Improving Mass Transfer to Soften Tissues by Pulsed Electric Fields: Fundamentals and Applications

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Abstract

The mass transfer phenomenon occurs in many operations of the food industry with the purpose of obtaining a given substance of interest, removing water from foods, or introducing a given substance into the food matrix. Pretreatments that modify the permeability of the cell membranes, such as grinding, heating, or enzymatic treatment, enhance the mass transfer. However, these techniques may require a significant amount of energy and can cause losses of valuable food compounds.

Pulsed electric field (PEF) technology is a nonthermal processing method that causes permeabilization of cell membranes using low energy requirements and minimizing quality deterioration of the food compounds. Many practical applications of PEF for enhancing mass transfer in the food industry have been investigated. The purpose of this chapter is to give an overview of the state of the art of application of PEF for improving mass transfer in the food industry.

INTRODUCTION: MASS TRANSFER IN FOOD INDUSTRY

Mass transfer exists in the migration of a substance between two phases under the influence of a concentration gradient in order to reach a chemical equilibrium (Welti-Chanes et al. 2005). This phenomenon occurs in many operations of the food industry in which the aim is extracting a given substance of interest (fruit juices, sugar, colorants, antioxidants, etc.), removing water from foods (drying), or introducing a given substance into the food matrix (osmotic dehydration, salting, or curing).

The structure of most animal- and plant-based foods consists of tissues that are composed of cells with a basic eukaryotic organization (Aguilera & Stanley 1999). Eukaryotic cells contain a nucleus, a cytoplasm, and different subcellular organelles enclosed in a cytoplasmic membrane that defines their boundaries. These membranes are bilayers composed of phospholipids that contain proteins inserted within the lipid matrix. In the case of plant cells, a cellulosic cell wall surrounds the cytoplasmic membrane.

The mass transfer rate depends on different factors, such as the difference of concentration of the substance between the two phases or the resistance that the substance finds in migrating from one phase to another. The presence of an intact cytoplasmic membrane, which acts as a semipermeable barrier, influences the migration of substances into or from the food tissues. Mass transfer in food material composed by cells mainly depends on the diffusion through the cell membranes. It has been estimated that the average diffusion coefficient of a small solute in a membrane is often approximately a million times lower than that in the adjacent aqueous solutions (Nobel 1999).

Industrial interest in increasing the velocity of the mass transfer and as a consequence in reducing the operation time is based on increasing productivity, preserving the nutritional or physiological value of the food components and reducing the economic cost of the process. As the diffusion barrier of the intact cytoplasmic membranes is lost when they are deteriorated, in many cases food materials are pretreated by mechanical grinding, heat, or enzymes to enhance the mass transfer rate by increasing the permeability of the cell membranes (Toepfl et al. 2006a). However, these techniques may require a significant amount of thermal or mechanical energy and can cause losses of valuable food compounds. Treatment of food material by pulsed electric fields (PEFs) could replace these conventional techniques. This technology has been proved as an effective method for irreversible permeabilization of cell membranes in plant and animal tissues without increasing temperature and at low operating cost (Toepfl et al. 2006b).

Although PEF technology is regarded as novel for the food industry, early studies concerning the use of direct and alternating electrical current for increasing the permeability of plant tissues in order to improve the extraction of different intracellular compounds, such as juice from fruits and vegetables and sugar from beets, were conducted by Russian researchers in the middle of the past century (Flaumenbaum 1949). Later, the first applications of PEFs for cell permeabilization were described by Doevenspeck (1960). In the 1980s, based on Doevenspeck's observations, the German company Krupps attempted to commercialize a PEF process called ELCRACK for cell disintegration, but implementation of the technology in the industry failed (Toepfl & Heinz 2007a). Since the beginning of the 1990s, interest in application of PEFs for food processing has revived. The area in which most efforts have been initially dedicated was microbial inactivation for enhancing food safety and stability without affecting nutritional and sensorial characteristics of foods. However, in recent years, an increasing number of research works have been focused on PEF-induced membrane permeability of eukaryote cells for improving mass transfer in different processes of the food industry (Donsi et al. 2010b, Knorr et al. 2011, Vorebiev & Lebovka 2008).

The purpose of this review is to give a general overview of the state of the art on the applications of PEFs for improving mass transfer in the food industry. The first part of the review deals with fundamental aspects concerning the PEF technology, the mechanisms of electroporation, the procedures proposed to monitor membrane permeabilization, and factors affecting permeabilization. Different applications of PEFs to improve mass transfer are described in the next part. Finally, the industrial feasibility of PEF technology and the cost estimation of the treatments required to permeabilize the cell membranes are discussed.

PULSED ELECTRIC FIELD TECHNOLOGY

Definition and Process Parameters

PEF is a treatment that involves the application of direct current voltage pulses for very short periods of time, in the range between microseconds to milliseconds, through a material placed between two electrodes. This voltage results in an electric field, the intensity of which depends on the gap between the electrodes and the voltage delivered. Although there is not a formal definition, field strengths of $E < 0.1$ kV cm^{-1} can be considered to be low-intensity electric fields, those in the range of 0.1 to 1 kV cm^{-1} to be moderate electric fields, and those with strengths of $E > 1$ kV cm^{-1} to be high-intensity electric fields (Asavasanti et al. 2010). Permeabilization of eukaryote cells for improving mass transfer can be achieved at low and moderate electric fields at treatment times in the range of 100 to 10,000 μs or at electric fields in the range or 1 to 10 kV cm^{-1} for shorter treatment times (<100 μs).

The basic components of an apparatus for application of PEF are the pulse generator and the treatment chamber (de Haan 2007) (**Figure 1**). The pulse generator consists of a charger that converts the AC to DC current and charges an energy storage apparatus, such as a capacitor or an inductor. The discharge of the electrical energy in the treatment chamber is controlled by a switch that is the most critical component in a pulse generator for industrial applications because it must turn on and off a circuit at high voltages and large current in a fraction of microseconds. If the voltage in the capacitor is not sufficiently high, a pulse transformer is used to step it up. The treatment chamber that contains the material to be treated consists of two electrodes separated by an isolating material. For basic studies aimed to get a fundamental understanding of the membrane permeabilization or factors affecting this phenomenon, static treatment chambers with parallel electrodes are generally used. However, recently there has been considerable progress in the development of continuous flow treatment chambers for PEF processing that are essential for scaling up the technology for industrial applications (Huang & Wang 2009). Although several different designs have been developed in the past few years, the three most important treatment chamber designs that presently are considered for commercial application of PEFs are parallel electrode, coaxial, and colineal configurations (**Figure 1**).

The most typical process parameters that characterize PEF technology are electric field strength, pulse shape, pulse width, number of pulses, pulse specific energy, and frequency (Barssoti et al. 1999) (**Figure 1**). The distance between the electrodes of the treatment chamber and the voltage delivered define the strength of the electric field. While in treatment chambers with parallel electrode configurations, the electric field strength between the electrodes is uniform, in colineal and coaxial configurations the electric field strength is not uniform and it changes depending on the location (Gerlach et al. 2008). The most generally applied pulse shapes used are exponential decay and square waveform. Square waveform geometry has been determined to be the ideal pulse shape for PEF processing because the electric field intensity remains constant for the pulse duration. The treatment time for a PEF application is defined as a function of the duration pulse width

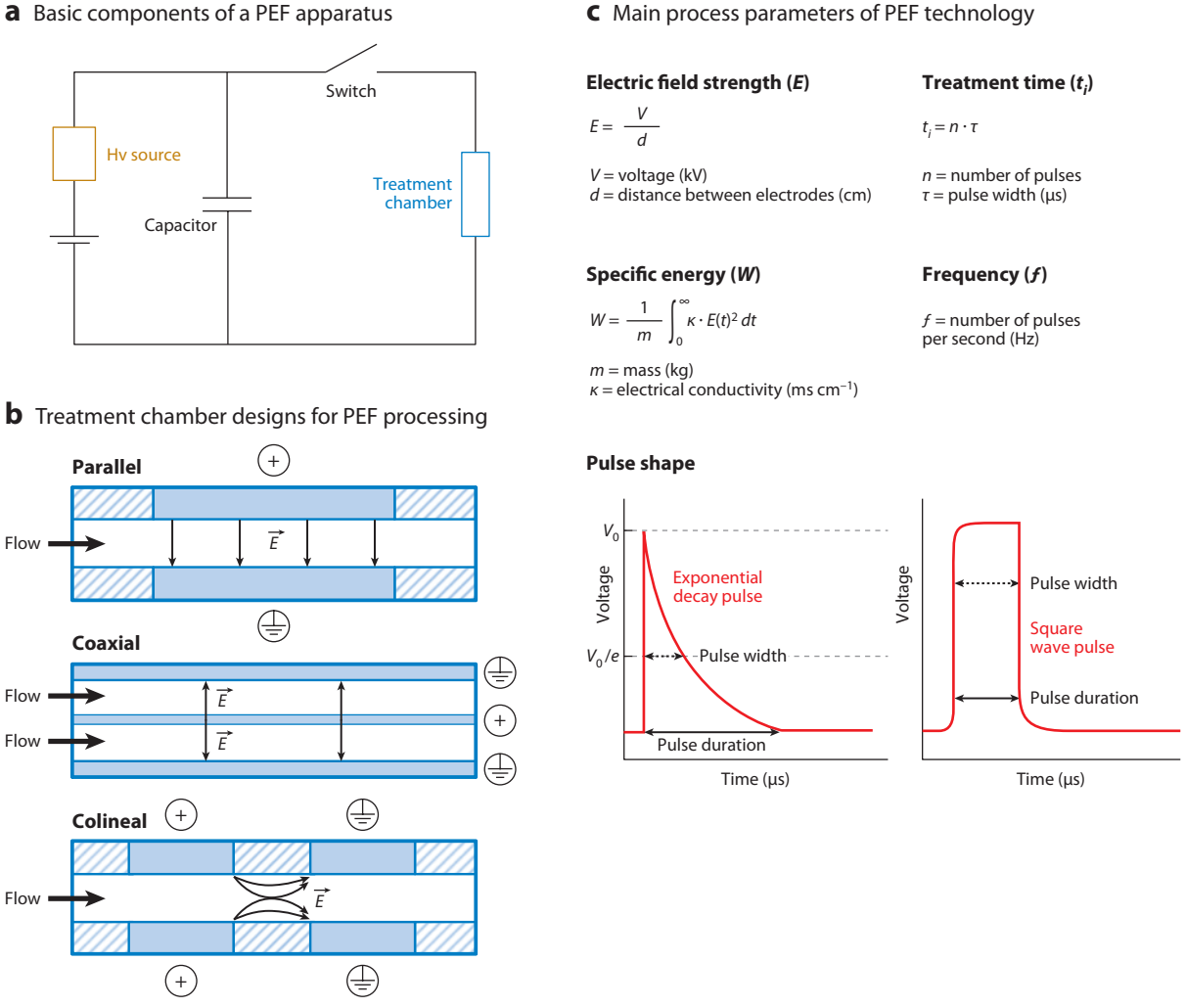

Figure 1

Electrical aspects of pulsed electric field (PEF) technology: (*a*) Basic components of a PEF apparatus, (*b*) treatment chamber designs for PEF processing, (*c*) main process parameters of PEF technology.

and number of pulses applied. In square waveform pulses, pulse width corresponds to the duration of the pulse, but in exponential decay pulses the time required for the input voltage to decay to 37% of its maximum value has been adopted as the effective pulse width. The specific energy of the pulse depends on the voltage applied, treatment time, and resistance of the treatment chamber that varies according to the geometry and conductivity of the material treated (Heinz et al. 2001). This parameter makes it possible to know approximately the increment of the temperature of the material as a consequence of the application of the treatment, to evaluate energy costs of the process and, consequently, to compare the PEF treatment efficiency with other technologies. Finally, the frequency indicates the number of pulses applied by unit of time, and it is often reported in Hz (pulses per second).

Mechanisms of Cell Membrane Permeabilization

The increment on the permeability of a cell membrane when external electric field pulses of duration of a micro-millisecond are applied is well known (Tsong 1991). This phenomenon, called electroporation or electropermeabilization, is a procedure routinely used in molecular biology to gain access to the cytoplasm in order to introduce different molecules. More recently, it has been proposed as an effective way of inactivating microorganisms at temperatures below those used in thermal processing and enhancing mass transfer (Teissié et al. 2002). However, the molecular mechanisms supporting the induction of permeabilization in the membranes are not fully understood.

The effects on a cell when exposing to an external electric field can be described in four steps (Saulis 2010): (*a*) increase of the transmembrane potential of the cytoplasmic membrane, (*b*) initiation of the pore formation, (*c*) changes in the number and/or size of the created pores during the PEF treatment, and (*d*) after finishing the PEF treatment, the electroporation can be reversible, in which the viability of the electroporated cell can be preserved by recovering the membrane integrity, or irreversible, leading to leakage of intracellular compounds or entrance of extracellular substances.

Several theories have been proposed in order to explain the mechanisms of initiation of cell electroporation (Ho & Mittal 1996, Teissié et al. 2005, Tsong 1991, Weaver & Chizmadzhev 1996). Electromechanical theories trying to explain the electroporation of biological membranes assume that the external electric field applied causes a membrane compression leading to a membrane rupture when the electrical force exceeds the elastic restoring force (Zimmermann et al. 1974). When the cell is exposed to external electric field strength, a time- and position-dependent transmembrane potential is induced across the cytoplasmic membrane due to the accumulation of oppositely charged ions at both sides of the nonconductive membrane (Teissié et al. 2002). The attraction between these ions causes membrane thickness reduction and formation of pores. From an electrical point of view, because of the low electrical conductivity of a membrane as compared with the surrounding liquid, a cell can be considered like a spherical capacitor. Transmembrane potential (U_m) generated by an external electric field for a spherical cell, assuming several simplifying restrictions, can be calculated as:

$$U_m = 1.5 \cdot r \cdot E \cdot \cos\theta \cdot \left(1 - e^{\left(\frac{t}{\tau}\right)}\right), \quad (1)$$

where r is the radius of the cell, E is the external electric field applied, θ is the angle between the normal to the membrane and the direction of the electric field, t is the time, and τ is the membrane capacitance treatment time. As the transmembrane potential depends on an angular parameter, the external electric field induces a position-dependent potential difference linearly related to the intensity of the applied electric field.

A critical value of the external electric field is required to induce a transmembrane potential (0.2−1.0 V) that leads to the formation of reversible or irreversible pores in the membrane (Saulis 2010). Although reversible electroporation that preserves viability of the cell is required for biotechnological applications of PEF, microbial inactivation and improving mass transfer by PEF in the food industry requires irreversible electroporation. When the applied external electric field is near the critical value, reversible electroporation occurs, allowing the cell membrane to recover its structure and functionality. Irreversible electroporation resulting in membrane disintegration and loss of cell viability occurs when electric field strengths higher than the critical value are applied.

According to Equation 1, the larger the cell, the greater the transmembrane potential that is generated across its cytoplasmic membrane. Because the size of eukaryotic cells is around

10 to 100 times higher than the size of microorganisms, the electric field strength required for electroporation of plant or animal cells (0.1–5 kV cm^{-1}) is lower than that required for microbial inactivation (10–35 kV cm^{-1}) (Donsi et al. 2010b).

Other electroporation theories assume that electroporation in a cell membrane occurs both in protein channels and in the lipid domain by the combined effects of the Joule heating and the external electric field (Tsong 1991). Application of an external electric field causes reorientation of lipid molecules of the membrane, creating hydrophilic pores that conduct current. This current generates local Joule heating that induces thermal phase transition of the lipid bilayer. Molecular dynamics of these events involve changes in conformation of the lipid molecule and rearrangement of the lipid bilayer by expanding the existing pores, creating new hydrophobic pores and forming structurally more stable hydrophilic pores. However, as the opening/closing of many protein channels is dependent on transmembrane potentials, it is expected that when a PEF is applied, many voltage sensitive channel proteins are opened. Once these channels are opened, they experience higher current than they are designed to conduct. As a result, these channels become irreversibly denatured by Joule heating or electrical modification of their functional groups.

The proposed theories to explain electropermeabilization are based on experiments on model systems, such as liposomes, or on individual eukaryote cells. However, in food materials, cells are part of tissues that have a complex structure, are highly inhomogeneous and have properties that are spatially dependent. In this case, the distribution of the local electric field is a complex function of the electrical properties of material constituents, porosity, and structure, and it changes during the PEF treatment (Vorobiev & Lebovka 2006). As a result, limited information is available regarding membrane permeabilization in real food systems as a consequence of the application of PEF. A fundamental understanding of these phenomena is essential for establishing the critical process parameters required for using PEF treatments to improve mass transfer in the food industry.

Procedures to Detect Membrane Permeabilization

Different methods have been used for testing cell membrane permeability and/or integrity in multicellular tissues. Microscopic observations, measurement of the liquid release, evaluation of the conductivity of the exuded liquid, measurements of the electrical impedance of the tissues or analyzing textural parameters of treated tissues are the most conventional methods used to assess electroporation (Donsi et al. 2010b).

The objective of some of these methods is quantifying the degree of permeabilization that has been defined as the ratio of electroporated cells to the total number of cells (Lebovka et al. 2002). A method that provides a precise and rapid measurement of the degree of permeabilization in a short time and whose measurements were correlated with the improvement of the mass transfer in different operations of the food industry would be a very useful tool to select the optimum PEF treatment conditions for a given application and to monitor PEF processing during industrial applications.

Microscopic visualization is a direct method to demonstrate the occurrence of cell permeabilization. This method is generally based on the uptake and active retention of dyes in living cells with an intact membrane or passive staining of the contents of cells with dyes that penetrate through permeabilized membranes (Fincan & Dejmek 2002, Janositz & Knorr 2010). Microscopic analysis of tissues before, during, and after PEF treatments has been revealed as a useful tool to gain insight into the permeabilization mechanisms of plant cells. However, quantification of the degree of permeabilization using these methods requires the use of image processing software.

When the membrane that surrounds the cell is permeabilized, the intracellular components and liquid content inside the cell diffuse outside the cell. It is possible to estimate the degree

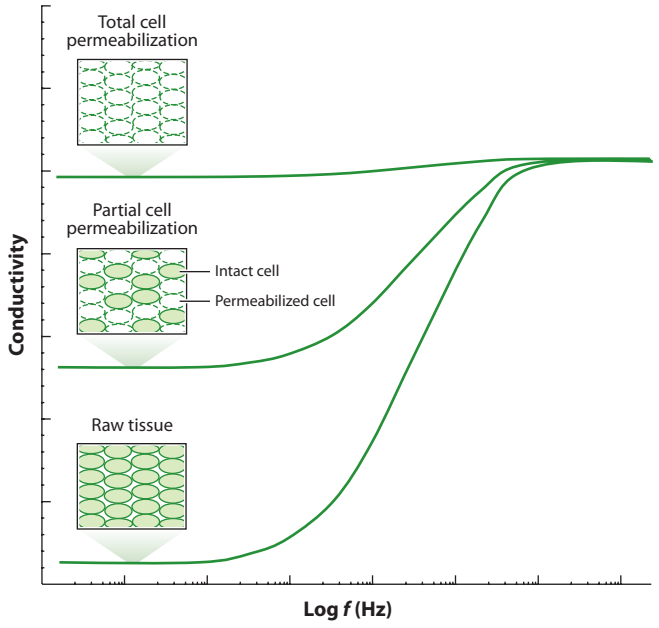

Figure 2

Typical frequency-conductivity spectra of plant tissue with intact, partially permeabilized, and totally permeabilized cells in the frequency range of the measured current of 1 kHz to 50 MHz (based on Knorr & Angersbach 1998).

of cell rupture by measuring the quantity of liquid released or the change in conductivity of the extracellular liquid. Quantification of the liquid liberated from a material treated by PEFs, subjecting the material to a given centrifugal force at which no liquid release occurs from untreated tissue, has been proven to be useful to determine the optimum PEF treatment conditions for permeabilization of potato cells (Knorr & Angersbach 1998). The increment of the conductivity of a solution that contains the cell tissue treated by PEFs as a consequence of the ion efflux from the cells has also been used as an indirect measurement of the intactness and permeability of cell membranes of onion tissues (Asavasanti et al. 2010, Ersus et al. 2010). However, some limitations of this method include the lack of selectivity regarding the contribution of different ions and the time required for ion or liquid leakage.

Measurement of the changes in the conductivity in PEF-treated biological tissues has been suggested as a simple and reliable method to obtain a measurement of the degree of cell damage (Angersbach et al. 1999). This method is based on the analysis of the frequency dependency of the electrical conductivity of biological cells. A typical evaluation of the conductivity spectra after different PEF treatment is show in **Figure 2**. At low frequency ranges, the conductivity of the cell tissue increases as a result of the irreversible electroporation of membranes. However, changes in conductivity at high frequency ranges are practically the same for intact and permeabilized cells because within this frequency range, the cell membrane does not present resistance to the measured electrical current. Based on the frequency dependency of the conductivity of intact and permeabilized tissues, a cell disintegration index was developed:

$$Z_p = 1 - \left(\frac{K_b}{K_b'}\right) \cdot \frac{(K_b' - K_l')}{(K_b - K_l)}; 0 \leq Z_p \leq 1, \qquad (2)$$

where K_l, K'_l are the electrical conductivities of untreated and treated material, respectively, at a low-frequency field (1–5 kHz); K_h, K'_h are the electrical conductivities of untreated and treated material, respectively, at a high-frequency field (3–50 MHz). This index characterizes the proportion of permeabilized cell in a material. For intact tissues, $Z_p = 0$; for a tissue with all the cells permeabilized, $Z_p = 1$.

The cell disintegration index has been proved to be a useful tool for establishing optimal PEF treatment conditions for improving mass transfer in different processes, such as dehydration of coconuts and red bell peppers or extraction of rapeseed oil and sugar from sugar beets (Ade-Omowaye et al. 2000, 2003b; Eshtiaghi & Knorr 2002; Guderjan et al. 2007).

The cell disintegration index was defined slightly differently by Lebovka et al. (2002) as

$$Z_p = \frac{K'_l - K_l}{K_l^d - K_l}, \tag{3}$$

where K_l, K'_l and K_l^d are the electrical conductivity of untreated, treated, and totally destroyed material, respectively, measured at a low-frequency field (1−5 kHz). Similarly to the former definition, the equation gives $Z_p = 0$ for intact tissues and $Z_p = 1$ for totally permeabilized cells.

The presence of an intact cytoplasmic membrane maintains an osmotic difference between the inside and outside of the cell that is balanced by a positive hydrostatic pressure within the cells, referred to as turgor pressure. This cellular turgor is an important component of the rigidity and firmness of plant materials. As a consequence of the disruption of the cell membranes caused by PEFs, the cellular turgor is removed, and there is a significant influence of the viscoelastic properties of plant tissues. Therefore, texture measurements can be used as an indicator of the integrity of the cell and the tissue (Fincan & Dejmek 2003, Lebovka et al. 2004). Measurement of the modification of the texture of tissues after PEF treatment has been proposed for evaluation of membrane integrity and for differentiation of the effect of different PEF treatment conditions (Asavasanti et al. 2010).

Recently, a sophisticated method using nuclear magnetic resonance relaxometry has been proposed to estimate cell permeabilization. The main advantages of this methodology are that it is a nondestructive measurement and it offers the possibility of being used in-line for optimizing PEF processing parameters during the treatment (Ersus et al. 2010).

Factors Affecting Membrane Permeabilization by Pulsed Electric Fields

The effectiveness of PEFs to permeabilize cell membranes depends on several factors that can be classified by means of process parameters (electric field strength, treatment time, specific energy, pulse shape, pulse width, frequency and temperature), physicochemical characteristics of the treated matrix (pH and conductivity), and characteristics of the treated cells (size, shape, membrane, and envelope structure). Whereas the influence of these factors on microbial inactivation by PEFs has been widely investigated (Álvarez et al. 2006), studies on factors affecting permeabilization of eukaryotic cells to improve mass transfer have been mainly focused on influence of process parameters on the efficacy of the process.

Electric field strength is the most relevant processing factor influencing membrane permeabilization of plant and animal cells. To induce membrane electroporation, it is necessary to apply a minimum electric field strength called critical electrical field strength (Asavasanti et al. 2010). Determination of this threshold is important for fundamental studies on the mechanisms of permeabilization and also for optimal process design. It has been observed that the critical electric field strength value necessary to initiate membrane permeabilization can depend on the method used to detect permeabilization and on the range of intensity of the electric field strength

applied. For different plant tissues, the critical electric field strength required has been reported to be in the range of 0.05–0.5 kV cm^{-1}, depending on the type of tissue, but the duration of the treatments at these low to moderate electric field strengths were in the range of 100–10,000 μs (Asavasanti et al. 2010, Vorobiev & Lebovka 2006). However, when shorter duration times are required (<100 μs), membrane electroporation needs the application of electric field strengths higher than 0.5 kV cm^{-1} (López et al. 2009a, Schilling et al. 2007). In the case of onion tissues, in the range of low to moderate intensities, it has been demonstrated that the critical electric field strength for the permeabilization of the cytoplasmic membrane (0.0067 kV cm^{-1} for 10 pulses of 100 μs) was lower than the critical electric field strength necessary to permeabilize the tonoplast, which is the membrane that surrounds the vacuole (0.2 kV cm^{-1} for 10 pulses of 100 μs or 0.133 kV cm^{-1} for 100 pulses of 100 μs) (Asavasanti et al. 2010). The vacuole is an intracellular organelle that can occupy 80% to 90% of the total cell volume; it is filled with water and can contain other compounds of interest, such as colorants, antioxidants, and flavors.

Generally, studies on PEF treatments to improve mass transfer have been done at room temperature. However, it has been demonstrated that temperature also may influence the damage induced by PEF in plant tissues. At electric field strengths below 0.1 kV cm^{-1}, an electroporation effect in potatoes was noticeable after treatment durations on the order of 10–1,000 s, but at 50°C treatment times were in the range of 0.01–1 s (Lebovka et al. 2005). The influence of the temperature on PEF permeabilization has been attributed to changes in the cell membrane fluidity and to structural changes in the cell walls. Microscopic analyses have demonstrated that the removal of the cell wall, which protects the cytoplasmic membrane from electroporation, increases the cell's sensibility to PEFs (Janositz & Knorr 2010).

Application of electric field strengths higher than the critical value results in an increment in the membrane electroporation and as a consequence, an improvement in mass transfer that depends on the electric field intensity and the treatment duration. However, additional increments in field strength or treatment duration above a threshold value do not cause any improvement of mass transfer, indicating that the maximum permeabilization is reached (Knorr & Angersbach 1998, Praporscic et al. 2004). As this maximum permeabilization can be obtained by applying different combinations of electric field strengths and number of pulses, from a process design point of view it is essential to identify the combinations of both parameters that require the minimum amount of total specific energy consumption.

Contradictory results have been obtained by different authors when the influence of pulse duration on the efficiency of PEF treatment for permeabilization of plant tissues has been investigated. When samples of sugar beet and apple tissues were exposed to the same treatment time, results showed higher cell permeabilization for the longer pulse duration (10–1,000 μs) (De Vito et al. 2008). However, no significant effect on the increment of permeabilization of onion tissues was observed by increasing pulse widths (20–1,000 μs) or frequency (0.5–8 Hz) (Ersus et al. 2010).

APPLICATIONS OF PULSED ELECTRIC FIELD TECHNOLOGY TO IMPROVE MASS TRANSFER

Juice Expression

Juice is the liquid that is naturally contained in cells of fruits or vegetables. One of the first steps for obtaining juices generally involves the application of mechanical pressure, which induces, among other effects, the breakdown of the cell membranes and consequently facilitates the release of the liquid phase of the interior of the cells (Bouzrara & Vorobiev 2003). Traditionally, the increase of the yield in the juice extraction has been one of the most important priorities for the

industry. As juices are often consumed for their perceived health benefits, procedures to improve yield should be gentle techniques that do not cause losses in nutritionally and physiologically valuable compounds. Treatment of mashed fruit prior to pressing with a complex cocktail of carbohydrase enzymes called pectinases is the most usual pretreatment to improve the extraction of soluble solids from fruit (Alkorta et al. 1998). However, PEF techniques have potential to improve the yield in fruit and vegetable juice processing.

The use of PEF to assist the juice expression by pressure has been deeply studied for different plant-based materials, such as carrot, grape, potato or apple tissues (Bazhal et al. 2001; Bazhal & Vorobiev 2000; Bouzrara & Vorobiev 2003; Grimi et al. 2011; Lebovka et al. 2003, 2004; McLellan et al. 1991; Praporscic et al. 2007; Schilling et al. 2007, 2008). However, variable improvements in extraction yield have been reported. McLellan et al. (1991) did not observe any significant increase in the release of apple juice after PEF treatments. However, laboratory tests demonstrated that PEF (1–5 kV cm^{-1}; 1.1–27 kJ kg^{-1}) increased apple juice yields in the range of 1.7% to 7.7%, whereas enzymatic treatment of the mash resulted in an improvement of 4.2% (Schilling et al. 2007). When a similar study was conducted by the same authors at the pilot scale, a PEF treatment of 10 kJ kg^{-1} at 3 kV cm^{-1} led to an enhancement in the release of nutritionally valuable polyphenols into the juice, but total juice yield was decreased rather than increased after application of PEFs to the apple mash (Schilling et al. 2008). These authors reported that in contrast to enzymatic mash maceration, genuine pectin quality was retained after PEF processing, which creates an additional commercial benefit.

Grimi et al. (2011) observed that the effect of PEF treatment on apple juice yield depended on whether the treatment was applied to the whole apples or to apple slices. Although the juice yield was higher when the PEF treatment was applied to the apple slices (64%) than when applied to the whole apples (58%), the treatment of the whole apples required lower energy consumption, improved juice characteristics, decreased juice turbidity, and increased antioxidant capacity.

Extraction of Intracellular Valuable Compounds

The use of PEFs has been investigated for improving the extraction of different compounds located in the cytoplasm or organelles of plant cells, such as pigments, sucrose, polyphenols, and oils.

Colorants. In recent years, there has been an increasing interest in the food industry in replacing synthetic colorants with natural pigments from fruit and vegetable extracts. This trend can be attributed to the safety and health benefits of natural pigments and to a strong consumer demand for more natural products (Cai et al. 2005). Natural colorants include chlorophylls, carotenoids, betalains, and flavonoids (especially anthocyanins). Depending on their solubility, these compounds are extracted by employing organic or inorganic solvents. Owing to their special sensibility to different factors, such as light, temperature, and oxygen, only gentle thermal processing or pressing techniques are usually applied to improve the extraction of these natural colorants (Delgado-Vargas et al. 2000).

It has been demonstrated that the application of a PEF treatment prior to the extraction facilitates the release of chlorophylls (Koehler et al. 2005), carotenoids (Grimi et al. 2007, Kim et al. 1999, Koehler et al. 2005), betalains (Chalermchat et al. 2004, Fincan et al. 2004, López et al. 2009b), and anthocyanins (Corrales et al. 2008, Gachovska et al. 2010). In all cases, PEF treatment led to important improvements in the extraction yield, revealing that this is a technology to consider for the industry. For instance, Koehler et al. (2005) studied the use of PEF for the extraction of chlorophyll and carotenoids from *Chlorella vulgaris*, obtaining improvements of 80% and 52.2%, respectively, after the application of a PEF treatment of 100 kJ kg^{-1} at 15 kV cm^{-1}.

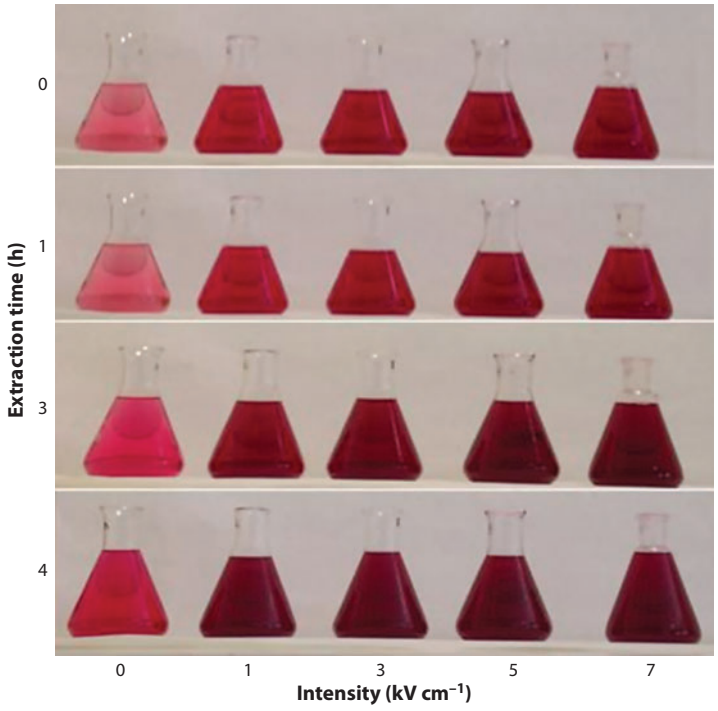

Figure 3

Color of extraction media after different extraction times (0, 1, 3, and 4 hours) containing red beetroots untreated (0 kV cm^{-1}) and treated by pulsed electric fields at different intensities (1, 3, 5, and 7 kV cm^{-1}).

López et al. (2009b) reported that the application of five pulses at 7 kV cm^{-1} (0.24 kJ kg^{-1}) created four times the total amount of betalain extracted from red beetroot (**Figure 3**). With respect to anthocyanins, Gachovska et al. (2010) determined that a PEF treatment of 16.63 kJ kg^{-1} at 2.5 kV cm^{-1} enhanced total anthocyanin extraction in water from red cabbage by 2.15 times. Similar improvements were described by Corrales et al. (2008) for winemaking byproducts after applying a treatment of 10 kJ kg^{-1} at 3 kV cm^{-1}.

Sucrose from sugar beets. The traditional process of extraction of sucrose from sugar beets involves cutting the beet into cossettes and posterior water extraction at high temperatures (70–75°C) during extracting times of 1–1.5 hours (Van der Poel et al. 1998). Unfortunately, the unspecific thermal destruction of the cells results in the release of nondesirable substances, such as pectin, into the extract, subsequently requiring a costly and complicated purification process. Furthermore, the water heating also implies significant energy consumption (approximately 175 kJ kg^{-1}), and the high temperatures favor the growth of thermophilic flora, which degrades sucrose, thus reducing the yield (Schultheiss et al. 2002).

Improvement of the sucrose extraction from sugar beets by PEFs has been one of the more deeply studied applications of this nonthermal technology (Bouzrara & Vorobiev 2000, El-Belghiti & Vorobiev 2004, El-Belghiti et al. 2005, Eshtiaghi & Knorr 2002, Jemai & Vorobiev 2006, Lebovka et al. 2007, López et al. 2009a). Eshtiaghi & Knorr (2002) found a similar disintegration level after the application of 60 pulses at 2.4 kV cm^{-1} as after a thermal treatment of 15 minutes at 75°C. El-Belghiti et al. (2005) developed a model to describe the sucrose extraction kinetics by

PEF, finding an optimum treatment of 250 pulses at 0.67 kV cm^{-1}. Moreover, the authors found that PEF treatment allowed them to obtain an extract with better global quality than did thermal treatment.

PEF has also been proposed to reduce the extraction temperature of sucrose from sugar beets. López et al. (2009a) studied the influence of PEF (0–7 kV cm^{-1}; 0.01–0.19 kV cm^{-1}) and the temperature extraction (20–70°C), determining that, for an extraction yield of 80%, the temperature could be reduced from 70°C to 40°C while applying a pretreatment of 20 pulses at 7 kV cm^{-1}.

Winemaking. Wine is an alcoholic beverage resulting from the fermentation of fresh grape juice. During red winemaking, the must of red grapes undergoes fermentation together with the grape pomace in a step called fermentation-maceration. In this step, yeasts convert most of the sugars of the grape juice into ethanol, but also phenolic compounds are extracted from the grape skin. These compounds play an essential role in the product quality, especially in red wine, making a fundamental contribution to the organoleptic attributes, particularly to color, bitterness, astringency, and mouthfeel, but also to red wine's well-known health-promoting characteristics, due to their antioxidant and free radical–scavenging properties (Boulton 2001, He & Giusti 2010, Rice-Evans et al. 1996). Owing to their particular significance, different winemaking practices, such as thermovinification, grape freezing, and the use of maceration enzymes, have been proposed to facilitate the extraction of these compounds during winemaking and, therefore, obtaining red wines with a high phenolic content (Bautista-Ortín et al. 2007, Sacchi et al. 2005).

The improvement of the phenolic extraction during red winemaking by treating the grapes by PEF before the fermentation-maceration step is one of the most investigated applications of this technology (Puértolas et al. 2010b). Initial studies conducted in 2005 at the University of Zaragoza demonstrated that a PEF treatment (2–10 kV cm^{-1}; 0.4–6.7 kJ kg^{-1}) to grapes previous to fermentation-maceration increased and accelerated the phenolic extraction in different varieties, such as Tempranillo, Graciano, Mazuelo, Garnacha, and Cabernet Sauvignon (López et al. 2008a,b; 2009c). The improvements obtained in color intensity, anthocyanins, and phenolic concentration for each variety by applying a PEF treatment ranged from 19% to 62%, from 18% to 43%, and from 14% to 45%, respectively. Similar results were obtained by Donsi et al. (2010a) in two Italian grape varieties, Aglianico and Piedirosso.

The feasibility of processing red grapes by PEF at the pilot scale (118 kg h^{-1}) was demonstrated later on, obtaining similar results to those obtained at lab scale (Puértolas et al. 2009a,b). The better chromatic characteristics and the higher phenolic content of the wine obtained from PEF pretreated grapes after the fermentation-maceration step were retained during aging in bottles or in oak barrels (Puértolas et al. 2010c,d). First sensory analysis carried out showed that the PEF treatment did not cause any strange taste or off-flavors. The wine obtained from PEF-treated grapes was similar to the control wine from a sensory point of view (Puértolas et al. 2010a). Recently, the potential of PEF treatment for reducing the temperature and time of maceration during elaboration of rosé wine has also been demonstrated (Puértolas et al. 2011).

Vegetable oils. Oils from plants are usually contained in seeds such as soybeans, sunflower seeds, corn kernels, and rapeseeds or fruits such as palm or olive. The extraction of oil from seeds generally involves mechanical extraction by pressing and extrusion followed by solvent extraction (Xu & Diosady 2003). However, there is an increasing demand for techniques with reduced organic solvent. Processes in which oil is obtained from seeds only by pressing operations are being used in the industry. In the case of virgin olive oil, the product is extracted from olives using only mechanical systems. The most usual procedure for obtaining this product involves crushing the

olives, after which the paste is stirred slowly in a mixer, and finally, after malaxation, the oil is separated from the paste by centrifugation. The increment of the paste temperature in the mixer increases the olive oil extraction yield, but it may affect the quality of the olive oil.

The use of PEF as a pretreatment to improve the oil extraction by conventional or novel techniques in different seeds or fruits, such as maize, soybeans, rapeseeds, and olives, has been investigated. Guderjan et al. (2005) experimented with the influence of the application of 120 pulses at 3 kV cm^{-1} on the posterior extraction of maize oil by pressing, supercritical carbon dioxide, and hexane. With respect to the controls, the PEF treatment allowed an increased extraction yield of 25.2%, 14.9%, and 27.8%, respectively. Moreover, the extracted oils presented a phytosterol concentration higher than that of the controls (up to 32.4%). An increment of 55% in the extraction yield in rapeseed oil production was obtained by applying a PEF treatment of 120 pulses at 7 kJ kg^{-1} (Guderjan et al. 2007). Likewise, higher concentrations of tocopherols, polyphenols, total antioxidants, and phytosterols were measured in the oils produced using PEF. Recently, Sánchez-Gimeno et al. (2010) reported that the application of a PEF treatment to the olive paste after grinding increased the percentage of oil extracted by 8.3% when the malaxation was conducted under cold processing conditions (15°C), improving the sensory characteristics of the product.

Dehydration

The basic aim of dehydration processes is to remove the moisture from foods in order to reduce their water activity, thus increasing their shelf life. Moreover, this water elimination decreases the weight and the volume of the products, facilitating transport and storage. In the food industry, the production of dehydrated foods is mostly accomplished by thermal dehydration or hot drying. These conventional techniques involve high energy costs (4–6 MJ kg^{-1}) and also affect the physical and biochemical status of foods, leading to shrinkage and change of color, texture, and taste (Ade-Omowaye et al. 2001). One of the techniques proposed to improve the process is osmotic dehydration, which consists of introducing the food material into a suitable hypertonic solution (Rastogi et al. 2002). As a consequence, partial water elimination from food and a diffusion of the solute to the product occur. Generally, osmotic dehydration is a slow process, suggesting the need for improving the process without negatively affecting the food quality.

Different studies have demonstrated that the increase of cell permeability by a previous PEF treatment enhances conventional and osmotic dehydration. For example, it has been reported that PEF treatment of several vegetables, such as potatoes, red peppers, and coconuts, reduced the conventional dehydration time by 20% to 30% without increasing the temperature above 60°C (Ade-Omowaye et al. 2000, 2003b; Knorr & Angersbach 1998). In the case of osmotic dehydration of vegetables, such as carrots, apples, and peppers, researchers have reported a higher release of water (up to 30%) and an increase of the dehydration rate in PEF pretreated samples as compared with the controls (Ade-Omowaye et al. 2002, 2003a; Amami et al. 2006, 2007; Rastogi et al. 1999; Taiwo et al. 2002, 2003). However, it has been observed that PEF did not cause an increment in the uptake of solutes during osmotic drying of samples pretreated by PEF.

Concerning the influence of PEF in the final product quality, the results depend on the application. For conventional dehydration, the PEF treatment led to a decrease in the processing temperature, conserving better sensory characteristics (Ade-Omowaye et al. 2001). On the contrary, the effect of PEFs in the osmotic dehydration seems unclear. An enhancement of the color was observed, but different studies have showed that PEFs could negatively influence the concentration of determined metabolites of interest, such as vitamin C, as an indirect effect of the increment on the water release (Taiwo et al. 2003). In any case, it is necessary to remark that the products pretreated by PEFs presented better rehydration capacity than did the controls.

Curing and Marination of Meat and Fish

Disintegration of animal cellular tissue by PEF treatment might also be utilized to enhance processes in which an uptake of substances is required, such as marination or curing of fish and meat products. In these cases, different salts and spices are added to preserve and to develop specific tastes and aroma profiles. Until now, the studies conducted on the use of PEFs for this application are scarce, possibly because of the necessity of processing solid structures of considerable dimensions and to the high conductivities of the animal tissues. Gudmundsson & Hafsteinsson (2001) determined an increase from 17% to 22% of the weight of brine-marinated cod fillets, after the application of 300 pulses at 3 kV cm^{-1}. Toepfl & Heinz (2007b) studied the effect of PEF treatment on ham curing time. The authors described that a PEF treatment of 20 kJ kg^{-1} at 4 kV cm^{-1} allowed shortening the time necessary to remove 30% of the meat's weight from 310 hours to only 60 hours.

Related to the scaling of the technology, the first system developed to apply treatments in a continuous flow was designed in the German Institute of Food Technology (Toepfl & Heinz 2007b). However, more studies are needed on the effects of PEF on animal tissues, especially related to the quality of the final products.

INDUSTRIAL FEASIBILITY AND COST ESTIMATION

Although a lot of research has been conducted for years on the potential applications of PEF to mass transfer, industrial application still remains unrealized. One of the major concerns for commercialization of PEF technology has been the generation of high voltage pulses with sufficient peak power to satisfy the industrial requirements (Puértolas et al. 2010b). In recent years, different pilot-scale equipment and industrial prototypes have been developed, especially for sucrose extraction, apple juice processing, and winemaking (Sack et al. 2010b).

Based on the promising results accomplished in bench-scale experiments, the first industrial prototype designed for sucrose extraction from sugar beets was developed in the Karlsruher Institut für Technologie using Marx generators (Sack et al. 2010b). In a sugar factory, 10–50 tons of sugar beets are processed per hour. This system allowed applying PEF treatments (3–5 kV cm^{-1}) to whole sugar beets at a throughput of approximately 10 tons per hour.

In 2006, based on Marx generators also, a PEF system for enhanced apple juice expression was developed (Sack et al. 2010b). With a throughput of 10 ton h^{-1}, the device has been installed onsite at an apple juice producer. Nevertheless, to the best of our knowledge there are still no commercially available apple juices produced by PEF technology.

Concerning the application of PEF for improving phenolic extraction in red winemaking, the first promising tests at wineries using industrial prototypes have been carried out by researchers at Karlsruhe Institute of Technology using electric field strengths higher than 30 kV cm^{-1} and a flow rate of 900 kg^{-1} h (Sack et al. 2010a) and by researchers at University of Zaragoza at lower electric field strengths (<5 kV cm^{-1}) and higher flow rate (2,000 kg h^{-1}) (**Figure 4**).

The most comprehensive approach to the economic cost of PEF treatments for improvement of mass transfer to soften tissues has been done by Toepfl et al. (2006a). In this study, the costs of the use of PEFs on juice extraction with the employment of maceration enzymes were compared in detail. Using the assumption of a current price of 0.1 € kWh as a base, the authors concluded that for applying a PEF treatment of 10 kJ kg^{-1} at 1–2 kV cm^{-1} and using a throughput of 10 ton h^{-1} (3 kWh ton^{-1}), the cost of the treatment can be estimated at 0.33 € ton^{-1}. For a conventional enzymatic maceration, the treatment costs can be estimated at 7.50 € ton^{-1}, 22.7

Figure 4

Pulsed electric field system developed at University of Zaragoza for treating grapes to improve the extraction of phenolic compounds during the fermentation-maceration step of red winemaking.

times the cost of PEF treatment. Using the same estimations and based on the data published, it has been estimated that the cost of PEF treatment (2–7 kV cm^{-1}; 0.56–6.76 kJ kg^{-1}) to improve the phenolic extraction in winemaking could be approximately 0.01–0.2 € ton^{-1} (Puértolas et al. 2010b). Concerning the use of PEFs for sucrose extraction, Frenzel et al. (2005) reported that the electric energy necessary for the electroporation is only 1–1.5 kWh ton^{-1} of sugar beets, which is only 3% of the total electric energy consumption of a sugar factory.

Owing to the low treatment costs, the major concerns for implementation of PEF technology in the food industry are the investment cost and the development of industrial equipment (currently in progress). Considering the general processing parameters reported for mass transfer (1–7 kV cm^{-1}; 2–50 kJ kg^{-1}), a generator with an average load power and voltage of 30 kW and 30 kV, respectively, is required. The estimated cost of this equipment is 100–200 k€. It is expected that the low cost per ton of the treatments might amortize this investment within a very short period of time. For instance, Sack et al. (2010b) determined that for apple juice processing, the increase of 6% of unfiltered juice obtained would compensate for the investment costs after only 1.5 years. Moreover, it is expected that when the technology is commercialized, the cost of the equipment will strongly decay.

The capacity of PEFs as an effective treatment to improve mass transfer in different processes of the food industry is demonstrated above. The low energy consumption and the short processing times required for permeabilization of eukaryote cells are key advantages for the immediate implementation of PEF technology at the industrial level.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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