is the radius, and θ is the angle between the site on the cell membrane where u_m is measured and the direction of the vector E .

$$E_m = 1.5 \cdot r_{cell} \cdot E \cdot \cos(\theta) \quad (1)$$

The highest drop of potential occurs at the cell poles ($\theta = 0, \pi$), and decreases to 0 at $\theta = \pm\pi/2$. That is why the maximum membrane damage probability occur at the poles of the cell exposed to the electric field facing the electrodes (Fig. 1). Being the membrane thickness h (≈ 5 nm) significantly smaller than the plant cell radius (≈ 100 μm), a selective concentration of the electric field on the membrane occurs, creating a trans-membrane electric field, $E_m = u_m/h$, which is about 10^5 times higher than the applied field strength (Vorobiev and Lebovka, 2008; Weaver and Chizmadzhev, 1996).

If a critical value of the field strength E_c is exceeded, a critical trans-membrane potential can be induced (typically 0.2-1.0 V for most cell membranes) that leads to the formation of reversible or irreversible pores in the membrane (Zimmermann and Neil, 1996). The occurrence of reversible or irreversible permeabilization of the cell membranes depends on the intensity of the external electric fields, pulse energy and number of pulses applied. The greater the value of these parameters, the higher is the extent of the membrane damage (Angersbach et al., 2002). When a mild PEF treatment is applied, either because the electric

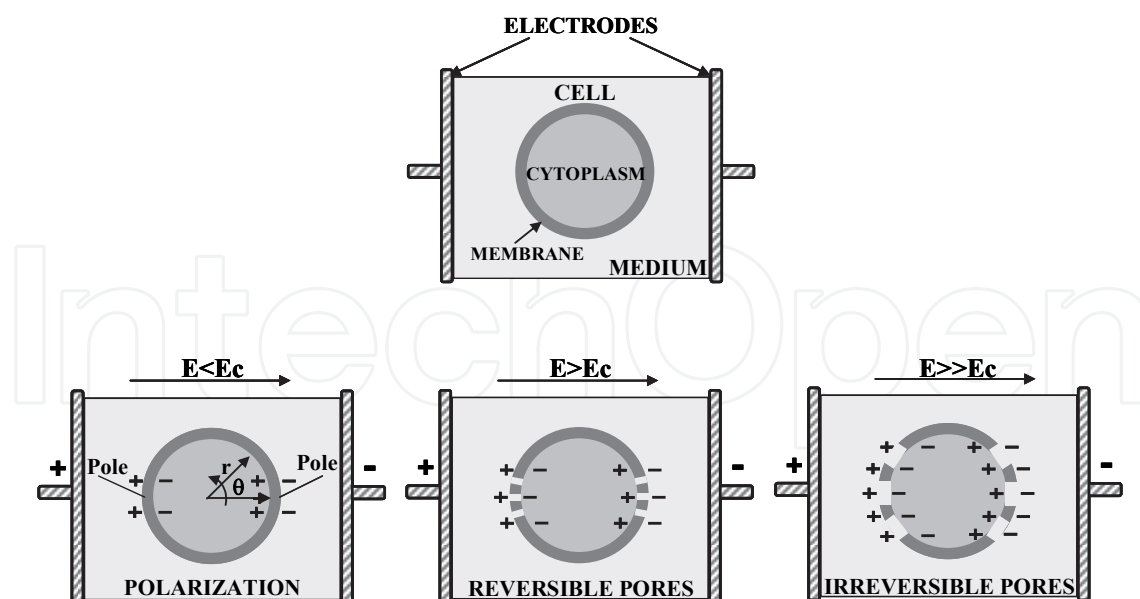


Fig. 1. Schematic depiction of the permeabilization mechanism of a biological cell membrane exposed to an electric field E . Electroporated area is represented with a dashed line. E_c : critical electric field strength.

field applied is below the critical value E_c or the number of pulses is too low, reversible permeabilization occurs, allowing the cell membrane to recover its structure and functionality over time. On the contrary, when more intense PEF treatment is applied, irreversible electroporation takes place, resulting in cell membrane disintegration as well as loss of cell viability (Zimmerman, 1986). According to Eq. 1, the external electric field to be applied in order to reach the critical trans-membrane potential decreases with the cell radius increasing. Being the plant tissue cells rather larger ($\approx 100 \mu\text{m}$) than microbial cells ($\approx 1\text{-}10 \mu\text{m}$), the electric field strength required for electroplasmolysis in plant cells ($0.5\text{-}5 \text{ kV/cm}$) (Knorr, 1999) is lower than that required for inactivation microorganisms ($10\text{-}50 \text{ kV/cm}$) (Barbosa-Canovas et al., 1999). However, modifications of the properties of the cell membranes occurring during the PEF treatment cause the critical electric field, required to cause disruptive effects on biological cells, to decrease. Experimental results have demonstrated that the rupture (critical) potential of the lipid-proteins membranes ranges from 2 V at 4°C to 1 V at 20°C and 500 mV at $30\text{-}40^\circ\text{C}$ (Zimmermann, 1986). The increase in temperature promotes greater ions mobility through the cell membranes, which become more fluid, and decreases their mechanical resistance (i.e. elastic modules) (Coster and Zimmermann, 1975).

Overall, the electroporation process consists of different phases. The first of them, which does not contribute to molecular transport, is the temporal destabilization and creation of pores (reported as occurring on time scales of 10 ns), during the charging and polarization of the membranes. The charging time constant ($1 \mu\text{s}$), defined as the time between electric field application and the moment when the membrane acquires a stable electric potential, is a parameter specific for each treated vegetable or animal tissue, which depends on cellular size, membrane capacitance, the conductivity of the cell and the extracellular electrolyte (Knorr et al., 2001). The second phase is a time-dependent expansion of the pores radii and aggregation of different pores (in a time range of hundred of microseconds to milliseconds, lasting throughout the duration of pulses). The last phase, which takes place after electric

pulse application, consist of pores resealing and lasts seconds to hours. Molecular transport across the permeabilized cell membrane associated with electroporation is observed from the pore formation phase until membrane resealing is completed (Kandušer and Miklavčič, 2008). Therefore, in PEF treatment of biological membranes, the induction and development of the pores is a dynamic and not an instantaneous process (Angersbach et al., 2002).

3. Detection and characterization of cell disintegration in biological tissue

The first studies on the degree of cell membrane permeabilization were based on quantifying the release of intracellular metabolites (i.e. pigments) from vegetable cells after electroporation induced by the application of PEF (Brodelius et al., 1988; Dörnenburg and Knorr, 1993). The irreversible permeabilization of the cells in vegetable tissue was demonstrated for the first time for potato tissue (exposed to PEF treatment), determining the release of the intracellular liquid from the treated tissue using a centrifugal method. A liquid leakage from the tissue of PEF-treated samples was detected, while no-release occurred from the control samples. This leakage was therefore interpreted as a consequence of the cellular damage by the electrical pulses inside the cells of the tissue (Angersbach and Knorr, 1997). However, in order to obtain a quantitative measure of the induced cell damage degree P , defined as the ratio of the damaged cells and the total number of cells, several methods have been defined. The direct estimation of the damage degree can be carried out through the microscopic observation of the PEF-treated tissue (Fincan and Dejmek, 2002). However, the procedure is not simple and may lead to ambiguous results (Vorobiev and Lebovka, 2008). Therefore, experimental techniques based on the evaluation of the indicators that macroscopically register the complex changes at the membrane level in real biological systems have been introduced. For example, the value of P could be related to a diffusivity disintegration index Z_D estimated from diffusion coefficient measurements of PEF-treated biological materials during the following extraction process (Jemai and Vorobiev, 2001; Lebovka et al., 2007b), where D is the measured apparent diffusion coefficient, with the subscript i and d referring to the values for intact and totally destroyed material, respectively.

$$Z_D = \frac{D - D_i}{D_d - D_i} \quad (2)$$

The apparent diffusion can be determined from solute extraction or convective drying experiments. Unfortunately, diffusion techniques are not only indirect and invasive for biological objects, but they may also have an impact on the structure of the tissue. Furthermore, also the validity of the Eq. 2 is still controversial (Vorobiev et al., 2005; Lebovka et al., 2007b).

Measurements of the changes in the electrophysical properties such as complex impedance of untreated and treated biological systems have been suggested as a simple and more reliable method to obtain a measurement of the extent of damaged cells (Angersbach et al., 2002). Intact biological cells have insulated membranes (the plasma membrane and the tonoplast) which are responsible for the characteristic alternating current-frequency dependence on the biological material's impedance. These membranes are faced on both sides with conductive liquid phases (cytosol and extracellular liquid), as illustrated in Fig. 2. Therefore, the electrical behavior of a single intact plant cell is equivalent to an ohmic-capacitive circuit in which insulated cell membranes can be assumed to be a capacitor

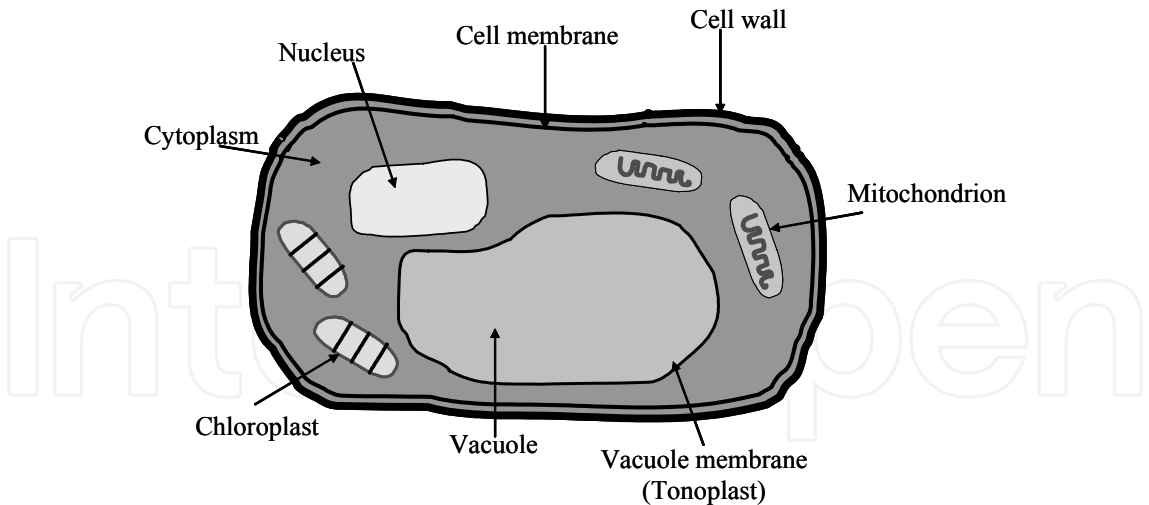


Fig. 2. Simplified scheme of anatomy of plant cells.

connected in parallel to a resistor, while the conductive liquid on both sides of the membranes can be introduced to this circuit as two additional resistors (Fig. 3a) (Angersbach et al., 1999). Hence, the electrophysical properties of cell systems, as characterized by the Maxwell-Wagner polarization effect at intact membrane interfaces, can be determined on the basis of impedance measurements in a frequency range between 1 kHz and 100 MHz, which is called β -dispersion (Angersbach et al., 2002). The complete disintegration of the cytoplasm membranes and tonoplast of plant cells reduces the equivalent circuit to a parallel connection of three ohmic resistor, formed by electrolyte of the cytoplasm, the vacuole, and the extracellular compartments, respectively (Fig. 3b).

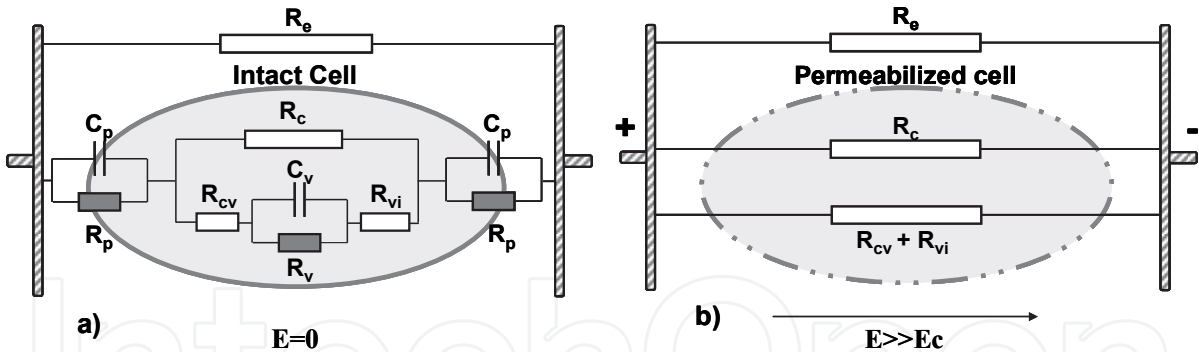


Fig. 3. Equivalent circuit model of (a) an intact and (b) ruptured plant cell. R_p, R_v , plasma and vacuole membrane (tonoplast) resistance; C_p, C_v , plasma and vacuole membrane (tonoplast) capacitance; R_c , cytoplasmic resistance surrounding the vacuole in the direction of current; R_{cv} , cytoplasmic resistance in vacuole direction; R_{vi} , resistance of the vacuole interior; R_e , resistance of the extracellular compartment (Adapted from Angersbach et al., 1999).

The impedance-frequency spectra of intact and treated samples are typically determined with an impedance measurement equipment in which a sample, placed between two parallel plate cylindrical electrodes, is exposed to a sinusoidal or wave voltage signal of alternative polarity with a fixed amplitude (typically between 1 and 5 V peak to peak) and frequency (f) in the range of 3 kHz to 50 MHz. However, the range of characteristic low and high frequencies used depends on the cell size in relation to the conductivity of cell liquid and neighboring fluids, as shown in Table 1 (Angersbach et al., 2002).

Biological material	Low frequency (kHz)	High frequency (MHz)
Large cells		
Animal muscle tissue	≤3	≥15
Fish tissue (mackerel or salmon)	≤3	≥3
Plant cells (apple, potato, or paprika)	≤5	≥5
Small cells		
Yeast cells (<i>S. cerevisiae</i>)	≤50	≥25

Table 1. Characteristic low and high frequency values for different biological material.

Electrical impedance is determined as the ratio of the voltage drop across the sample and the current crossing it during the test. The complex impedance $Z(j\omega)$ is expressed according to Eq. 3, where j is the imaginary unit, $\omega = 2\pi f$ is the angular frequency, $|Z(j\omega)|$ is the absolute value of the complex impedance, and φ the phase angle between voltage across the sample and the current through it.

$$Z(j\omega) = |Z(j\omega)| \cdot e^{j\varphi}$$

(3)

As the complex impedance $Z(j\omega)$ depends on the geometry of the electrode system, the specific conductivity $\sigma(\omega)$ can be instead used (Knorr and Angersbach, 1998; Lebovka et al., 2002; Sack and Bluhm, 2008). For the plate electrode system it has been calculated according to Eq. 4, where l_s is the length of the sample and A_s is the area perpendicular to the electric field.

$$\sigma(\omega) = \frac{l_s}{A_s |Z(j\omega)|}$$

(4)

The results of numerous experiments indicate that the impedance or conductivity-frequency spectra of intact and processed plant tissue in a range between 1 kHz and 50 MHz can typically be divided into characteristic zones (Angersbach et al., 1999). Fig. 4a shows a typical frequency-impedance spectra for artichoke bracts and the transition from an intact to ruptured state in the frequency range of the measured current of 100 Hz to 10 MHz. The results show that the absolute value of the impedance of the intact biological tissue is strongly frequency dependent. This is because in the low frequency field the cell membrane acts as a capacitor preventing the flow of the electric current in the intracellular medium (ohmic-capacitive behavior). Upon increasing the frequency, the cell membrane becomes less and less resistant to the current flow in the intracellular liquid. At very high frequency values, the membrane is totally shorted out and the absolute value of the complex impedance is representative of the contribution of both extra and intracellular medium (pure ohmic behavior). Thus, the tissue permeabilization induced by an external stress such as PEF treatment, is detectable in the low frequency range. In the high frequency range, because the cell membrane does not show any resistance to the current flow, there is practically no difference between the impedance of intact cells and cells with ruptured membranes. As PEF treatment intensity (field strength and energy input) increases, the extent of membrane permeabilization also increases, thus leading to a significant lowering of the impedance value. When the cells are completely ruptured, the impedance reaches a constant value, exhibiting no frequency dependence (pure ohmic

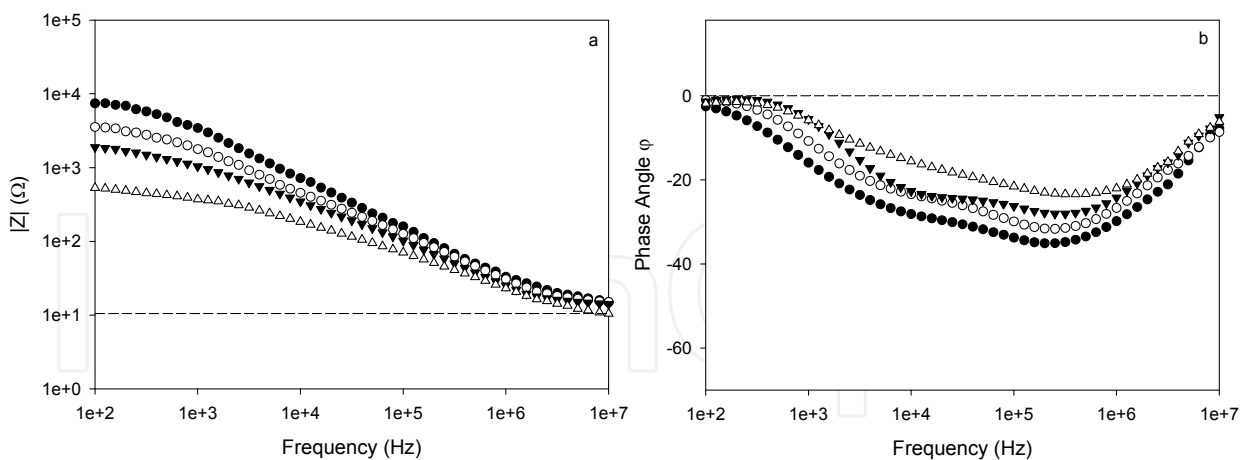


Fig. 4. (a) Absolute value ($|Z|$) and (b) phase angle (ϕ) of the complex impedance of control and PEF-treated artichoke bracts as a function of frequency (Unpublished data). (●) Control; (○) 3 kV/cm, 1 kJ/kg; (▼) 3 kV/cm, 10 kJ/kg; (△) 7 kV/cm, 10 kJ/kg; (---) theoretical trend of completely ruptured cells.

behavior) (Battipaglia et al., 2009; Pataro et al., 2009). However, the typical electrical behaviour of intact and processed plant tissue can be also analysed in terms of frequency-phase angle spectra (Pataro et al., 2009; Battipaglia et al., 2009; Sack and Bluhm, 2008; Sack et al., 2009). Fig. 4b shows a typical frequency-phase angle spectra for artichoke bracts and the transition from intact to ruptured state in the frequency range of the measured current of 100 Hz to 10 MHz. According to the ohmic-capacitive behavior of intact biological tissue, a negative value of the phase angle is detected. In particular, at characteristic low and high frequencies, the imaginary component of the cell impedance is equal to zero (Angersbach et al., 1999; Angersbach et al., 2002). Hence, the phase angle between voltage and current approaches zero, which is the typical behavior of a pure ohmic system.

At medium frequencies, the influence of the capacitive current through the cell membranes on the phase angle is quite high and a minimum value of the phase angle is detected. As reported in Table 2, the minimum phase angle varies with the type of plant material. During the PEF treatment, the capacitances of the cell membranes become more and more shortened, and the increase of the phase angle can be taken as a measure for the degree of electroporation. If all cells are opened completely, the phase angle approaches zero in the ideal case (Pataro et al., 2009; Sack et al., 2009).

In order to quantify the cellular degree of permeabilization, a coefficient Z_p , the cell disintegration index, has been defined on the basis of the measurement of the electrical complex conductivity of intact and permeabilized tissue in the low ($\approx 1\text{--}5$ kHz) and high (3–50 MHz) frequency ranges (Angersbach et al., 1999), as shown in Eq. 5, where σ is the electrical conductivity, the superscripts i and t indicate intact and treated material, respectively, and the subscripts l and h the low and high frequency field of measurement, respectively.

$$Z_p = \frac{(\sigma_h^i / \sigma_h^t) \sigma_l^t - \sigma_l^i}{\sigma_h^i - \sigma_l^i}$$

(5)

Biological material	Frequency (kHz)(*)	Reference
Apple	50	(Sack et al. 2009)
Carrots	100	(Sack et al. 2009)
Potato	90	(Sack et al. 2009)
Artichoke	200	(Battipaglia et al. 2009)
Sugar beet	50	(Sack and Bluhm 2008)
Pinot noir grapes	100	(Sack et al. 2009)
Alicante grapes	400	(Sack et al. 2009)
Aglianico grapes	300	(Donsi et al., 2010a)
Piediroso grapes	900	(Donsi et al., 2010a)
Muskateller mash	300	(Sack et al. 2009)
Riesling mash	700	(Sack et al. 2009)

Table 2. Typical frequency value of minimum phase angle for different biological material.

The disintegration index characterizes the proportion of damaged (permeabilized) cells within the plant product (Knorr and Angersbach, 1998). It is the average cell disintegration characteristic in the sample and describes the transition of a cell from an intact to ruptured state (Ade-Omowaye et al., 2001). For intact cells, $Z_p=0$; for total cell disintegration, $Z_p=1$. Another definition of the cell disintegration index Z_p was given by Lebovka et al. (2002), based on the work of Rogov and Gorbатов (1974) according to Eq. 6, where σ is the measured electrical conductivity value at low frequencies (1–5 kHz) and the subscripts i and d refer to the conductivities of intact and totally destroyed material, respectively

$$Z_p = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i}$$

(6)

Therefore, σ_i and σ_d can be estimated as the conductivity value of untreated material in low frequency range and the conductivity value of treated material in the high frequency range, respectively (Donsi et al., 2010b). As in the previous case, $Z_p=0$ for intact tissue and $Z_p=1$ for totally disintegrated material. This method has proved to be a useful tool for the determination of the status of cellular materials as well as the optimization of various processes regarding minimizing cell damage, monitoring the improvement of mass transfer, or for the evaluation of various biochemical synthesis reactions in living systems (Angersbach et al., 1999; Angersbach et al., 2002). Unfortunately, there exists no exact relation between the disintegration index Z_p and damage degree P , though it may be reasonably approximated by the empirical Archie’s equation (Eq. 7) (Archie, 1942), where exponent m falls within the range of 1.8-2.5 for biological tissue, such as apple, carrot and potato (Lebovka et al. 2002).

$$Z_p \approx P^m$$

(7)

In summary, electroporation of biological tissue and the consequent mass transfer process are complex functions of material properties which, in turn, are spatially dependent and highly inhomogeneous. The use of methods based on the evaluation of macroscopic indicators, such as those described above, can help to better understand the complex

changes occurring at the membrane level during the electroporation processes as well as clarify how the subsequent leaching phenomena are affected by the degree of membrane rupture. However, all these methods are indirect and do not allow the exact evaluation of the damage degree. In addition, it should be also considered that, depending on the type of process and on the food matrices used, not all the indicators are able to accurately quantify the release of intracellular metabolites from plant tissue in relation to the cell damage induced by PEF. Probably, the use of multiple indicators such as those evaluated by the simultaneous diffusion and electrical conductivity measurements during solid-liquid leaching process assisted by PEF, should be used to provide a more simple and effective way of monitoring the extraction process.

4. Influence of PEF process parameters

According to electroporation theory, the extent of cell membrane damage of biological material is mainly influenced by the electric treatment conditions. Typically, electric field strength E , pulse width τ_p and number of pulses n_p (or treatment time $t_{PEF} = \tau_p n_p$) are reported as the most important electric parameters affecting the electroporation process. In general, increasing the intensity of these parameters enhances the degree of membrane permeabilization even if, beyond a certain value, a saturation level of the disintegration index is generally reached (Lebovka et al., 2002). For example, the disintegration index of potato tissue was reported to be markedly increased when increasing either the field strength or the number of pulses (Angersbach et al., 1997; Knorr and Angersbach, 1998; Knorr, 1999). The effect of the applied field strength (between 0.1 and 0.4 kV/cm) and pulse width (between 10 and 1000 μ s) on the efficiency of disintegration of apple tissue by pulsed electric fields (PEF) has also been studied (De Vito et al., 2008). The characteristic damage time τ , estimated as a time when the disintegration index Z_p attains one-half of a maximal value, i.e. $Z_p = 0.5$ (Lebovka et al., 2002), decreased with the increase of the field strength and pulse width. In particular, longer pulses were more effective, and their effect was particularly pronounced at room temperature and moderate electric fields ($E = 0.1$ kV/cm). However, Knorr and Angersbach (1998), utilizing the disintegration index Z_p for the quantification of cell permeabilization of potato tissue, found that, at a fixed number of pulses, the application of variable electric field strength and pulse width, but constant electrical energy per pulse W , resulted in the same degree of cell disintegration. Thus, the authors suggested that the specific energy per pulse should be considered as a suitable process parameter for the optimization of membrane permeabilization as well as for PEF-process development.

For exponential decay pulses, W (kJ/kg pulse) can be calculated by Eq. 8, where E_{max} is the peak electric field strength (kV/m), k is the electrical conductivity (S/m), τ_p is the pulse width (s), and ρ is the density of the product (kg/m³).

$$W = \frac{kE_{max}^2\tau_p}{\rho} \quad (8)$$

The relationship between W and cell permeabilization was evaluated systematically by examining the variation of specific energy input per pulse (from 2.5 to 22000 J/kg) and the number of pulses ($n_p = 1-200$; pulse repetition = 1 Hz). The Z_p value induced by the treatment increased continuously with the specific pulse energy as well as with the pulse numbers.

Theoretically, the total cell permeabilization of plant tissue was obtained by applying either one very high energy pulse or a large number of pulses of low energy per pulse (Knorr and Angersbach, 1998). Based on these results, the total specific energy input W_T , defined as $W_T = W n_p$ (kJ/kg), should be used, next to field strength, as a fundamental parameter in order to compare the intensities of PEF- treatments resulting from different electric pulse protocols and/or PEF devices. In addition, the use of the total energy input required to achieve complete cell disintegration for any given matrix also provides an indication of the operational costs. Utilizing the disintegration index Z_p evaluated by Eq. (6) for the quantification of cell membrane permeabilization of the outer bracts of artichokes heads, the relationship between total specific energy input ranging from 1 to 20 kJ/kg and cell permeabilization, evaluated for different field strength applied in the range from 1 to 7 kV/cm, is reported in Fig. 5.

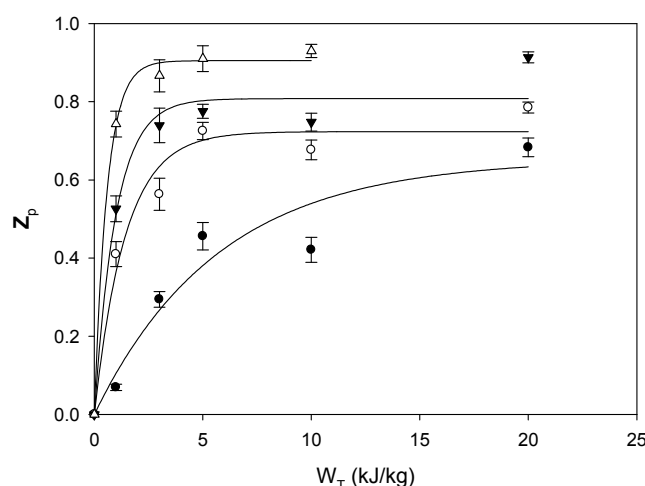


Fig. 5. Disintegration index Z_p of outer bracts of artichoke head versus total specific energy input at different electric field strength applied: (●) 1 kV/cm; (○) 3 kV/cm; (▼) 5 kV/cm; (△) 7 kV/cm (unpublished data).

The extent of damaged cells grows with both energy input and field strength applied during PEF treatment. However, for each field strength applied, the values of Z_p usually reveal an initial sharp increase in cell disintegration with increasing in energy input, after which any further increase causes only marginal effects, being a saturation level reached. The higher is the field strength applied, the higher the saturation level reached. In particular, as clearly shown by the results reported in Fig. 5, the energy required to reach a given permeabilization increases with decreasing the field strength applied. The characteristic electrical damage energy $W_{T,E}$, estimated as the total specific energy input required for Z_p to attain, at each field strength applied, one-half of its maximal value, i.e. $Z_p=0.5$, is presented in Fig. 6. The $W_{T,E}$ values decrease significantly with the increase of the electric field strength from 1 to 3 kV/cm and then tend to level-off to a relatively low energy value with further increase of E up to 7 kV/cm. Based on these results, the use of higher field strength should be preferred in order to obtain the desired degree of permeabilization with the minimum energy consumption. However, the estimation of the optimal value of the electric field intensity must take into account that beyond a certain value of E no appreciable reduction in the energy value required to obtain a given permeabilization effect can be achieved. From

the results reported in Fig. 6, an electric field intensity in the range between 3-4 kV/cm can be estimated as optimal (E_{opt}), from the balance between the maximization of the degree of ruptured cells in artichoke bracts tissue and the minimum energy consumption, which impacts on the operative costs, at the minimum possible electric field intensity, which impacts on the investment costs.

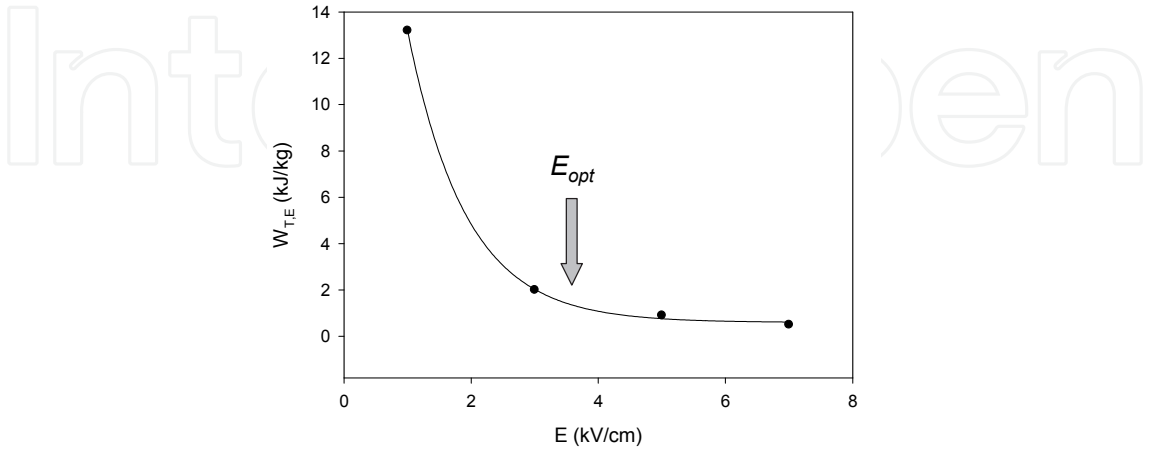


Fig. 6. Characteristic electrical damage energy W_{TE} of outer bracts of artichoke versus electric field strength applied (unpublished data).

A further criterion for energy optimization, based on the relationship between the characteristic damage time τ and the electric field intensity E , has been proposed by Lebovka et al. (2002). A PEF treatment capable of achieving a Z_p value of 0.5, is characterized by a duration t_{PEF} corresponding to the characteristic damage time $\tau(E)$, which is in turn a function of the electric field. Therefore, the energy input required will be proportional to the product $\tau(E) \cdot E^2$, as shown by Eq. 8. Since the $\tau(E)$ value decreases by increasing the electric field intensity E , the product of $\tau(E) \cdot E^2$ goes through a minimum (Fig. 7). Criteria of energy optimization require a minimum of this product. This minimum corresponds to the minimum power consumption for material treatment during characteristic time $\tau(E)$. A further increase of E results in a progressive increase of the product $\tau(E) \cdot E^2$ and of the energy input, but gives no additional increase in conductivity disintegration index Z_p . An

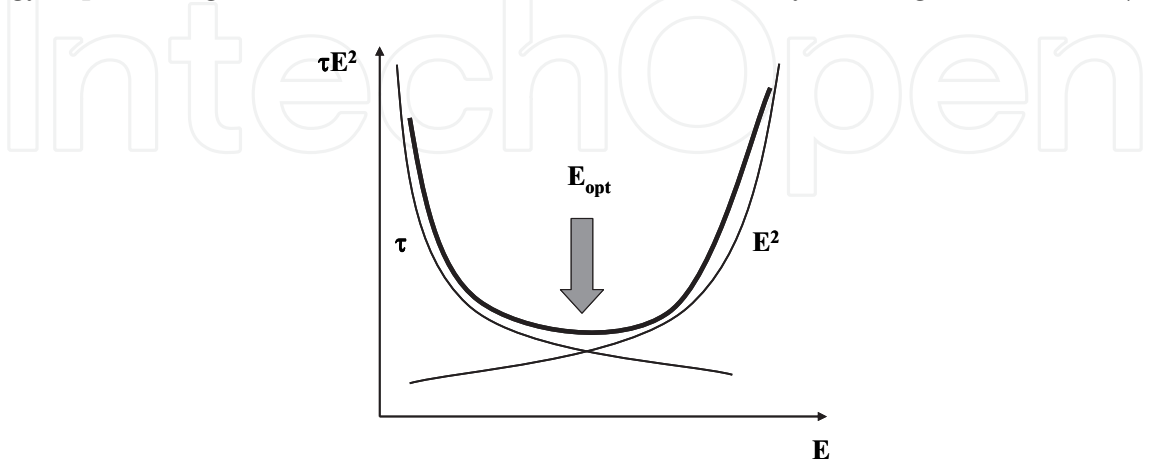


Fig. 7. Schematic presentation of optimization product $\tau(E) \cdot E^2$ versus electric field intensity E dependence (adapted from Lebovka et al., 2002).

optimal value of the electric field intensity $E_{opt} \approx 400$ V/cm, that results in maximal material disintegration at the minimal energy input, was estimated for apple, carrot and potato tissue. Based on this value the characteristic time τ was estimated as $2 \cdot 10^{-3}$ s for apple, $7 \cdot 10^{-4}$ s for carrot and $2 \cdot 10^{-4}$ s for potato and the energy consumption decreased in the same order: apple \rightarrow carrot \rightarrow potato (Lebovka et al., 2002).

5. Effect of PEF treatment of mass transfer rate from vegetable tissue

5.1 Models for mass transfer from vegetable tissue

Mass transfer during moisture removal for shrinking solids can be described by means of the Fick's second law of diffusion, reported in Eq. 9, also when PEF-pretreatment was applied to increase tissue permeabilization (Arevalo et al., 2004; Lebovka et al., 2007b; Ade-Omowaye et al., 2003). In Eq. 9, ω is the average concentration of soluble substances in the solid phase as a function of time (ω_0 is the initial concentration) and D_{eff} (m^2/s) is the effective diffusion coefficient.

$$\frac{\partial \omega}{\partial t} = D_{eff} \frac{\partial^2 \omega}{\partial x^2} \quad (9)$$

The most commonly used form of the solution of Eq. 9 is an infinite series function of the Fourier number, $Fo = (4 D_{eff} t)/L^2$, which can be written according to Eq. 10 (Crank, 1975). The solution of Eq. 10 is based on the main assumptions that D_{eff} is constant and shrinkage of the sample is negligible (Ade-Omowaye et al., 2003).

$$\vartheta = \frac{\omega}{\omega_0} = \frac{8}{\pi} \sum_{n=0}^{\infty} \frac{1}{(2n+1)} \exp \left[-(2n+1)^2 \pi^2 F_o \right] \quad (10)$$

The application of Eq. 10 to the drying of PEF-treated vegetable tissue, was reported for the ideal case of an infinite plate (disks of tissue with diameter \gg thickness), according to the form of Eq. 11 (Arevalo et al., 2004), where $M_r = (M - M_e)/(M_0 - M_e)$ is the adimensional moisture of the vegetable tissue at time t , M_0 is the initial moisture content, M_e is the equilibrium moisture content, M is the moisture content at any given time, D_{eff} is the effective coefficient of moisture diffusivity (m^2/s), t is the drying time (s), and L is half-thickness of the plate (m).

$$M_r = \frac{M - M_e}{M_0 - M_e} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-(2n+1)^2 \frac{\pi^2 D_{eff} t}{L^2} \right] \quad (11)$$

For long drying times, Eq. 11 is expected to converge rapidly and may be approximated by a one-term exponential model, reported in Eq. 12, which can be used for the estimation of the moisture effective diffusivity (Arevalo et al., 2004; Ade-Omowaye et al., 2003).

$$M_r = \frac{M - M_e}{M_0 - M_e} = \frac{8}{\pi^2} \exp \left[-\frac{\pi^2 D_{eff} t}{L^2} \right] \quad (12)$$

In other cases, the first five terms of the series of Eq. 11 were used for the estimation of the moisture effective diffusivity, by means of the least square fitting of the experimental data

(Loginova et al., 2010; Lebovka et al., 2007b). Due to the simplifying assumptions taken, the solution reported in Eq. 10 applies well to the extraction of soluble matter from PEF-treated vegetable tissue, which is considered to be dependent on an effective diffusion coefficient D_{eff} , but also takes into account the maximum amount of extractable substances. Eq. 13 represents the modified form of the Crank solution that was applied to the extraction of soluble matter from vegetable tissue (Loginova et al., 2010).

$$1 - \frac{y}{y_{\infty}} = \frac{8}{\pi^2} \alpha \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-(2n+1)^2 \frac{\pi^2 D_{eff} t}{L^2} \right] \quad (13)$$

In Eq. 13, y is the solute concentration in the extracting solution, y_{∞} is the concentration at equilibrium ($t=\infty$) and α is the solid/liquid ratio. The values of the effective diffusion coefficient D_{eff} exhibit a strong dependence on the temperature, at which the mass transfer process, such as drying, extraction or expression, occurs. In particular, the dependence of D_{eff} on temperature can be expressed through an Arrhenius law, reported in Eq. 14, where D_{∞} is the effective diffusion coefficient at an infinitely high temperature (m^2/s); E_a is the activation energy (kJ/mol), R is the universal gas constant ($8.31 \cdot 10^{-3} \text{ kJ/mol K}$) and T is the temperature (K) (Amami et al., 2008).

$$D_{eff} = D_{\infty} \exp \left[-\frac{E_a}{RT} \right] \quad (14)$$

Frequently, the kinetics of extraction of PEF treatments was expressed through a simplified form of Eq. 12, which is reported in Eq. 15 and which can be used for the estimation of a kinetic constant of extraction k_d . The kinetic constant k_d includes the diffusion coefficient of the extracted compound, the velocity of the agitation, the total surface area, the volume of solvent and the size and geometry of solid particles (Lopez et al., 2009a; Lopez et al., 2009b). In Eq. 15, y is again the solute concentration in the extracting solution and y_{∞} is the concentration at equilibrium ($t=\infty$).

$$\frac{y}{y_{\infty}} = [1 - \exp(-k_d t)] \quad (15)$$

Some authors reported that mass transfer from vegetable tissue subjected to extraction, pressing or osmotic dehydration may occur according to two different regimes, corresponding to convective fluxes of surface water and diffusive fluxes of intracellular liquids (Amami et al., 2006). The convective or “washing” regime occurs in the initial stages of the mass transfer process and is associated to higher mass fluxes, with its importance further increasing for the tissue that is humidified electrically. The pure diffusion regime is instead characterized by a lower rate of transfer and becomes significant when the washing stage is completed (El-Belghiti and Vorobiev, 2004). The mathematical model that can be used to describe the combination of the washing and pure diffusion regimes is reported in Eq. 16 (El-Belghiti and Vorobiev, 2004; Amami et al., 2006).

$$\frac{y}{y_{\infty}} = \frac{y_w}{y_{\infty}} [1 - \exp(-k_w t)] + \frac{y}{y_{\infty}} [1 - \exp(-k_d t)] \quad (16)$$

In Eq. 16, y represents is the solute concentration in the solution at any time during the extraction process, y_{∞} is the equilibrium solute concentration, y_w is the final solute concentration in the solution due to the washing stage alone, y_d is the final solute concentration in the solution due to the diffusion stage alone. Moreover, k_w and k_d represent the rate constants for the washing stage and for the diffusion stage, respectively and give indications about the characteristic times $\tau_w = 1/k_w$ and $\tau_d = 1/k_d$ of the two phenomena.

5.2 Effect of PEF pretreatment on mass transfer rates during drying processes

The reported effect of PEF treatment on mass transfer rates during drying of vegetable tissue is typically an increase in the effective diffusion coefficient D_{eff} . For example, Fig. 8 reports the D_{eff} values estimated from drying data of untreated and PEF-treated potatoes (Fig. 8a) and bell peppers (Fig. 8b). In particular, Fig. 8a shows the Arrhenius plots of $\ln(D_{eff})$ vs. $1/T$ for convective drying of intact, freeze-thawed and PEF-treated potato tissue. In the Arrhenius plot, the activation energy can be calculated from the slope of the plotted data, according to Eq. 17.

$$\ln D_{eff} = \ln D_{\infty} - \frac{E_a}{R} \frac{1}{T} \quad (17)$$

Remarkably, PEF treatment did not significantly affected the activation energy E_a in comparison to untreated potato samples ($E_a \approx 21$ and 20 kJ/mol, respectively), but caused a significant reduction of the estimated D_{∞} values (intercept with y-axis). In comparison, freeze-thawed tissue exhibited a significantly different diffusion behavior, with the D_{eff} value being similar to that of the PEF-treated tissue at low temperature (30°C) and increasing more steeply at increasing temperature ($E_a \approx 27$ kJ/mol) (Lebovka et al., 2007b). Similarly, the application of PEF increased the effective water diffusivity during the drying of carrots, with only minor variations of the activation energies. More specifically, a PEF treatment conducted at $E = 0.60$ kV/cm and with a total duration $t_{PEF} = 50$ ms, increased the values of D_{eff} , estimated according to Eq. 11, from $0.3 \cdot 10^{-9}$ and $0.93 \cdot 10^{-9}$ m²/s at 40 to 60°C drying temperatures, respectively, for intact samples, to $0.4 \cdot 10^{-9}$ and $1.17 \cdot 10^{-9}$ m²/s at the same temperatures for PEF-treated samples. In contrast, the activation energies, estimated from Eq. 14, were only mildly affected, being reduced from ≈ 26 kJ/mol to ≈ 23 kJ/mol by the PEF treatment (Amami et al., 2008).

The increase of PEF intensity, achieved by applying a higher electric field and/or a longer treatment duration, causes the D_{eff} values to increase until total permeabilization is achieved. For example, Fig. 8b shows the D_{eff} values estimated from fluidized bed-drying of bell peppers, PEF treated with an electric field ranging between 1 and 2 kV/cm and duration of the single pulses longer than the duration applied in the previous cases ($400 \mu\text{s}$ vs. $100 \mu\text{s}$). The total specific applied energy W_T was regulated by controlling the number of pulses and the electric field applied. Interestingly, the D_{eff} values increased from $1.1 \cdot 10^{-9}$ to an asymptotic value of $1.6 \cdot 10^{-9}$ m²/s when increasing the specific PEF energy up to 7 kJ/kg, probably corresponding to conditions of complete tissue permeabilization. As a consequence, further PEF treatment did not cause any effect on D_{eff} values (Ade-Omowaye et al., 2003).

5.3 Effect of PEF on mass transfer rates during extraction processes

In the case of extraction of soluble matter from vegetable tissue, the PEF treatments affected the mass transfer rates not only by increasing the effective diffusion coefficient D_{eff} , but also

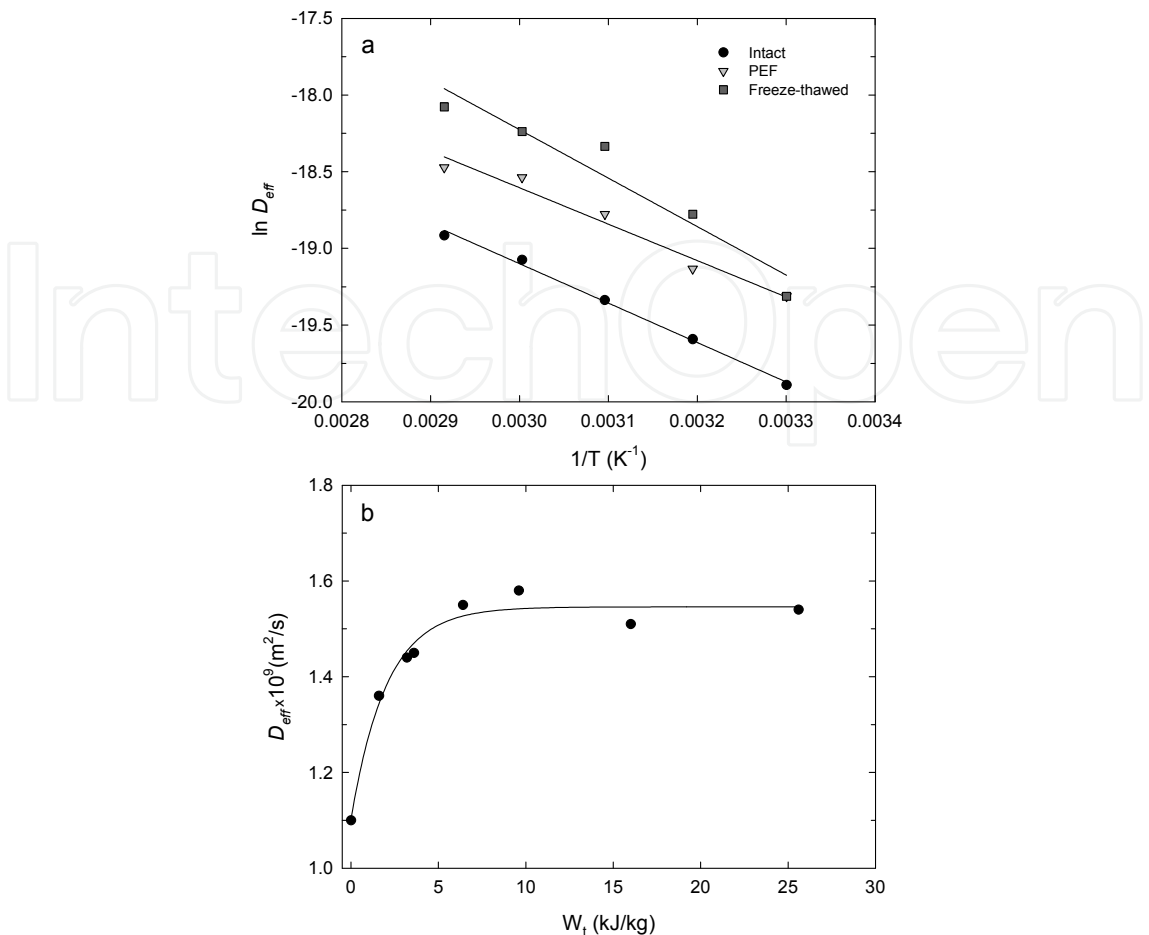


Fig. 8. Dependence of diffusion coefficients of PEF-treated samples on drying temperature and on the specific PEF energy. (a) Dependence on temperature of diffusion coefficients during drying of untreated, freeze-thawed and PEF treated potatoes. PEF treatment conditions were $E=0.4$ kV/cm and $t_{PEF} = 500$ ms. Drying was carried at variable temperature in a drying cabinet with an air flow rate of $6\text{ m}^3/\text{h}$ (Lebovka et al., 2007b). (b) Dependence on the specific applied energy of PEF treatment of diffusion coefficients during drying of bell peppers. PEF treatment conditions were $E=1\text{-}2$ kV/cm and $t_{PEF} = 4\text{-}32$ ms. Drying was carried at $60\text{ }^\circ\text{C}$ in a fluidized bed with air velocity of 1 m/s (Ade-Omowaye et al., 2003).

inducing a significant decrease in the activation energy E_a , which translates in smaller dependence of D_{eff} on extraction temperature. Fig. 9a reports the activation energies of intact, PEF-treated and thermally-treated apple slices, estimated from the data of sugar concentration in the extraction medium through Eq. 13 and 14. Apple samples treated by PEF ($E=0.5$ kV/cm and $t_{PEF} = 0.1$ s) exhibited an intermediate activation energy ($E_a \approx 20$ kJ/mole), which was significantly lower than for intact samples ($E_a \approx 28$ kJ/mole) and measurably higher than for samples that were previously subjected to a thermal treatment at $75\text{ }^\circ\text{C}$ for 2 min ($E_a \approx 13$ kJ/mole). Moreover, PEF treatment also induced an increase of the D_{eff} value in comparison to untreated tissue for all the different temperatures tested (Jemai and Vorobiev, 2002). For example, at $20\text{ }^\circ\text{C}$ D_{eff} estimated from PEF-treated samples ($3.9 \cdot 10^{-10}\text{ m}^2/\text{s}$) was much closer to the D_{eff} value of denatured samples ($4.4 \cdot 10^{-10}\text{ m}^2/\text{s}$) than to the D_{eff} of intact tissue ($2.5 \cdot 10^{-10}\text{ m}^2/\text{s}$). In addition, at $75\text{ }^\circ\text{C}$ the D_{eff} value of PEF-treated samples was $13.4 \cdot 10^{-10}\text{ m}^2\text{ s}^{-1}$, compared with $10.2 \cdot 10^{-10}\text{ m}^2/\text{s}$ for thermally denatured

samples, indicating that the electrical treatment had a greater effect on the structure and permeability of apple tissue than the thermal treatment (Jemai and Vorobiev, 2002).

PEF treatment of sugar beets affected the diffusion of sugar through the cell membranes by decreasing the activation energy of the effective diffusion coefficients. Fig. 9b shows the Arrhenius plots of the effective sugar diffusion coefficient D_{eff} of PEF treated sugar beets from two independent experiments (Lebovka et al., 2007a; El-Belghiti et al., 2005). For example, PEF treatment conducted at $E=0.1$ kV/cm and $t_{PEF} = 1$ s caused the reduction of the activation energy from ≈ 75 kJ/mol (untreated sample) to ≈ 21 kJ/mol, with the D_{eff} values being always larger for PEF treated samples (Lebovka et al., 2007a). Interestingly, a different experiment resulted in similar values of the activation energy (≈ 21 kJ/mol) of D_{eff} for sugar extraction from sugar beet after a PEF treatment conducted at $E = 0.7$ kV/cm and $t_{PEF} = 0.1$ s. Similarly, the values of the effective diffusion coefficient D_{eff} , estimated for extraction of soluble matter from chicory, were significantly higher for PEF-treated samples ($E = 0.6$ kV/cm and $t_{PEF} = 1$ s) than for untreated samples in the low temperatures range, while at high temperature (60 – 80 °C) high D_{eff} values were observed for both untreated and PEF-pretreated samples. In particular, the untreated samples exhibited a non-Arrhenius behavior, with a change in slope occurring at ≈ 60 °C. For $T > 60$ °C, the diffusion coefficient activation energy was similar to that of PEF treated samples, while for $T < 60$ °C the activation energy was estimated as high as ≈ 210 kJ/mol, suggesting an abrupt change in diffusion mechanisms. In particular, the authors proposed that below 60 °C, the solute matter diffusion is controlled by the damage of cell membrane barrier and is therefore very high for untreated samples (≈ 210 kJ/mol) and much smaller for PEF treated samples (≈ 19 kJ/mol). Above 60 °C, the extraction process is controlled by unrestricted diffusion with small activation energy in a chicory matrix completely permeabilized by the thermal treatment (Loginova et al., 2010).

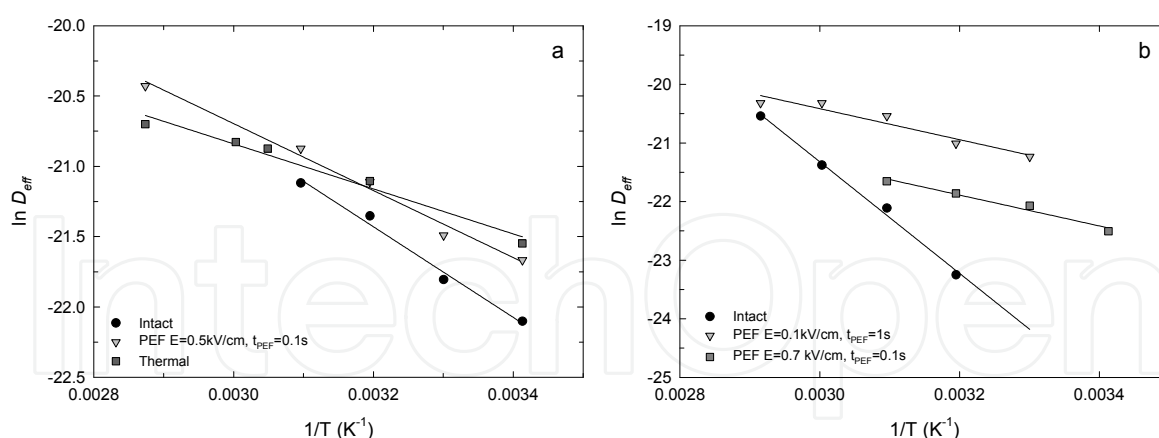


Fig. 9. Dependence on temperature of diffusion coefficients during extraction of soluble matter. (a) Diffusion of soluble matter from untreated, thermally treated (75 °C, 2 min) and PEF treated apples. PEF treatment conditions were $E=0.5$ kV/cm and $t_{PEF} = 0.1$ s (Jemai and Vorobiev, 2002). (b) Diffusion of sugar from sugar beets. PEF treatment conditions were $E=0.1$ kV/cm and $t_{PEF} = 1$ s (Lebovka et al., 2007a) and $E=0.7$ kV/cm and $t_{PEF} = 0.1$ s (El-Belghiti et al., 2005).

Apparently, the intensity of the PEF treatment may significantly affect the D_{eff} values and the equilibrium solute concentration. Fig. 10 shows the values of the effective diffusion

coefficients D_{eff} (Fig. 10a) and the equilibrium sugar concentration y_{∞} (Fig. 10b), estimated through data fitting with Eq. 15 and 13, for a PEF treatment significantly different from those reported in Fig. 8 and 9, due to the electric field being significantly higher (up to 7 kV/cm) and the treatment duration shorter (40 μ s) (Lopez et al., 2009b).

Interestingly, for low temperature extraction (20 and 40 °C), both D_{eff} and y_{∞} values significantly increased upon PEF treatment. In particular, most of the variation of both D_{eff} and y_{∞} occurred when increasing the applied electric field from 1 to 3 kV/cm, with $E = 1$ kV/cm only mildly affecting the mass diffusion rates, suggesting that for $E \geq 3$ kV/cm the sugar beet tissue was completely permeabilized. At higher extraction temperature (70 °C), both D_{eff} and y_{∞} values are independent on PEF treatment, being the thermal permeabilization the dominant phenomenon (Lopez et al., 2009b).

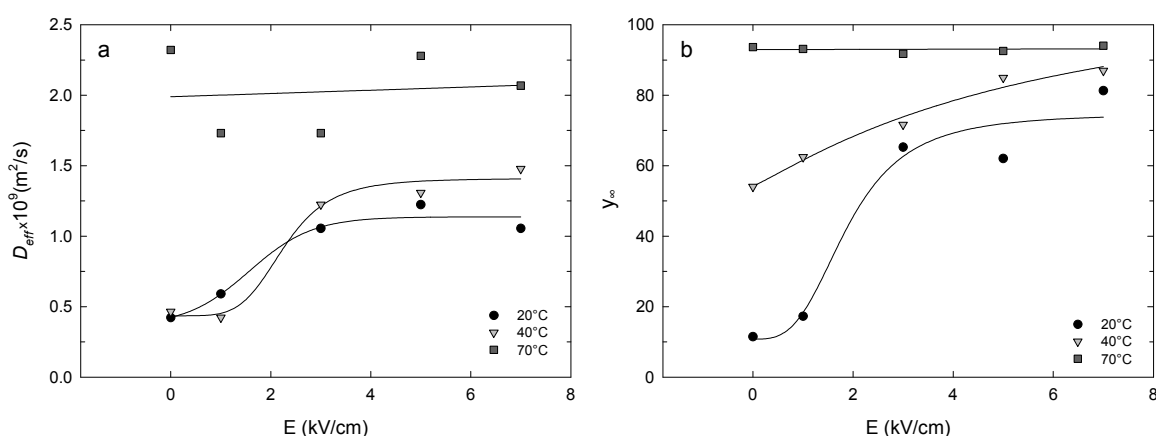


Fig. 10. Dependence on PEF treatment intensity of diffusion coefficient D_{eff} (a) and maximum sugar yield y_{∞} (b) during sugar extraction from sugar beets. PEF treatment conditions were $E=0\text{--}7$ kV/cm and $t_{PEF} = 4 \cdot 10^{-5}$ s (Lopez et al., 2009b).

6. A case study - red wine vinification

A promising application of PEF pretreatment of vegetable tissue is in the vinification process of red wine. Grapes contain large amounts of different phenolic compounds, especially located in the skin, that are only partially extracted during traditional winemaking process, due to the resistances to mass transfer of cell walls and cytoplasmatic membranes. In red wine, the main phenolic compounds are anthocyanins, responsible of the color of red wine, tannins and their polymers, that instead give the bitterness and astringency to the wines (Monagas et al., 2005). In addition, polyphenolic compounds also contribute to the health beneficial properties of the wine, related to their antioxidant and free radical-scavenging properties (Nichenametla et al., 2006).

The phenolic content and composition of wines depends on the initial content in grapes, which is a function of variety and cultivation factors (Jones and Davis, 2000), but also on the winemaking techniques (Monagas et al., 2005). For instance, increasing fermentation temperature, thermovinification and use of maceration enzymes can enhance the extraction of phenolic compounds through the degradation or permeabilization of the grape skin cells (Lopez et al., 2008b). Nevertheless, permeabilization techniques suffer from some drawbacks, such as higher energetic costs and lower stability of valuable compounds at higher temperature (thermovinification), or the introduction of extraneous compounds and

general worsening of the wine quality (Spranger et al., 2004). Therefore, PEF treatment may represent a viable option for enhancing the extraction of phenolic compounds from skin cells during maceration steps, without altering wine quality and with moderate energy consumption.

From a technological prospective, great interest was recently focused on the application of PEF for the permeabilization of the grape skins prior to maceration. The enhancement of the rate of release of phenolic compounds during maceration offers several advantages. In case of red wines obtained from grapes poor in polyphenols, it can avoid blending with other grape varieties richer in phenolic compounds, or use of enzymes. Moreover, it can reduce significantly the maceration times (Donsì et al., 2010a; Donsì et al., 2010b).

The main effect of PEF treatment of grape skins or grape mash is the increase of color intensity, anthocyanin content and of total polyphenolic index with respect to the control during all the vinification process on different grape varieties (Lopez et al., 2008a; Lopez et al., 2008b; Donsì et al., 2010a). Furthermore, it was reported that PEF did not affect the ratio between the components of the red wine color (tint and yellow, red and blue components) and other wine characteristics such as alcohol content, total acidity, pH, reducing sugar concentration and volatile acidity (Lopez et al., 2008b). In particular, Fig. 11 shows the evolution of total polyphenols concentration in the grape must during the fermentation/maceration stages of two different grape varieties, Aglianico and Piedirosso. Prior to the fermentation/maceration step, the grape skins were treated at different PEF intensities ($E = 0.5 - 3 \text{ kV/cm}$ and total specific energy from 1 to 25 kJ/kg), with their permeabilization being characterized by electrical impedance measurements. Furthermore, the release kinetics of the total polyphenols were characterized during the fermentation/maceration stage by Folin-Ciocalteu colorimetric methods. It is evident that on Aglianico grape variety the PEF treatment caused a significant permeabilization that enhanced the mass transfer rates of polyphenols through the cellular barriers. Moreover, higher intensity of PEF treatment resulted in both faster mass transfer rates and higher final concentration of polyphenols (Fig. 11a). In contrast, the PEF treatment of Piedirosso variety did not result in any effect on the release kinetics of polyphenols, with very slightly differences being observable between untreated and treated grapes (Fig. 11b).

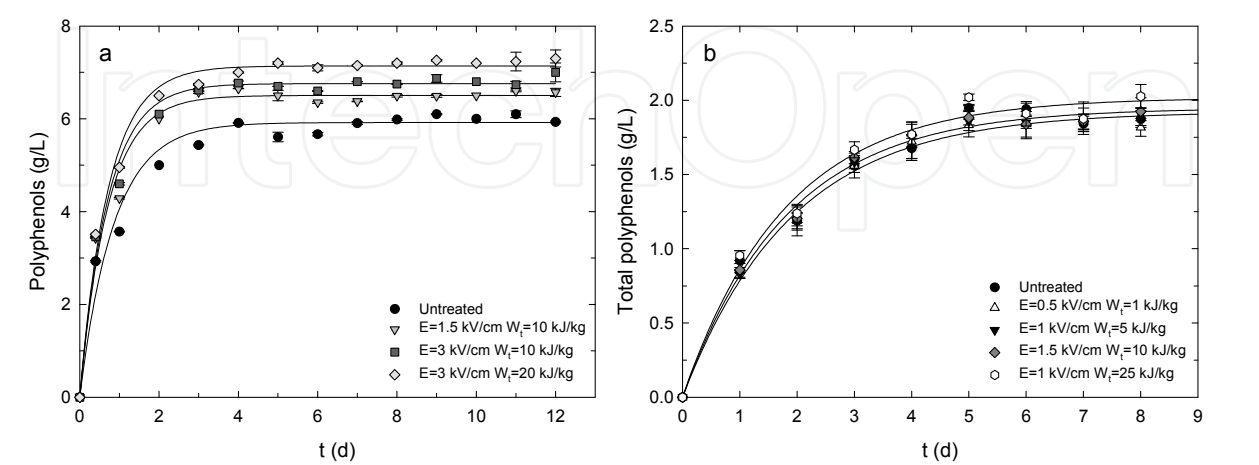


Fig. 11. Evolution over time of total polyphenols concentration in the grape must during fermentation/maceration of two Italian grape varieties: Aglianico (a) and Piedirosso (b) (Donsì et al., 2010a).

This is particularly evident in Fig. 12, where the kinetic constant k_d (Fig. 12a) and the equilibrium concentration y_∞ (Fig. 12b) are reported as a function of the total specific energy delivered by the PEF treatment. While both k_d and y_∞ increased for Aglianico grapes at increasing the specific energy, for Piedirosso the estimated values of both k_d and y_∞ remained constant and independent on the PEF treatments. This is even more remarkable if considering that PEF treatments, under the same operative conditions, caused a significant increase of the permeabilization index Z_p on both grape varieties, as shown in Fig. 12c. In particular, for a total specific energy $W_T > 10$ kJ/kg a complete permeabilization ($Z_p \approx 1$) was obtained for Piedirosso and an almost complete permeabilization for Aglianico ($Z_p \approx 0.8$).

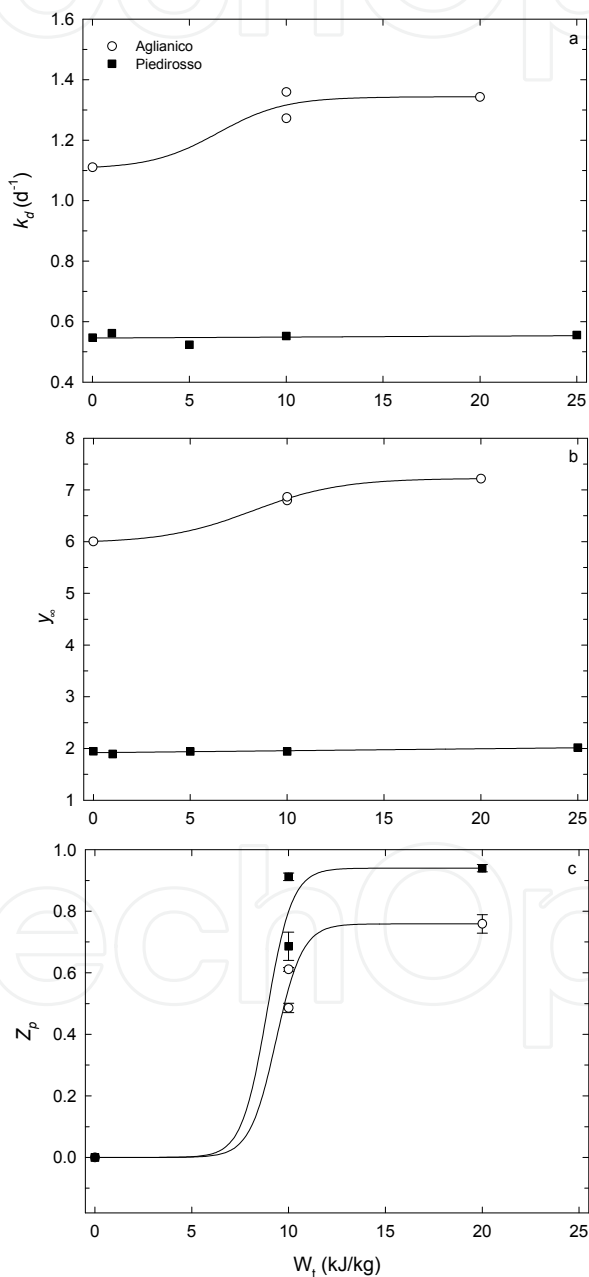


Fig. 12. Kinetic constant k_d (a), equilibrium polyphenolic concentration y_∞ (b) estimated through Eq. 15 from maceration data and permeabilization index Z_p (c) of different untreated and PEF-treated grape varieties, Aglianico and Piedirosso (Donsì et al., 2010a).

Fig. 13, which reports a scheme of a grape skin cell, may help in clarifying the discrepancies observed between measured permeabilization and mass transfer rates in the case of Piediroso and to explain the mechanisms of PEF-assisted enhancement of polyphenols extraction. Polyphenols and anthocyanins are mainly contained within the vacuoles of the cells, and therefore their extraction encounters two main resistances to mass transfer, which are formed respectively by the vacuole membrane and the cell membrane. PEF treatment causes permanent membrane permeabilization provided that a critical trans-membrane potential is induced across the membrane by the externally applied electric field (Zimmermann, 1986). Since for a given external electric field the trans-membrane potential increases with cell size (Weaver and Chizmadzhev, 1996), the critical value of the external electric field E_{cr} required for membrane permeabilization will be lower for larger systems. Therefore, it can be assumed that the critical electric field for cell membrane permeabilization, E_{cr1} , will be lower than the one for vacuole membrane permeabilization, E_{cr2} . Therefore, in agreement with the reported data, it can be assumed that the applied electric field $E > E_{cr1}$ already at $E = 1 \text{ kV/cm}$ and that the extent of cell membrane permeabilization depends only on the energy input. Whereas, in the case of the vacuole membrane permeabilization, the critical value E_{cr2} is probably in the range of the applied electric field, and the increase of the intensity of E (from 0.5 to 3 kV/cm) can also increase the permeabilization of the membrane of smaller vacuoles. For the above reasons, it can be concluded that the permeabilization index Z_p takes into account the permeabilization of the cell membrane and therefore suggests that cell permeabilization occurred both for Aglianico and Piediroso grapes.

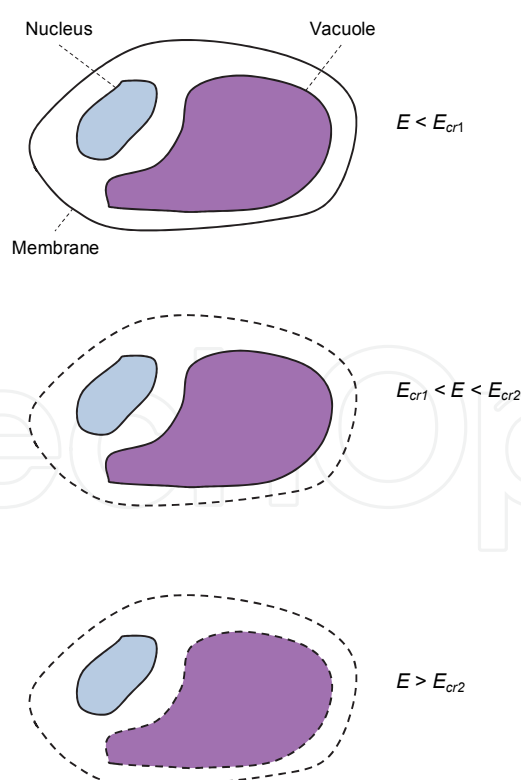


Fig. 13. Simplified scheme of the effect of PEF treatments with electric field intensity E on the structure of a grape skin cell. E_{cr1} : critical electric field for cell membrane permeabilization; E_{cr2} : critical electric field for vacuole membrane permeabilization.

Assuming that the resistance to mass transfer through the vacuole membrane is the rate determining step, the fact that the mass transfer rates are enhanced only for Aglianico and not for Piediroso can be explained only inferring that, due to biological differences, the applied PEF treatments were able to permeabilize the vacuole membrane only of Aglianico grape skin cells and not of Piediroso grape skin cells.

In summary, PEF treatments of the grape skins resulted able to affect the content of polyphenols in the wine after maceration, depending on the grape variety. For Piediroso grapes, the PEF treatment did not increase the release rate of polyphenols. On the other hand, PEF treatment had significant effects on Aglianico grapes, with the most effective PEF treatment inducing, in comparison with the control wine, a 20% increase of the content of polyphenols and a 75% increase of anthocyanins, with a consequent improvement of the color intensity (+20%) and the antioxidant activity of the wine (+20%). Moreover, in comparison with the use of a pectolytic enzyme for membrane permeabilization, the most effective PEF treatment resulted not only in the increase of 15% of the total polyphenols, of 20% of the anthocyanins, of 10% of the color intensity and of 10% of the antioxidant activity, but also in lower operational costs. In fact, the cost for the enzymatic treatment is of about 4 € per ton of grapes (the average cost of the enzyme is about 200 €/kg, and the amount used is 2 g per 100 kg of grapes), while the energy cost for the PEF treatments, calculated as (specific energy) · (treatment time) · (energy cost), was estimated in about 0.8 € per ton of grapes (with the energy costs assumed to be 0.12 €/kWh) in the case of the most effective treatment (Donsì et al., 2010a).

7. Conclusions and perspectives

PEF technology is likely to support many different mass transfer-based processes in the food industry, directed to enhancing process intensification. In particular, the induction of membrane permeabilization of the cells through PEF offers the potential to effectively enhance mass transfer from vegetable cells, opening the doors to significant energy savings in drying, to increased yields in juice expression, to the recovery of valuable cell metabolites, with functional properties, or even to the functionalization of foods. For instance, PEF treatment of the grape pomaces during vinification can significantly increase the polyphenolic content of the wine, thus improving not only the quality parameters (i.e. color, odor, taste...) but also the health beneficial properties (i.e. antioxidant activity). Furthermore, PEF treatments can also be applied to enhance mass transfer into the food matrices, by permeabilization of the cell membranes and enhanced infusion of functional compounds or antimicrobial into foods, minimally altering their organoleptic attributes.

In consideration of the fact that energy requirements for PEF-assisted permeabilization are in the order of about 10 kJ/kg of raw material, it can be concluded that PEF pretreatments can represent an economically viable option to other thermal or chemical permeabilization techniques. However, further research and development activities are still required for the optimization of PEF technology in process intensification, especially in the development of industrial-scale generators, capable to provide the required electric field.

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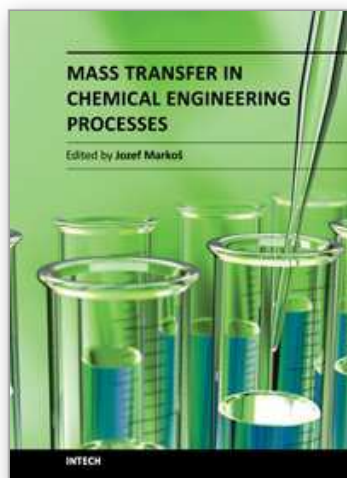
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This book offers several solutions or approaches in solving mass transfer problems for different practical chemical engineering applications: measurements of the diffusion coefficients, estimation of the mass transfer coefficients, mass transfer limitation in separation processes like drying, extractions, absorption, membrane processes, mass transfer in the microbial fuel cell design, and problems of the mass transfer coupled with the heterogeneous combustion. I believe this book can provide its readers with interesting ideas and inspirations or direct solutions of their particular problems.

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