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Electroporation in Food Processing and Biorefinery

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Abstract Electroporation is a method of treatment of plant tissue that due to its nonthermal nature enables preservation of the natural quality, colour and vitamin composition of food products. The range of processes where electroporation was shown to preserve quality, increase extract yield or optimize energy input into the process is overwhelming, though not exhausted; e.g. extraction of valuable compounds and juices, dehydration, cryopreservation, etc. Electroporation is—due to its antimicrobial action—a subject of research as one stage of the pasteurization or sterilization process, as well as a method of plant metabolism stimulation. This paper provides an overview of electroporation as applied to plant materials and electroporation applications in food processing, a quick summary of the basic technical aspects on the topic, and a brief discussion on perspectives for future research and development in the field. The paper is a review in the very broadest sense of the word, written with the purpose of orienting the interested newcomer to the field of electroporation applications in food technology towards the pertinent, highly relevant and more in-depth literature from the respective subdomains of electroporation research.

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Introduction

The Electroporation Phenomenon

Electroporation (also termed electropermeabilization or pulsed electric field treatment) is a process in which induced transmembrane potential by means of an externally applied electric field of sufficient strength causes an increase in cell's plasma membrane conductivity and permeability. This increase is attributed to creation of aqueous pathways i.e. pores in the lipid bilayer and has been demonstrated by experiment on lipid bilayers, cells in suspension, monolayers and biological tissues. See e.g. (Krassowska and Filev 2007a; Neu and Neu 2009; Kotnik et al. 2012a) as essential reading in fundamentals of electroporation. Although other terminology is also in use, such as electropermeabilization and PEF treatment, we will continue to refer to the process in the remainder of this paper as electroporation.

Depending on the duration of cell's exposure to the electric field, the local field strength (i.e. the maximum amount of energy deliverable to the membrane via the external electric field), and the rate of membrane recovery, there are three possible outcomes of electric field application. If the field strength and exposure time are insufficient, there is no electroporation, and cell's permeability and viability remain unaffected. See (Kotnik et al. 2012a) for the basic principles of the electroporation phenomena. If the field strength exceeds what is known as reversible threshold and exposure is of sufficient duration, so-called

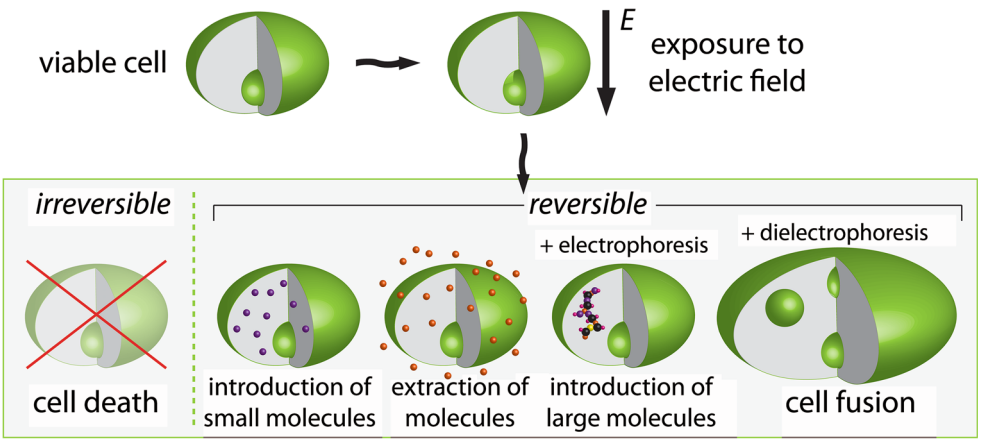

Fig. 1 A schematic representation of cell electroporation with possible outcomes depending on the pulsing protocol (amplitude, shape, duration of pulses) and additional cell manipulation techniques, e.g. (di)electrophoresis. Schema redrawn based on Fig. 16.1 in (Rebersek and Miklavcic 2010)

reversible electroporation occurs (Rols and Teissié 1990; Phoon et al. 2008; Čorović et al. 2012); the membrane is permeabilized and remains in a state of higher permeability for a period of time, but is eventually able to spontaneously return to its original state by means of membrane resealing, a process in which the pores close and the cell restores its normal transmembrane potential. We should point out that this is only possible provided the environmental conditions stay favourable for cell survival and function. If the field strength and amount of delivered energy are too high, however, irreversible electroporation occurs (Davalos et al. 2005; Al-Sakere et al. 2007), resulting in loss of cell homeostasis (and possibly in a complete breakdown of the plasma membrane), effectively killing the cell. Figure 1 is a schematic representation of some of electroporation applications depending on the outcome of the treatment and on whether additional low-voltage electric fields are used in combination with electroporation facilitating other phenomena, such as electrophoresis and dielectrophoresis (Kanduser et al. 2009; Usaj et al. 2010; Hu et al. 2013; Rems et al. 2013).

Both reversible and irreversible electroporation have found their applications in fields such as biomedicine, biotechnology and the environmental sciences (Haberl et al. 2013; Yarmush et al. 2014). In biomedicine, reversible electroporation is used in electrochemotherapy to introduce cytotoxic drugs into tumour cells in a process known as electrochemotherapy (Miklavcic et al. 2012; Mali et al. 2013), for gene and transdermal drug delivery (Gehl 2003; Denet et al. 2004; Pavselj and Miklavcic 2008; Chiarella et al. 2013), cell fusion (Hu et al. 2013; Rems et al. 2013), as well as inserting proteins into the plasma membrane (Raffy et al. 2004), while non-thermal irreversible electroporation (NTIRE—non-thermal irreversible electroporation) is being used as a means of tissue ablation for cancer treatment (Davalos et al. 2005; Garcia et al. 2011).

In food processing, electroporation has shown promising results for extraction of juices and other valuable compounds from plant tissue and microorganisms, such as microalgae (Fincan et al. 2004; Vorobiev and Lebovka 2008; Donsi et al. 2010; Puertolas et al. 2012; Vanthoor-Koopmans et al. 2013), tissue dehydration (Ade-Omowaye et al. 2001; Lebovka et al. 2007b; Shynkaryk et al. 2008; Jaeger et al. 2012) and nonthermal preservation and sterilization by microbial inactivation (Qin et al. 1996; Jayaram 2000; Yeom et al. 2002; Wesierska and Trziszka 2007; Mosqueda-Melgar et al. 2012; Bermudez-Aguirre et al. 2012; Marsellés-Fontanet et al. 2012). Reversible electroporation may also help in creating new methods for cryopreservation of biological tissues (Phoon et al. 2008; Shayanfar et al. 2013), and plant metabolism stimulation (Sabri et al. 1996; Ye et al. 2004; Saw et al. 2012; Dymek et al. 2012; Straessner et al. 2013). Among the environmental applications, we find wastewater treatment (Rieder et al. 2008; Gusbeth et al. 2009; Junfeng et al. 2013) and biofuel production (Vanthoor-Koopmans et al. 2013; Zbinden et al. 2013; Eing et al. 2013; Grimi et al. 2014), both currently under intensive research and development as promising electroporation applications.

This paper focuses on the applications of electroporation in the field of food processing and valuable compounds recovery (biorefinery). We first give a brief overview of potential electroporation applications in the food processing industry, followed by a more detailed review of the current state–of-the-art within the scope of each of the applications. We then present some of the problems encountered and common challenges facing researchers in the field. Thereon, we continue by describing approaches taken when transferring theoretical knowledge to practical implementations and conclude with electroporation in food processing and environmental technologies in light of perspectives for future research and development. For more comprehensive reviews on electroporation as an emerging

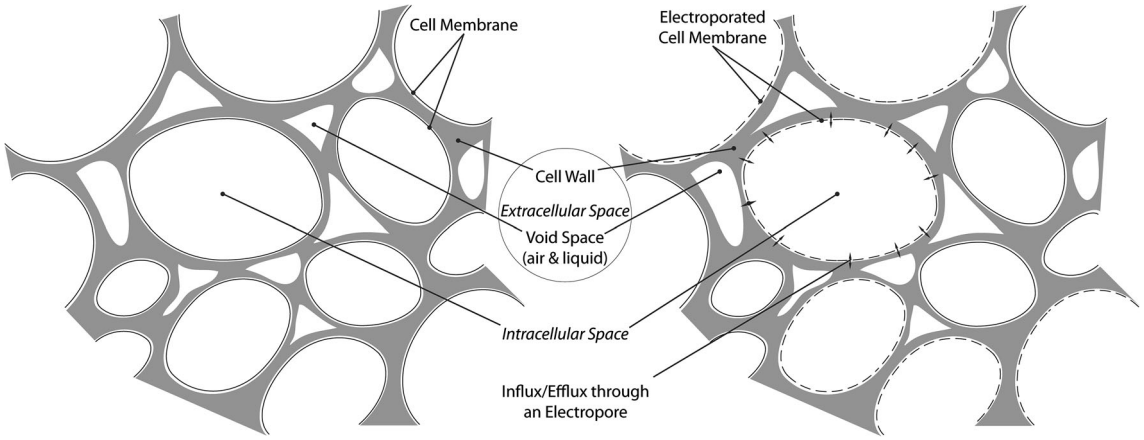

Fig. 2 A schematic representation of plant tissue before (*left*) and after electroporation (*right*). The pores created in the plasma membrane facilitate influx and/or efflux of water and solutes into the cells from the extracellular space or from the cells into the extracellular space. Redrawn based on Fig. 17 in (Halder et al. 2011)

technology in food processing, see e.g. (Vorobiev and Lebovka 2010; Knorr et al. 2011).

Overview of Fundamentals and Applications of Electroporation in Food Processing

As shown by the schematic representation of plant tissue in Fig. 2 (left-hand part) in plant tissue cells are embedded into a structure formed by the extracellular matrix—the cell wall. The cell wall provides support to the plasma membrane, enabling it to withstand enormous pressure differences across the membrane due to turgor and gives shape to the cell. The cell wall is a selective filter that allows water and ions to diffuse freely, but is a limiting factor in diffusion or convective flux of large molecules of more than 20 kDa in size. However, molecules of up to 10 kDa can pass between cells of some higher plants through structures known as plasmodesmata, i.e. specialized cell–cell junctions that extend through the cell wall (Lodish et al. 2008). Note that these junctions are not shown in Fig. 2. Although in intact plant tissue the plasma membrane is the structure providing the largest resistance to intercellular (i.e. cell-to-cell) transport, as well as to transport between intracellular and extracellular space, we must bear in mind also the hindrance and filtering behaviour of the cell wall when considering mass transport in plant tissues (Buttersack and Basler 1991), as opposed to animal tissues where cells are not embedded in a porous extracellular structure akin to the cell wall. The cell wall thus presents an additional complexity and must be considered when studying electroporation and related mass transport phenomena in plants (Janositz and Knorr 2010; Janositz et al. 2011).

Figure 2 (right-hand part) gives a simplified schematic representation of plant tissue that has been electroporated. The plasma membrane has increased permeability due to electroporation (depicted by the dashed line representing the permeabilized membrane) and has lost the ability to selectively control influx and efflux of water and of those solutes that are, depending on their hydrodynamic size, able to pass through the membrane. According to current knowledge, see e.g. (Galindo et al. 2008; Ganeva et al. 2014; Stirke et al. 2014), electroporation may also affect the cell wall and either decrease or increase its permeability (results of recent studies seem to be rather contradictory, and further studies are needed). The effect of electroporation on cell wall appears to depend on cell configuration (cells in suspension, monolayers or tissues), species of organism (yeast, plants) and treatment protocol. However, the increase in permeability and conductivity of electroporation-treated plant tissue is by general consensus attributed predominantly to the increased permeability of the cell membrane. As mentioned in "The Electroporation Phenomenon" section, electroporation may cause the cell membrane to break down completely (irreversible electroporation), in which case the cytosol, parts of disintegrated membrane, and the intracellular material remain trapped in the extracellular matrix.

The described phenomenon of plant cell membrane increased permeability or destruction via electroporation opens up a plethora of interesting possibilities from the food processing point of view. The mass transport facilitated by the disrupted plasma membrane and promoted by concentration gradients (diffusion) or pressure gradients (process is often referred to as *expression*), is of interest in many applications in food industry. In Table 1, we list applications where the interest is predominantly in mass transport enhancement by electroporation, also identifying the primary mechanism or objective of processing improved by electroporation, as well as the required field strength and specific energy consumption per unit mass of the treated material.

Table 1 Electroporation applications to food materials with focus on improving mass transport

Application	Electric field strength[a] (kV/cm)	Energy consumption[a] (kJ/kg)	Primary mechanism of action	Typically studied (model) materials
Juice extraction	0.1–5	1–15	Extraction of intracellular water with solutes	Apple, carrot, potato, sugarbeet
Valuable compounds recovery	0.1–10	0.5–90	Extraction of intracellular compounds e.g. phenols	Apple, carrot, chicory, rapeseed, red beetroot, sugarbeet
Wine quality enhancement	2–10	0.4–10	Release of polyphenols into grape juice	Cabernet sauvignon, Riesling, Aglianico, and other local varieties
Dehydration	0.1–3	0.5–25	Extraction of water	Apple, carrot, mango, pepper, potato, strawberry, green biomass
Biorefinery[b]	1.5–50	10–60	Lipid, protein extraction, release of other extractives	Microalgae, yeast, alfalfa, grapes, rapeseed
Cryopreservation[c]	App. 0.5	n/a	Reversible electroporation, infusion of cryopreservant	Spinach leafs
Meat processing (meat curing)[d]	2–3	10	Infusion of curing agent (e.g. salts)	Pork (ham, sausages, minced meat)

Consolidated from data given in (Donsi et al. 2010), except where otherwise indicated

[a] A range of averages is given to consolidate findings from most of the reviewed studies. See reference(s) for details

[b] See e.g. (Gachovska et al. 2006; Liu et al. 2012, 2013; Grimi et al. 2014)

[c] See e.g. (Phoon et al. 2008)

[d] See e.g. (Toepfl 2006; Toepfl and Heinz 2008)

In case of water or solute introduction into or extraction out of tissue, we are applying the electric treatment via a series of trains of electric pulses of moderate field strength[1] (MEF—moderate electric field), i.e. several 100 V/cm up to 1–2 kV/cm (see Table 1; Puertolas et al. 2012). However, the augmenting effect of electroporation to mass transport has also been observed in applications of low intensity electric fields to the biological material, on the order of less than 100 V/cm (Asavasanti et al. 2010). If the applied electric pulses are of high intensity (HPEF—high pulsed electric field), on the order of about 5–50 kV/cm, they may cause irreversible damage to cells of bacteria, yeasts and moulds, and can therefore be used to preserve material through inactivation and destruction of these microorganisms; the objective in this case is to pasteurize or sterilize the biological liquids: foods or sludge (Álvarez et al. 2006; Mosqueda-Melgar et al. 2008a, 2008b; Guerrero-Beltran et al. 2010; Bermudez-Aguirre et al. 2012). This is often achieved through a combination of electroporation treatment and other traditional techniques of food preservation—e.g. thermal, chemical or pressure based (Martín-Belloso and Sobrino-López 2011)—as many of the harmful microorganisms, such as bacterial spores, prove to be hard to destroy by pulsed electric fields alone (Shin et al. 2010; Bermudez-Aguirre et al. 2012) and are considered as one of the hurdles in pasteurization and sterilization processes. Table 2 summarizes the applications where electroporation is employed primarily with intent to inhibit microbial activity in the treated liquid. Additional information in terms of the required field strengths and energy consumption is also given. By comparing data in Table 2 with data presented in Table 1, note the higher field requirements and specific energy consumption in microbial inactivation applications. To understand why microbial inactivation generally requires significantly higher field strengths and energy consumption in comparison with e.g. extraction applications, it is important to understand the mechanisms of action of electroporation and related phenomena. The process of exposing a viable cell to an external electric field can be divided into several stages (Saulis 2010). First, the transmembrane potential increases with time due to charging of the plasma membrane by the electric field. The amplitude of the transmembrane potential reached is determined by cell size and shape, membrane and medium conductivities (intra and extracellular) and pulse parameters (shape, duration, field strength). A critical transmembrane potential is required in order for pore initiation stage to begin, when a population of small metastable hydrophilic pores appears in the plasma membrane. This population evolves in time, during which the number of pores as well as their sizes change. Following the electric treatment, in the post-treatment stage lasting

[1] Though no formal definition exists, the electric field strengths are often referred to in the literature as per the defined ranges or abbreviations, and these designations do not necessarily represent the actual local electric field strength the cells are exposed to.

Table 2 Electroporation applications with focus on microbial inactivation

Application	Electric field strength[a] (kV/cm)	Energy consumption[a] (kJ/kg)	Reference(s)
Juice preservation	2–30	5–250	(Toepfl 2011)
Solid and semisolid food products preservation[b]	Comparatively higher than in juice preservation	Comparatively higher than in juice preservation	(Gudmundsson and Hafsteinsson 2005)
Enzyme deactivation	10–90	100–2500	(Mañas and Vercet 2006; Buckow et al. 2013)
Sludge disintegration (wastewater treatment)	10–20	50–200	(Toepfl et al. 2006)

Typical field strengths and energy consumption

[a] A range of averages is given to consolidate findings from most of the reviewed studies. See reference(s) for details

[b] The subject of electroporation-assisted microorganism deactivation in solid products lacks reliable studies. It has been found that high field strengths required for inactivation (higher than for juices) have a detrimental effect on texture and structure, rendering the treatment application to these materials for preservation unrealistic (Gudmundsson and Hafsteinsson 2005)

from milliseconds to hours, leakage of intracellular compounds, entrance of substances present in the extracellular space into the cell and ultimately pore resealing or cell death as well as other processes, occur. The result of electroporation treatment, either a viable or a dead cell, is thus determined by conditions and processes that are occurring during all stages of electroporation treatment. These conditions can vary considerably for different applications. Considering an exemplary microorganism—a pathogenic bacterial species for example—as a typical prokaryotic organism only several micrometres in length, the induced transmembrane voltage is calculated to be an order of magnitude lower at the same external electric field strength, as compared to a small eukaryotic cell several tens of micrometres in diameter. The presence (and structure) of cell wall should also be considered (Aronsson et al. 2005; Golberg et al. 2012). For a more thorough and in-depth treatment of microbial inactivation from the point of view of electroporation theory, see for instance (Saulis 2010). We also give further references in discussions of pasteurization and sterilization by electroporation, found within "Inactivation and Destruction of Microbial Contaminants, Parameters Influencing the Success of Electroporation Treatment Application and Methods of Evaluating Their Importance, and Pasteurization and Sterilization of Liquid Foods: A New Arms Race?" sections.

In addition to the described enhanced mass transport or membrane destruction and applications based on these effects of electroporation, pulsed electric fields may also be used at low intensities (less than 100 V/cm) for induction of stress response in plant and yeast cells (Ye et al. 2004; Dymek et al. 2012; Straessner et al. 2013). Though a very limited body of research is available in this field of electroporation, we will briefly discuss the subject in the succeeding chapter, in which we examine the state of the art of electroporation research in food processing.

Figure 3 gives a schematic representation of exposure of a biological cell to an external electric field and applications of electroporation in food, biomaterials and wastewater processing.

Electroporation in Food Processing

Extraction of Juices

Juice is the liquid naturally contained in cells of fruits and vegetables and is generally extracted from tissue via application of one or several pre-treatment procedures, followed by pressing or passive diffusion. The pre-treatment processes, such as mechanical, thermal, chemical, enzymatic, etc. (Lebovka et al. 2011), are designed to damage the biological tissue (cells and the extracellular matrix), and are followed by application of pressure, which further facilitates the release of the liquid phase of cell interior by exerting force causing cell membrane breakdown. The pre-treatment may also be followed by suspending tissue in a solvent (e.g. water), thus facilitating extraction via diffusion that occurs due to the concentration gradient which is established as tissue with high concentration of solutes in juice is submerged into the solvent of higher purity (Bouzrara and Vorobiev 2003; Bazhal et al. 2003; Lopez et al. 2009).

For the food industry, optimizations in juice yield, quality and energy consumption have traditionally been at the forefront of interest. Since juices are often consumed for their favourable organoleptic properties and perceived benefits to health, it is highly undesirable that these properties should be lost during the pre-treatment and extraction processes (Puertolas et al. 2012). Enhancing hydraulic pressing efficiency, diffusion rate and increasing the yield in production of fruit and vegetable juices through

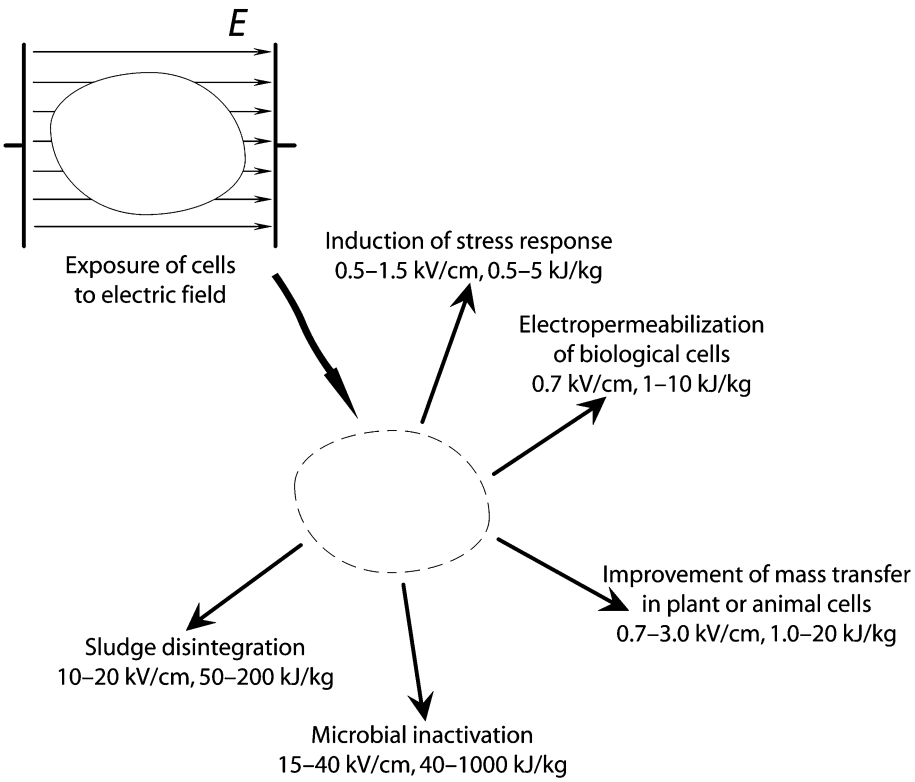

Fig. 3 A schematic representation of the exposure of a biological cell to an external electric field and resulting applications of electroporation in food, biomaterials and wastewater processing. Reproduction based on a figure given in (Toepfl et al. 2005)

membrane electroporation and damage are some of the beneficial effects of applying electroporation to plant tissues as a pre-treatment.

Initial attempts at electroporating plant tissue for improving juice extraction suffered from uncontrolled increase of food temperature and thus product quality deterioration due to high-intensity electric fields applied. In recent years, however, effective electroporation of plant cell membranes has been demonstrated at moderate electric pulse amplitude, avoiding excessive temperature increase and opening possibilities for numerous applications for juice extraction enhancement.

Some of the products for which efficiency of electroporation has been shown are alfalfa, apples, carrots, peppers, potatoes and sugar beet (Gachovska et al. 2006; Donsi et al. 2010; Vorobiev and Lebovka 2010). Electroporation-assisted extraction was shown to give increased juice yields of up to 70 % when applied as the sole pre-treatment technique, improving the quality of juice in terms of purity (as compared to thermal treatment), while also increasing concentration of valuable juice solutes such as proteins, minerals, β-carotene and others up to 60 %. In sugar beet, a 100 % increase in total soluble solids in juice was obtained with electroporation followed by pressing (Mhemdi et al. 2012). Synergistic effects between electroporation and ohmic heating have also been demonstrated, giving juice extraction yields up to 85 % higher as compared to traditional thermal or enzymatic pre-treatments. See e.g. (Vorobiev and Lebovka 2008, 2010; Puertolas et al. 2012) for a comprehensive review of the studies in electroporation-assisted juice extraction.

Recovery of Valuable Compounds

Extraction of several valuable metabolites that may be found in plant cells and inside cell organelles such as vacuoles is of great commercial interest. Electroporation in this context has been studied as means to enhance extraction of colourants (pigments), antioxidants (e.g. polyphenols), flavonoids and other secondary metabolites, sugars and lipids (e.g. oils) from plants and microalgae.

According to recent reviews in (Donsi et al. 2010; Vorobiev and Lebovka 2010; Puertolas et al. 2012), it has been demonstrated that applying electroporation treatment prior to extraction enhances the release of chlorophylls, carotenoids, betalains and flavonoids, especially anthocyanins. In a study with microalgae of species *Chlorella vulgaris* for instance, improvement in chlorophyll and carotenoids extraction by 80 and 52 %, respectively, was obtained after application of electric pulses. In a similar study with betanin extraction from red beetroot, 90 % of total red colourant was extracted from electroporated sample, in comparison with less than 5 % from the untreated control. Since these organic compounds are normally sensitive to light, temperature and oxidation, traditional extraction techniques are mild in nature (mild

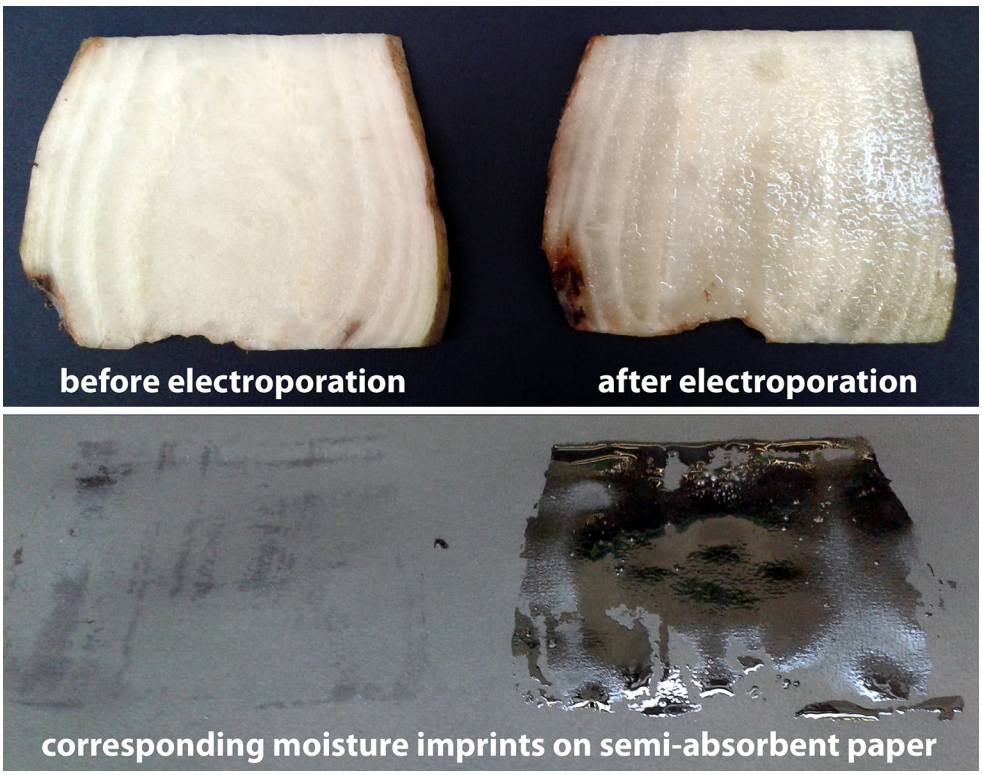

Fig. 4 *Upper half* Two slices of sugar beet before (*left*) and after (*right*) treatment with electroporation. *Lower half* The moisture imprints left on the semi-absorbent paper by the sugar beet slices (shown above), illustrating increased spontaneous (i.e. no pressure or mechanical treatment) release of sugar beet juice from the electroporated sugar beet slice. (Source: authors' own photograph)

thermal or pressing), and their efficiency can be greatly increased through combination with electroporation pretreatment.

In sugar from sugar beet extraction, traditional process involves slicing the roots into cossettes (beet cuts) followed by extraction by diffusion in hot water at 70–75 °C. This unfortunately leads to release of pectin into the juice, which requires complicated and costly purification. The combination of lower temperature and electroporation allows obtaining comparable results in terms of sugar yield, but at a much higher juice purity and hence lower energy and material costs for purification (Bouzrara and Vorobiev 2000; Eshtiaghi and Knorr 2002; Bluhm and Sack 2009). Figure 4 shows a cut through sugar beets before and after treatment by electroporation together with the corresponding moisture imprints left by the samples on a semi-absorbent sheet of coloured paper. The yield of juice obtained by cold pressing of electroporated sugar beet is considerably higher as compared to the control, i.e. non-treated sugar beet tissue. In addition to increased yield of juice, higher transparency of juice indicating higher purity has also been observed (Bluhm and Sack 2009).

Market-driven development of foods with health benefits promotes interest of the food and pharmaceutical industries in extraction of health-protecting compounds such as polyphenols, well known for their antioxidant, anti-inflammatory and anti-tumour properties. Since these bioactive compounds are sensitive to thermal and/or chemical processing, electroporation has been studied in relation with enhancement of polyphenols extraction. At high amounts of energy delivered during electroporation by the electric pulses, some deterioration and degradation of valuable compounds was observed, however, in general, electroporation treatment results in only a marginal reduction of bioactive compounds available for extraction and presents a promising method of increasing food quality with respect to health (Donsi et al. 2010). Recently, there has been an increase in scientific interest in recovery of valuable compounds from grape seeds and skins, waste by-products of the winemaking process, via the application of high-intensity electric fields, and even electric discharges, in order to extract the polyphenols from waste grape material (Boussetta et al. 2011).

Electroporation treatment has also been studied for enhancement of oil extraction from maize, olive, soybeans and rapeseed tissues. According to some studies, to achieve an observable benefit of electroporation application to oil yield, longer, high-energy pulses must be used with total energy delivered reaching as high as 90 kJ/kg. Even at these energies, however, the benefit to yield is only

marginal, while the treatment does, however, seem to improve the composition profile of the extracted oils; an increase in content of phytosterols and isoflavonoids in extracted oil after electroporation has been observed (Guderjan et al. 2005, 2007; Donsi et al. 2010). Other studies, summarized in (Puertolas et al. 2012) indicate an up to 30 % increase in oil yield from maize, and an increment of 55 % in the extraction yield in rapeseed oil production, as well as higher concentrations of tocopherols, polyphenols, antioxidants and phytosterols in oils produced using electroporation.

Electroporation in Winemaking

The potential of electroporation use for improvements in the wine making industry depends strongly on whether the treatment is applied in production of white or red wines. In white wine production, wine is made by fermenting the must obtained from crushing the white grapes. High-quality white wine is obtained from must with a low content of solid particles, low concentration of colour pigments and with reduced pressing time which limits the activity of enzymes that catalyse polyphenol oxidation causing must browning. Electroporation may be used to increase the yield of extracted grape juice of higher purity but with higher polyphenol content as opposed to juice obtained by traditional pressing method. Results of first laboratory-scale studies are promising, but need to be evaluated on the pilot plant scale (Praporscic et al. 2007; Luengo et al. 2012).

In red winemaking, however, wine is produced by fermenting must of red grapes together with grape skins. This step of the process is known as maceration. During maceration, polyphenolic compounds are extracted from grape skin and enhance the quality of the red wine by affecting the colour, flavour and ageing. Additionally, polyphenols are, as mentioned in the preceding section, associated with beneficial effects on health. Recently, a review giving sources to the essential reading material in the field of electroporation-enhanced polyphenol extraction in red winemaking was presented in (Luengo et al. 2012). The results of the studies reviewed indicate an up to 62 % increase in colour intensity, up to 43 % increase in anthocyanins content, and up to 45 % increase in total polyphenols during maceration as compared to the control samples, if the grape skins underwent electroporation prior to the start of the maceration process. When up-scaled from laboratory to a continuous-flow industrial-scale environment, studies show a 48-h decrease in maceration time and a higher content of polyphenols at the end of fermentation as compared to the control. From the sensory point of view, the wine produced by means of electroporation did not exhibit any unusual flavours or changes in taste. These results also come at a low energy cost (0.4–6.7 kJ/kg) and processing time (less than one second). For other recent studies on red grapes, see for instance (Delsart et al. 2014; Cholet et al. 2014).

As briefly mentioned in "Recovery of Valuable Compounds" section, electroporation-assisted extraction of polyphenols is also interesting in light of reuse of waste or by-products of winemaking industry. These by-products represent a vast and cheap source of polyphenols, and several authors have investigated the potential in harvesting these valuable compounds from biological waste. The energy requirements, however, appear to be higher in recovery of compounds from grape by-products as compared to those in applications for increasing polyphenol richness of must during maceration or fermentation (El Darra et al. 2013). The field of harvesting biological waste for valuable compounds is in its early research stages, and much more insight needs to be gained to evaluate its full industrial potential (Luengo et al. 2012).

Dehydration of Plant Material

The intact cell membranes in food materials represent a highly limiting factor (barrier) to water transport during drying of food matrices. Electroporation can, through increasing cell membrane permeability, enhance the mass transport and thus enable faster and more energy-efficient process of tissue dehydration. As shown in the Introduction in Fig. 2, cells and cell walls in plant tissue form the structure of a porous medium. Electroporation of cell membranes can thus greatly increase the porosity of tissue, increasing its hydrodynamic permeability as well as mass and heat transfer rates (Shynkaryk et al. 2008; Donsi et al. 2010; Jaeger et al. 2012; Mahnič-Kalamiza et al. 2014).

Drying of foods in the food industry is mostly accomplished through thermal or hot-air dehydration. Since these processes come at a high energy cost and affect the organoleptic properties of food material, osmotic dehydration has been proposed as an alternative solution. In osmotic dehydration, food material is introduced into a hypertonic solution, causing water to leave the material through osmosis and solute from the solution is introduced by diffusion into the biological tissue. This is, however, a slow process and not without negative effects to properties of dehydrated food (Amami et al. 2006; Puertolas et al. 2012). Several studies have been conducted where conventional thermal drying or osmotic dehydration were combined with electroporation. Recent reviews and summaries of the findings of these studies are found in (Donsi et al. 2010; Vorobiev and Lebovka 2010; Puertolas et al. 2012; Jaeger et al. 2012). In summary, products that were studied and positively responded to electroporation treatment with an increase in mass transport and thus drying rate includes

potatoes, carrots, bell pepper, okra, raisins, red beet, mangoes, strawberries, coconuts and apples. In conventional dehydration, electroporation decreased drying time by up to 30 % while not exceeding the drying temperature of 60 °C. In osmotic dehydration, an up to 30–50 % increase in water release as well as improved dehydration kinetics in samples pre-treated with electroporation were observed. Regarding the final product quality, in conventional thermal dehydration quality is enhanced due to lower drying temperature, while the results appear to be inconclusive for osmotic dehydration due to increased water release causing a proportional decrease in content of metabolites of interest, e.g. vitamin C.

Cryopreservation

Freezing is a fairly widespread method of food preservation. Formation of crystals during freezing and recrystallization after freezing often result in deterioration of frozen food characteristics such as texture and flavour. For this reason, attempts have been made to improve the resistance of raw food products to freezing damage through cryopreservation. This method involves infusing tissue with cryoprotectants, a group of substances that prevent formation of crystals during freezing. Sugars or other cryoprotectants (e.g. sugar alcohols such as mannitol) can be forced into the tissue via osmotic dehydration in hypertonic solution or by vacuum impregnation (Velickova et al. 2013). In intact tissue, however, these processes have limited effect since the intact plasma membrane is poorly permeable. The cryoprotectant is withheld mainly in the extracellular matrix and does not reach the intracellular space, which allows crystals to form during freezing (Phoon et al. 2008). Reversible electroporation, due to its transient increase in membrane permeabilization, enables introduction of ions and molecules into biological cells. This enables the cryoprotectant to efficiently permeate into the cells, protecting them from damage that would otherwise be incurred during freezing. Promising studies have been conducted on laboratory scale in recent years demonstrating the effectiveness of combining cryoprotectant with permeabilizing effects of pulsed electric fields for cryopreservation. Quality (texture, firmness, colour, etc.) of spinach leaves and potato strips was retained by impregnating the material with trehalose (Phoon et al. 2008; Shayanfar et al. 2013) or calcium chloride (Shayanfar et al. 2013).

Inactivation and Destruction of Microbial Contaminants

The most effective method of preserving food quality and assuring microbial safety of raw materials during production and processing is preventing contamination with microorganisms. Unfortunately, it is also the most difficult, if not impossible to achieve in industrial practice. The objective in food industry is thus to attempt and control the activity of microorganisms once the contamination has taken place (Álvarez et al. 2006), by either deactivating them temporarily (heating/cooling/freezing, addition of preservatives, changes in pH, changes in atmospheric composition, etc.) or destroying them completely. The idea of using electricity in food preservation has a long history, and application of pulsed electric fields for microbial inactivation has been studied for over 50 years (Barbosa-Canovas et al. 1997; Toepfl et al. 2007; Sobrino-Lopez and Martin-Belloso 2010; Saulis 2010; Knorr et al. 2011; Morales-de la Pena et al. 2011). During the last couple of decades, the potentials and aspects of electroporation in food preservation have been thoroughly studied, and a variety of microorganisms have effectively been inactivated in various aqueous solutions, e.g. juice, apple cider, milk, yakju, whole egg, pea soup, nutrient broths, model beer, algae extracts and miscellaneous model buffer solutions. In addition, synergistic effects between electroporation and other methods, e.g. nisin, acid treatment, mild heating, low temperature and high pressure, have been demonstrated (Toepfl et al. 2006; Saulis 2010).

The success of electroporation treatment of microorganisms strongly depends on a number of critical factors. Among others, these include processing parameters (e.g. treatment time, electric field strength, pulse number and duration), treatment medium characteristics (e.g. conductivity), as well as microbial characteristics (e.g. shape, size, cell wall structure and composition). The main process parameters that determine efficiency of treatment include electric field strength, shape and duration of the pulses, treatment time, pulse repetition frequency, energy delivered and temperature. Of these, field strength, treatment time and—on these two dependent—amount of energy delivered, are the most critical. Microbial characteristics of greatest importance were shown to be the type of microorganism, cell size and shape, and growth conditions. While yeast and bacterial cells are susceptible to high-energy electroporation treatment, bacterial spores seem to be largely resistant to electrical treatment. Therefore, the focus of studies and efforts in electroporation application for microbial inactivation can be found in pasteurization rather than sterilization (Álvarez et al. 2006).

Electric field strengths required for efficient inactivation of microbes in vegetable juices are within the range of 15–40 kV/cm, and energy requirements fall into the range between 50 and 1,000 kJ/kg (see Fig. 3). Treatment at these parameters causes electroporation of not only microbial, but also animal and plant cells, causing destruction of tissue integrity and structure, which are

highly undesirable in food processing industry. Thus, it appears that the technique is mostly applicable for achieving pasteurization of liquid foods, e.g. preservation of vegetable juice, milk, eggs, etc. (Toepfl et al. 2006).

The main strength of electroporation application in food industry for microbial inactivation seems to be in its ability to affect less and thus better preserve the nutritional and sensory properties of food material as compared to thermal treatment (Odriozola-Serrano et al. 2013). Also important are the observed synergistic effects of electroporation when combined with existing treatments (thermal for instance). There are also several other effects of electroporation that were noticed and might benefit its future widespread use in food industry and its acceptance with the consumers, such as sulphur dioxide and pesticide reduction in treated juices (Marsellés-Fontanet et al. 2012).

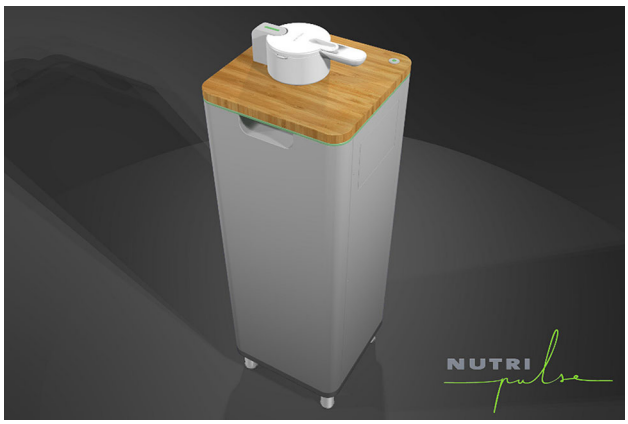

Fig. 5 NutriPulse® by IXL Netherlands B.V., an appliance marketed under claims of "cooking with the help of pulsed electric fields" (IXL Netherlands B.V. 2014)

Other Food Processing Applications and the Effect of Electroporation on Enzymes and Food Constituents

In addition to the applications of electroporation for food processing described in the preceding subsections, several other applications have been proposed, particularly in recent years. Inactivation capability of pulsed electric fields has been extended to attempts at controlling enzyme activity and metabolism activity with applications in food preservation (Mañas and Vercet 2006) and organism growth stimulation (Jaeger et al. 2008; Frey et al. 2011). The required pulse amplitudes for enzyme inactivation are even higher than for microbial inactivation due to higher resistance to treatment of enzymes as compared to microorganisms, and such treatment was not possible until introduction of powerful-enough generators. Since plant and fungi growth stimulation (metabolism control) requires low-energy fields as it is undesirable to negatively influence the organism's viability, pulses on the order of nanoseconds in duration and strengths of up to 50 kV/cm were used in some of the preliminary studies (Frey et al. 2011). Reversible electroporation has only recently gained interest among researchers as a stressing mechanism to promote production (biosynthesis) of metabolites, enabling large amounts of phytochemicals to be produced in bioreactors by stressing selected plant species using low-energy-pulsed electric fields (Jaeger et al. 2008; Mattar et al. 2013).

Several applications in preparation, curing and cooking of meat and vegetable products have also been proposed. While very limited literature exists on the subject, there is growing interest in the potential of electroporation application for meat and fish products. For instance, a weight increase of brine-marinated cod fillets has been observed following treatment by electroporation, and treatment impact on chicken muscle as well as salmon meat has been investigated. Electroporation has also been shown to accelerate fermentation of sausages by improving the availability of intracellular liquid for fermenting cultures (Toepfl et al. 2006). Although no mechanisms of action of electroporation on proteins have yet been proposed (Toepfl et al. 2006) and the subject invites further elucidation, a commercial "cooking" device, whose manufacturer advertises as capable of preparing food with help of electroporation (literally: "pulsed electric fields"), is in the testing phase (see Fig. 5, courtesy of IXL Netherlands B.V. 2014). Additionally, electroporation was shown to enhance peeling of some fruits (e.g. tomato, prune, mango), equalling the ease of skin removal to that of steam treatment, at the energy cost of an applied electroporation treatment of 1–7 kJ/kg (Toepfl 2012). Interesting changes in texture and viscoelastic properties of the treated tissues, presumably caused by the loss of turgor pressure during and following electroporation, were also observed (Grimi et al. 2009; Leong et al. 2014). These changes may facilitate easier handling of produce and more efficient processing (reduction in cutting force and wear of cutting implements).

Biorefinery (integrated biomass conversion processing) of microalgae for food, feed, chemicals and pharmaceuticals is a digression from existing and established attempts in environmental engineering to harvest microalgae for biofuel. Biofuel production, though facilitated by energy-efficient electroporation, is currently not in itself economically feasible (de Boer et al. 2012; Vanthoor-Koopmans et al. 2013). This is due to high energy demands required for mixing, concentrating and finally cracking the tough cell wall of the microalgae to obtain their products. Nevertheless, the application is interesting since harvesting microalgae constituents with high market value might, in combination with optimization of energy use, make

production and harvesting of these microorganisms economically viable. For this application, electroporation is investigated as a mild and effective mechanism of cell disruption, facilitating extraction of unaltered constituents at low energy costs (Vanthoor-Koopmans et al. 2013; Grimi et al. 2014; Flisar et al. 2014).

Valuables produced by microalgae include lipids, pigments, unsaturated fatty acids, proteins and polysaccharides. These compounds are stored intracellularly and a cell disintegration method has to be applied in order to extract this valuable material from within the algae (Goettel et al. 2013; Coustets et al. 2013). Up to now, different cell disruption methods have been investigated, including chemical, mechanical, thermal and enzymatic methods. These methods are, however, normally unable to preserve all of the valuable fractions in the obtained algae extract. This is due to the fact they are mostly focused on harvesting one of the valuable compounds, while inadvertently damaging the rest. Within this context, electroporation presents itself as an interesting mild or gentle extraction technique, preserving all extracted fractions and making them available for further biorefinery. Electroporation treatment thus enables an industrial-scale harvesting of the complete potential of microalgae (Vanthoor-Koopmans et al. 2013). Several studies have demonstrated that although electroporation treatment does not seem to cause a spontaneous increase in release of lipids, it does greatly enhance solvent extraction of lipids (Zbinden et al. 2013; Eing et al. 2013; Goettel et al. 2013). Use of electroporation is therefore envisioned as a part of a multi-stage process, where electric treatment would be used first as a cell disruption method, followed by two stages of biorefinery. During the first stage, high-value water-soluble compounds are extracted and dispatched for further processing. In the second stage, using an environmentally friendly organic solvent such as ethanol, the lipids and other compounds insoluble in water are extracted. This can be done with high efficiency in terms of both yield and energy, as the wet extraction of lipids during second stage of biorefinery does not require the conventional energy-consuming drying of biomass (Eing et al. 2013).

The number of technologies and applications harvesting the power of electroporation applied to biological material seems to be growing at an ever increasing rate. New ideas are constantly being put to the test as industrial processing is studied and optimized in every detail in light of what electroporation-based treatments can offer to the industry and the market. The task of reviewing the massive amount of knowledge accumulated in the field and the numerous applications currently under consideration is a difficult one, as we are continually surprised by the expanding field of electroporation applications in the food industry.

Challenges and Approaches to Problems Solving

This section is an attempt at giving a brief review of some of the more technical aspects of electroporation in food processing. We begin by presenting an established method of assessing changes in biological tissue due to electroporation, and continue with a brief overview of some of the parameters that determine success of electroporation application. Following, this is a short introduction to modelling in electroporation-assisted food processing, and we conclude the section with a glance at industrial equipment design and implementation, a major research domain within the field of electroporation.

Assessing the Degree of Electroporation in Biological Tissues

Commonly used methods for detecting cell membrane permeabilization range from microscopic observations of cells and tissues, often entailing the use of dyes, to macroscopic observations such as measuring release of liquid or the electrical conductivity of the released liquid, analysing the textural properties of treated tissues or measuring the electrical impedance of the tissues. The latter of these methods seems particularly suitable for quick, on-line and in-process evaluation of effects of electroporation in tissues and is the least invasive. A recent addition to this list of conventional methods is based on the nuclear magnetic resonance (NMR) technology, which is, due to its nature, a non-invasive technique of analysis.

Vorobiev and Lebovka (2008) defined the so-called damage degree P as the ratio of damaged (meaning electroporated) cells to the total number of cells in tissue. Studies have shown a possibility of estimating, approximately, the damage or electroporation degree P via measurements of effective diffusion coefficients of known solutes in biological materials (Jemai and Vorobiev 2002). In this case, P is approximated as $P \approx (D - D_\mathrm{i})/(D_\mathrm{d} - D_\mathrm{i})$, where D is the measured effective diffusion coefficient, and indices d and i refer to totally destroyed and intact cellular material, respectively. The effective diffusion coefficient D is measured via diffusion or drying experiments; however, these methods are indirect and invasive. Moreover, the measured effective diffusion coefficient captures not only the effect of membrane electroporation, but also the effect of electroporation of cells to the rate of solute diffusion through extracellular pathways in tissue (hence the coefficient is termed "effective"). As it is not easy to decouple these effects using a single measured parameter, this measure of tissue electroporation must, therefore, be considered as a rough estimate, but is nevertheless particularly useful for comparison of different treatment parameters and species of diffusing solute. A more practical measurement in terms of

invasiveness and speed of assessment is via the more conventionally used conductivity measurements. A *conductivity disintegration index* Z can be defined as $Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i)$, where σ is the electrical conductivity (the real component of complex admittance, measured at low frequency of about 1–5 kHz) and indices d and i refer to totally destroyed and intact cellular material, respectively (Lebovka et al. 2000). Thus, the defined index gives $Z = 1$ for completely electroporated tissue and $Z = 0$ for intact tissue. Although this method is useful, fast and relatively simple to use, it requires supplementary measurements to determine the conductivity of completely damaged tissue, using either freeze-thawed tissue samples or samples of electroporated tissue using very high electric field strengths and/or treatment times (Lebovka et al. 2007a). The connection between Z and P has not yet been explained theoretically. A phenomenological, experimentally determined empirical Archie's equation giving $Z \approx P^m$ has been proposed, with coefficient m determined experimentally for some food produce, e.g. potatoes, carrots and apples (Lebovka et al. 2002).

A more direct approach of assessing changes caused by electroporation in plant tissues in situ was proposed by Fincan and Dejmek (2002) and involves samples of tissue between electrodes arranged in a setup suitable for microscopy with the ability to record and analyse, post-experiment, the images during or after application of electroporation. In light of difficulties presented by preparing samples of quality suitable for microscopy, onion epidermis tissue presents a clear advantage in ease of sample preparation and was used in the cited study, with neutral red (NR) as the staining agent used to visually detect electropermeabilization of the cell membrane. Direct observation of NR-stained tissue was found useful in supporting previous permeabilization estimates based on conductivity measurements (index Z), as well as allowed for the detection and observation of individual permeabilized cells. Studies with microscopy and ion leakage measurements with electrically damaged plant tissues (particularly onions) and plant cells (e.g. tobacco), have recently experienced a renewal in interest from the scientific community (Asavasanti et al. 2010; Ersus and Barrett 2010; Janositz and Knorr 2010). The findings of these studies highlight and point at the complex behaviour of cells in biological tissues under exposure to electroporative pulses, in particular in relation to the structural inhomogeneity (e.g. variable cell size, shape, etc.) which is affecting the degree of cell membrane permeabilization. Ion leakage, alliin lyase enzyme activity, and textural property measurements were also used to determine critical field strengths required to reversibly and/or irreversibly electroporate cells in onion tissue (Asavasanti et al. 2010). A recent work with tobacco cells with and without cell walls studied under the microscope has shed more light on the impact of cell wall on the efficacy of electroporation of plant cells, evaluating sensitivity of cells to electric pulses with and without cell wall and measuring the resulting changes in cell size (Janositz and Knorr 2010). In a more recent study (Cholet et al. 2014), authors combined histocytological observations with a study of levels of polysaccharide fractions and total amounts of tannins in treated red grape skins to determine that a specific electroporation treatment substantially and profoundly modified the organization and composition of the skin cell walls. In another recent study, authors evaluated the suitability of NMR imaging for assessing cell membrane integrity in electroporated samples (Ersus et al. 2010).

Parameters Influencing the Success of Electroporation Treatment Application and Methods of Evaluating Their Importance

Parameters determining membrane permeabilization and thus the success of application of electroporation in treatment of food materials are related with all aspects of the produce and processing. This means that in addition to the physicochemical and electrical properties of the treated material (medium and cytosol conductivities, membrane composition, presence or absence of an exterior envelope, etc.), also the electrical treatment parameters used during electroporation (pulse shape, pulse amplitude, pulse duration, pulse repetition frequency, etc.), as well as other processing parameters (pH, temperature, etc.), all play an important role and determine the outcome of treatment (Barbosa-Canovas et al. 1997; Raso and Heinz 2010; Puertolas et al. 2012). For a review of the influences of various parameters on success of microbial inactivation by electroporation, see e.g. (Barbosa-Canovas et al. 1997; Álvarez et al. 2006; Sobrino-Lopez and Martin-Belloso 2010; Saulis 2010; Knorr et al. 2011). In the field of juice and valuable compounds extraction from plant materials or osmotic dehydration of vegetable tissues, work has been focused predominantly towards characterizing the damage degree index Z (as a relative measure of treatment performance) in relation to different parameters of the applied electric field (pulse duration, number, amplitude), various species and varieties of treated plants (e.g. potatoes, apples, carrots,…) and the combined effects of electroporation and other treatments, applied either concurrently or in succession. The treatments, often researched for their potential synergistic action with electroporation to enhance diffusion of solutes in tissue or expression of juices, are heat processing (temperature increase), pressure processing (pressing) and chemical processing (addition of enzymes, lime, lye, etc.). For relevant reviews and a listing of pertinent literature, see e.g. (Vorobiev and Lebovka 2008;

Donsi et al. 2010). For a recent study of pulse parameter effects on extraction from microalgae, see (Goettel et al. 2013).

To successfully electropermeabilize the membrane, the field strength experienced by the cell exposed to the electroporating pulses must achieve a certain minimum value, termed the *threshold of electroporation*, *critical field strength* or *reversible electroporation threshold* (Kotnik and Miklavcic 2000; Fincan and Dejmek 2002; Asavasanti et al. 2010), in order to induce the critical transmembrane voltage across the membrane required for the onset of increased permeability. Recent works, already mentioned in the preceding subsection on assessing the degree of electroporation, describe studies performed using onion tissue or tobacco cells as model materials (Asavasanti et al. 2010; Ersus and Barrett 2010; Janositz and Knorr 2010). Their findings indicate that the electroporation threshold or field strength required to reversibly permeabilize the membrane depends on the externally applied electric field strength, and on the method employed to detect permeabilization. The field strength reported as required for permeabilization falls in the range of 50–500 V/cm, with duration of treatment (treatment duration being an integral quantity given as the product of pulse duration and number of pulses) in range of 100–10,000 μs (Puertolas et al. 2012). When using pulses of shorter duration and/or shorter total treatment time (<100 μs), higher electric field strengths are required to permeabilize the membrane (Lopez et al. 2009). This is consistent with observations of Pucihar et al. (Pucihar et al. 2011a). Experiments with onion tissues reported by Asavasanti et al. indicate that higher electric field strengths are required also in order to permeabilize the tonoplast (membrane of the vacuole, an intracellular organelle), the permeabilization of which is necessary to release the solutes of interest, often contained within the cell's organelles (Asavasanti et al. 2010). The importance of cell wall presence has also been evaluated by microscopy (Janositz and Knorr 2010). These results suggest that the well-established descriptions of pulse protocol that are based on reporting only the total specific energy delivered to the material, as established by Knorr and Angersbach (Knorr and Angersbach 1998), might be insufficient to adequately describe the electrical treatment parameters. This consideration seems to be of greater importance in applications where the objective is the achievement of selective or reversible permeabilization of target cells, i.e. in applications of what could be referred to as 'gentle electroporation treatment'. A lot of insight on this subject is available in the biomedical field of electroporation research, which we will now briefly discuss.

A massive body of work done on eukaryotic cells is available in the biomedical domain of electroporation applications, where the primary interests most often lie in either molecule delivery, i.e. drug or gene delivery (Gehl 2003; Denet et al. 2004; Miklavcic et al. 2012; Mali et al. 2013; Chiarella et al. 2013; Zorec et al. 2013; Miklavčič et al. 2014), or in irreversibly permeabilizing cells causing tissue ablation (Davalos et al. 2005; Garcia et al. 2011). We illustrate this by citing two examples.

In a recent study of Pucihar et al., equivalent pulse parameters for achieving electroporation were sought for (Pucihar et al. 2011b). A calcium-sensitive dye was used to detect electroporation, and the relations between the amplitude and duration/number of pulses resulting in the same fraction of electroporated cells were determined. Pulse duration was varied between 150 ns and 100 ms, and number of pulses from 1 to 128. To achieve the same fraction of electroporated cells, the field strength had to be adjusted within range of 100 and 10,000 V/cm. These ranges with respect to pulse number, duration and electric field strength, are sufficient to capture conditions and parameters in many if not all electroporation applications for food processing, the most obvious exception being microbial inactivation, whose primary targets are predominantly prokaryotic cells susceptible only to high-intensity electric fields (Álvarez et al. 2006). The insights gained by the study should thus be applicable to materials and conditions encountered in food processing applications, e.g. in juice and valuable compounds extraction, tissue dehydration, cryopreservation, growth stimulation and others. The findings, if indeed applicable to plant materials, indicate that even when using different values of treatment parameters (pulse duration, pulse length, pulse number), if these values are carefully controlled and adjusted in relation to each other, we can arrive at the same degree of electropermeabilization. However, the relationship between pulse amplitude and pulse duration as well as the relationship between pulse amplitude and number of pulses were found to be highly nonlinear and best described using two-parameter power or three-parameter exponential functions. This implies that even though the energy input during treatment may be very high (for instance in using low-field-strength, long-duration pulses), it might not be as effective in permeabilizing the cells as a treatment protocol of same specific energy input, but using very high-intensity fields of shorter duration.

In a different, modelling and simulation study (Miklavcic and Towhidi 2010), the authors developed a chemical-kinetics and trapezium barrier models, supplemented with a molecular transport model across a permeabilized membrane to study the effects of varying pulse shape parameters (e.g. unipolar rectangular pulses with different rise/fall times, triangular, sinusoidal, unipolar rectangular modulated pulses, etc.). Their results show that rectangular pulses are more effective than sinusoidal or triangular pulses, but more importantly, that the rise and fall times of

unipolar rectangular pulses do not significantly affect the uptake of molecules by the cells. The work is significant because it provides a possible framework for evaluating effects of different pulse shapes and effects related with transitional effects in electric field generation in relation with cross-membrane mass transport, an issue which has not been theoretically studied in the field of electroporation applications for the food processing in much detail.

Additional insight may also be gained by experimenting with molecular dynamics (MD) simulations, whose feasibility has increased due to rapid progression in computing power especially over the last decade. In an MD simulation, a segment of the lipid bilayer representing the membrane of a biological cell is simulated in detail, meaning that physical interaction between its individual constituents (atoms and molecules) is described as an N-body problem using classical Newtonian physics. In addition to simulating occurrence, evolution and disappearance of pores in lipid bilayers induced by mechanical stress (Leontiadou et al. 2004), the influence of an externally superimposed field and the associated electrical stress can be also be accounted for in this manner. Recently, MD simulations on lipid bilayers were performed in order to, in example, evaluate the role of water in pore formation and evolution (Ho et al. 2013b), to examine pore conductance of monovalent salts—note the connection between membrane conductance and tissue electrical conductivity—(Ho et al. 2013a), and to determine electroporation thresholds with respect to the lipid bilayer composition (Polak et al. 2013). Although the segments of the simulated bilayer are (over)simplified and as of yet far from being complete digitized atom-by-atom replicas of living biological membranes, careful interpretation of results reported by the cited and related studies can significantly aid in corroborating or explaining experimentally obtained evidence, observed at the microscopic (macroscopic as compared to the scale of MD simulations) level (Delemotte and Tarek 2012).

Mathematical Modelling of Electroporation-Related Phenomena in Food Processing Assisted by Electroporation

Within the domain of electroporation in food processing, we can identify several directions in the published literature. A typical example would be modelling of a treatment chamber for purposes of thermal monitoring and control, optimizing treatment parameters or energy efficiency. Another purpose is to gain theoretical insight into the process-governing phenomena, but using food products as model materials. A good example of this is a through-pore solute diffusion model—the industrially relevant implications may be hinted at, but its main purpose lies in advancing knowledge in basic principles of phenomena and theoretically evaluating the importance of variations in parameters. These variations may result from either biological variability of material or differences in process or electrical treatment parameters.

With respect to the *method* of model construction, we encounter applications of purely phenomenological and empirically derived models, sometimes transferred to the field due to similarities in process physics from an unrelated scientific or engineering field. As an example, consider the empirical Archie's equation, briefly discussed in "Assessing the Degree of Electroporation in Biological Tissues" section. These models can be very useful, in particular if the purpose of their construction is of an engineering nature (optimization). Their drawback is that they are only valid within the range of parameters for which they were validated and may completely fail outside of this range. Also, their coefficients need to be experimentally determined directly or estimated based on comparable experiments. On the other hand, a model can be constructed using a combination of basic laws of thermodynamics (e.g. conservation of mass, energy, etc.), laws of classical electrodynamics (Maxwell's equations), some phenomenologically derived transport laws (e.g. Darcy's, Fourier's, Fick's, etc.), models of electroporation (the background of most of which are the thermodynamics of lipid bilayers) and if needed, biological laws (e.g. cell survival models). For examples, see works such as (Krassowska and Filev 2007b; Pucihar et al. 2008; Rauh et al. 2010; Golberg and Rubinsky 2010; Kotnik et al. 2010; Li and Lin 2011; Salengke et al. 2012; Kotnik et al. 2012b; Rems et al. 2013; Mahnič-Kalamiza et al. 2014).

These models can reach a high degree of complexity, are usually difficult to validate using experimental methods, and are seemingly not as attractive for use in industrial applications as are empirical models. In the electroporation-assisted food processing domain, these models have, however, gained a foothold in the subject of treatment chamber analysis and design, predominantly in the field of microbial inactivation research. They have yet to appear in problems of valuable compounds and juices extraction and dehydration (on the level of biological tissue) and microbial inactivation (on the level of cells).

As described in the applications review ("Electroporation in Food Processing" section), electroporation is being studied as a method of non-thermal food preservation, i.e. microbial inactivation, either as a single treatment or in combination with other processing techniques (e.g. thermal or high pressure) to achieve pasteurization or sterilization of liquid foods. During treatment, material as a liquid passes through a treatment chamber equipped with electrodes via which pulses are delivered. The flow of fluid can be described as a fluid dynamics flow field, with the

electrodes creating an electric field. The ohmic dissipation of energy within the fluid field, resulting from the application of electric pulses to conductive media, establishes also a thermal field (Joule heating). The challenge with utilization of these treatment chambers is in their optimal design and use of treatment parameters. This is in order to ensure that there are no dielectric breakdowns and under-treatment (electric field highs or lows) or overheating of the material (temperature highs), and that the treatment is efficient in addition to being effective (Rauh et al. 2010; Salengke et al. 2012). Numerical methods and models are used to describe the fluid, electric and thermal fields in the treatment chamber in order to facilitate design and parameter optimization. For a recent review on the coupled modelling approach, see e.g. (Gerlach et al. 2008), as well as individual studies for electric field and temperature distribution (Salengke et al. 2012) and differentiation between the electric and thermal field effects in the continuous-flow treatment chamber (Jaeger et al. 2010). For modelling of high pressure processing (HPP) in combination with electroporation and thermal effects, see for instance works of Rauh et al. (2010).

Saulis (2010) in his review on fundamentals of electroporation applications in food processing, focused predominantly on food pasteurization and sterilization, includes a listing with relevant sources of some of the often used models of the inactivation process by electroporation. As some of the most frequently employed models in order to describe the dependencies of the efficiency of inactivation on the different process variables, he cites the Weibull distribution, logarithmic, sigmoid, polynomial as well as other miscellaneous functions, adapted to approximate descriptions of experimental results. For more engineering perspectives and a more detailed review of models of microbial and enzymatic inactivation, see (Morales-de la Pena et al. 2011).

In the field of electroporation application for enhancing mass transport in treated material (valuable compounds and juices extraction, dehydration, winemaking, etc.), the models concern the effect of applied electroporative pulses on the diffusion rate of solutes, the transport rate of water molecules, or to the hydraulic permeability of cells and tissues if pressing is the chosen mode of intracellular compounds extraction. The mass transport models used in these cases to describe solute diffusion are often simple, analytically derived and based on known solutions of diffusion problems. For a comprehensive review of these models in electroporation-assisted extraction, see (Pataro et al. 2011).

The models used in analysing mass transport phenomena in electroporated biological tissues are based on either analytical solutions, or they are to some degree (or even completely) phenomenological in nature. As an example, see for instance (El-Belghiti et al. 2005) for an approach to a model study of two-stage solute extraction by diffusion, or (Lebovka et al. 2003) for a problem description with juice extraction from treated biological tissue. The phenomenological models of filtration–consolidation found in literature that describe juice extraction, are based on poromechanics approaches, found in theory of fractured rocks and soils. Their application to expression from biological material was already proposed by Lanoiselle et al. (1996) and can be found used in e.g. (Grimi et al. 2010). The diffusion models described above are considering material as homogeneous, i.e. not comprising cellular structure. Recently, some new or modified models were proposed (Petryk and Vorobiev 2013); Mahnič-Kalamiza et al. 2014; Mahnič-Kalamiza and Vorobiev 2014), rooted in the theory of porous media, introducing the cell membrane into the mass transport model. This allows the authors to couple the effects of electrical treatment to the cell membrane, thus offering an opportunity to theoretically study mass transport in relation to electroporation parameters. The importance and influence of these parameters have already been studied to a large degree not only in the food processing, but mainly in the biomedical field of electroporation applications. We believe it is necessary, in order to advance insights on the impact of electroporation treatment to mass transport in biological material, to couple a mass transport model with a model of pore evolution, e.g. (Krassowska and Filev 2007a). Furthermore, we should account for the effects of tissue as a packed multicellular structure (Essone Mezeme et al. 2012), material property discontinuities and inhomogeneity (Becker 2012), cell wall (Janositz and Knorr 2010), tissue electrical conductivity (Neal et al. 2012), effect of electrical field thresholds and conductivity changes (Sel et al. 2005; Corovic et al. 2013), pulse duration and number (Pucihar et al. 2011b) and electrokinetic effects such as electrophoresis (Li and Lin 2011; Li et al. 2013) and electroosmosis (Movahed and Li 2012).

Industrial Equipment Design, Implementation and Commercial Applications

An important aspect of an emerging processing technology is its integration and acceptance into the industrial environment. Ever since electroporation was first proposed as an application of relevance to the food industry in the 1960s, the fields of industrial equipment design for applying electroporation to food materials, as well as the implementation of this technology, have experienced interest from the scientific community and also several attempts from the industry to commercialize the process (Toepfl 2012). Historically, the major hurdles in implementation were posed by the poor reliability and performance of pulsed power switches required for continuous

delivery of high amounts of electrical energy and high electric field strengths required for industrial-scale applications. The scaling up from laboratory to industrial-level processing requires a corresponding scale-up from several kW to more than 100 kW in terms of power requirements (Toepfl 2011). The first commercial application in Europe was, therefore, introduced only relatively recently, in 2009, in the form of a juice preservation line (microbial inactivation) with processing capacity of 1,500 l/h. Encouraging results were obtained on an industrial pilot of 4.5 t/h for cider production (Turk et al. 2012a) and with a pilot for grape mash treatment (Sack et al. 2010a). This was followed in 2010 with the first industrial system for processing vegetables with capacity of 50 tons/h (Toepfl 2012). Recently, an industrial pilot for sugar beet processing (sucrose extraction and purification) was setup with the capacity of 10 tons/h (Vidal 2014).

Electroporation systems for food industry typically comprise a pulse power supply and a treatment chamber (Bluhm 2006; Sack et al. 2010b). Up to the last decade of the past century, spark gaps and vacuum tubes were predominantly employed for achieving power switching, but these systems were poorly reliable and of short lifetime. Recently, advances in high-performance semiconductor industry have provided interesting possibilities for the power switching applications on industrial scale with high-performance thyristors and transistors, though the implementation at present continues to pose challenges due to high power requirements. For this reason, recent designs are predominantly modular configurations allowing use of standard components, both for reasons of scalability, as well as for providing reliability and lower maintenance costs. An important issue that needs to be considered in the design is regulations that need to be followed, and demands pertaining to hygienic standards and operational safety in the industry, that need to be met (Toepfl 2012).

The selection or design of a suitable pulse generator is broadly determined by four parameters: the peak voltage required, which is highly dependent on the desired application (microbial inactivation vs. extraction in example, see Fig. 3); the peak current, determined by the product maximum conductivity and the geometry of the treatment chamber; the average power required, dependent on the desired processing capacity (tons, litres per hour); and the pulse waveform, which is typically either rectangular or exponentially decaying. Common generator designs comprise either setups of pulse transformers or semiconductor-based Marx generators. The typical average power these setups are capable of delivering falls within range of 30–400 kW. Although it is the average power level that determines the maximum treatment capacity, the peak power has been shown to present a greater challenge, since when using treatment chambers of large cross sections, required peak power can range up to several hundred MW (Toepfl 2012). At these extremely high currents and current densities, electrode erosion was studied as a possible concern. However, provided that stainless steel or in extreme cases titanium electrodes are used, electrochemical reactions and metal release to the product do not exceed acceptable limits (Morren et al. 2003; Roodenburg et al. 2005).

The other key component in addition to the power supply in a typical treatment system is the treatment chamber, the part in which electric pulses are delivered to the biological material. As opposed to the laboratory scale, in industrial-scale implementation setups capable of continuous processing are highly desirable. For batch or continuous processing, different chamber designs were proposed throughout the years, see e.g. (Huang and Wang 2009) for a review in treatment chamber designs as used for microbial inactivation. Broadly speaking, the parallel-plate, co-axial and co-linear designs are the most prevalent types. These different chamber designs present various advantages and drawbacks, mainly in relation to the homogeneity of the field to which the passing material is exposed, accessibility for cleaning and maintenance, and the average load resistance. The electric field distribution homogeneity is important in order to achieve uniform treatment of the product. This is especially important in microbial inactivation (in areas of field strength below critical the treatment may fail to achieve the necessary reduction in microorganism activity) and treatment of temperature-sensitive food materials (problem of local hotspots and overheating). The average load resistance is important because it determines, at a fixed peak pulse voltage, the maximum power (current) the power generator must be capable of delivering. In general, the low load resistance in parallel-plate configuration requires more powerful generators, but provides good field homogeneity, while co-linear and co-axial designs are easier to clean and have higher load resistance, thus lowering the current necessary to establish the required electric field strength (Morales-de la Pena et al. 2011). Researchers are working on improving the design of co-linear and co-axial chambers in terms of optimizing the homogeneity of electric field strength. For example, introduction of grids in the chamber as mechanic obstacles to the hydrodynamic flow of the particle suspension or juice, homogenizes the flow in the area where the electric field is applied, thus homogenizing the application of the treatment to the material. This prevents undesired overheating or insufficient treatment effects (Jaeger et al. 2009).

For applications where the material cannot be pumped (solid products treatment), a different design is required. For this purpose, belt or rotating systems were developed. Products are submerged in water (water is used as an

electrolyte, "extending" the electrodes to the product, delivering the energy to the target tissue) and conveyed through an electrode chamber by a belt. Parallel-plate electrodes apply the electroporating pulses, which are generally larger in comparison to those in tubular chamber designs for liquid food. Since the chamber has also a higher cross-section, resulting power consumption for equivalent field strength is thus significantly higher (as compared to tubular designs). Currently, systems with capacity up to 50 tons/h are available (Toepfl 2012). A special chamber with an added device for compaction of sliced particles and treatment of compacted slices without addition of liquid was recently patented (Vidal and Vorobiev 2011) and successfully tested in the sugar industry (Vidal 2014). Such a chamber permits electroporation of plant materials with no addition of liquid, thus significantly decreasing the energy consumption.

For additional reviews and more information on basic principles in designing generators of electric pulses and equipment used for electroporation, see for example (Bluhm 2006; Maged and Amer Eissa 2012; Rebersek et al. 2014), as well as (Sack et al. 2005, 2010a; Turk et al. 2012a, b) for some examples in implementation of electroporation devices on the industrial scale.

Perspectives for Future Research and Development

In this section, we indicate, in short, which direction the latest research and development into applications of electroporation for food processing seem to be pointing in. Along with these indications reflected in some of the more recent publications and some potential hurdles awaiting researchers in the long term, we also give a personal opinion on where we are currently at, and what we will most likely be reading about in the future in the field of electroporation-assisted food processing and biorefinery.

Modelling Electroporation-Related Phenomena in Electroporation of Food Materials and Environmental Applications

Comparing recent developments in modelling of electroporation-related phenomena in the biomedical domain with those in the field of food processing, we can roughly discern two modelling paradigms: the more theoretical, phenomenon-driven approach in biomedicine, focusing on modelling the effects of treatment protocol on biological material from the basics of the electroporation phenomena point of view, and the industry and engineering-oriented phenomenological and empirical models, prevalent in the food processing domain and used for more direct comparison or optimization of treatment parameter variations to the efficiency of the electroporation application. While empirical models may without argument present an invaluable source of information on the treatment efficacy and efficiency for the industry trying to implement this new technology into its processing lines, they may be—as already discussed (see "Mathematical Modelling of Electroporation-Related Phenomena in Food Processing Assisted by Electroporation" section)—reliably applied only within the ranges of parameters for which they were experimentally derived and validated. Moreover, if they are not based on the physics of the process, optimization must be performed on data obtained at the laboratory scale and transferred to the scale of operation and the applied treatment parameters, and this transfer and validation are normally performed through construction of and experimentation with setups at the level of pilot plants. More efforts need to be directed towards interdisciplinary collaboration, thus connecting researchers from disparate electroporation application domains working on modelling electroporation-related phenomena.

Modelling the phenomena of electroporation on the cell membrane, cell, and tissue levels, while including the mass transport, will allow for determination of necessary and/or optimal pulse parameters. From there, we can then derive what electrode chamber (providing local electric field) and finally what pulse generator is needed for a particular application. We can thus expect multi-scale models which incorporate electric, mass, temperature, and eventually chemical reactions models. This kind of models can then be directly coupled with electronics design software used in pulse generators design.

The Future of Electroporation in Valuable Compounds Recovery and Juice Extraction

As shown in the preceding sections of this paper, electroporation application for valuable compounds recovery and juice extraction has been extensively studied from the engineering and industrial points of view. There remains, however, some lack of understanding of basic principles of interaction of electric pulses, particularly of longer duration (order of ms) and high intensity (several kV/cm), with plant cells, tissues, and the bioactive compounds contained therein that are targeted for extraction.

Several recent works have shed more light on the subject, whilst opening a plethora of new questions into electroporation effects on cell structure. For instance, it was demonstrated that electric pulses of a given (sufficient) strength and duration modify not only the cell membrane, but also the cell wall and the internal membranes (of vacuoles and organelles). Electroporation can also modify molecular connections of intracellular components, thus modifying the properties of extracted components (Delsart et al. 2014; Cholet et al. 2014).

Another subject that beckons further elucidation is the influence of the pulse application frequency, particularly in the very high frequency range (nanosecond-scale pulses), a subject under intensive development in the biomedical field of electroporation applications, but poorly researched and understood in relation to food products and constituents under electric field treatment. Conversely, the low frequency, long pulse duration spectrum of potential effects and applications seem almost completely unexplored, i.e. there is a lack of research on electrokinetic effects in relation to mass transport (electroosmosis, electrophoresis). To again give analogy with biomedicine, use of electrophoretic low-voltage and long pulses post-permeabilization (after application of high-voltage, short-duration pulses) is an established method of increasing post-electroporation mass transport in transdermal drug and gene delivery (Satkauskas et al. 2002; Kanduser et al. 2009; Zorec et al. 2013). Given that enhanced mass transport in the electroporated material is the main objective of extraction applications, these augmenting effects of electrically driven transport must be evaluated also for the food processing applications.

Last but not least, following the trends in food processing applications of the recent decade or so, there is an increased interest and focus towards combining different treatments to food materials for achieving the given objective. In example, the combined (synergistic) effects of electroporation and heating (so-called thermo-electroporation) or of electroporation in combination with heating and high pressure treatment (so-called mano-thermo-electroporation) seem to be the directions of recent trends in research of electroporation for valuable compounds recovery and juice extraction, though they have traditionally been present for a much longer time in electroporation applications for microbial inactivation and sterilization.

Microalgae: A (Re)discovery of Bioreactors

Microalgae have been considered as a potential source of proteins, lipids as well as other valuable compounds (Becker 2007; Gouveia and Oliveira 2009; Wijffels et al. 2010; Chacon-Lee and Gonzalez-Marino 2010). They are considered to be extremely promising organisms for our sustainable future, as they can be grown on non-arable land and have high growth rate (Dismukes et al. 2008). In particular, microalgae can serve as a potential source of energy (Hannon et al. 2010), food (Draaisma et al. 2013), feed (Skrede et al. 2011; Kiron et al. 2012), cosmetics and pharmaceuticals (Pulz and Gross 2004; Gong and Jiang 2011), owing to their high photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops (Widjaja et al. 2009). On the other hand, algae are already a relevant model for wastewater treatment and CO_2 sequestration, which is connected to solution of global environmental problems (Lam et al. 2012). Aquatic microalgae are among the fastest growing photosynthetic organisms, having carbon fixation rates an order of magnitude higher than those of land plants, as microalgae utilize CO_2 as one of the main building blocks for their biomass. Concerning energy, microalgae can be converted directly into energy sources, such as biodiesel, and therefore it appears to be a promising source of renewable energy (Gouveia and Oliveira 2009). Moreover, microalgae cultivation allows for the production of biomass containing a variety of valuable products that generate revenues contributing to the return of the investment (Olaizola 2003; Spolaore et al. 2006). Concerning food, microalgae are an attractive food and food supplement source, since they are rich in proteins, peptides, carbohydrates, lipids, polyunsaturated fatty acids, trace elements and other essential nutrients with protective and detoxifying roles (vitamins, minerals, pigments) (Gong and Jiang 2011).

The main problems are digestibility and availability. Various extraction techniques have been proposed with limited success (Wiyarno et al. 2011; Adam et al. 2012; McMillan et al. 2013; Biller et al. 2013). There are few reports at this stage on use of electroporation as an extraction technique in microalgae for water-soluble proteins (Coustets et al. 2013; Grimi et al. 2014), where the authors report selective extraction by means of electroporation and an increased, but limited success has been reported so far in extraction of lipids (Goettel et al. 2013). Suitable choice of pulse parameters and combination with other extraction techniques may place electroporation on the stage also in microalgae research (Coustets et al. 2013; Flisar et al. 2014).

Pasteurization and Sterilization of Liquid Foods: A New Arms Race?

As discussed in the preceding sections, microbial inactivation by electroporation presents interesting new possibilities for extending the shelf-life of food products to the processing industry. However, as we are constantly and consistently reminded on all open fronts against microorganisms, evolution is an inescapable and tireless process, opening a new arms race with every new method applied for an attempted control of microorganism proliferation. For example, the widespread use of antibiotics for agricultural purposes has rendered much of the antibiotics arsenal useless against selected resistant strains of pathogens, harmful not only to livestock, but also to humans, creating an arms race between research into finding new antibiotics on one side and the adaptation of pathogens on the other (Mathew et al. 2007). As demonstrated in a recent study by Sagarzazu et al. (Sagarzazu et al. 2013),

application of electroporation for inactivation of microorganisms raises similar concerns. The authors of the study report of an acquisition of resistance to electroporation treatment in a *Salmonella* serovar (specifically in *S. typhimurium* SL1344). They consistently observed an increased resistance to electric treatment after repeated rounds of electroporation and outgrowth of survivors. In addition to the acquired resistance to the electric treatment, higher tolerance to acidic pH, hydrogen peroxide and ethanol were also found. It is possible to assume—based on the results of the cited study—that significant risks could be taken in case of wide-scale application of the technology. The risk is that microorganisms, exhibiting increased tolerance to electric treatment, could be released into the uncontrolled natural environment. This suggests that more research is needed in future to insure the long-term efficacy and safety of applying electroporation technology for microorganism inactivation in large scale industrial deployments (as opposed to controlled laboratory-scale experiments). Isolation of "resistant" strains on the other hand could provide valuable insight in exact mechanisms of microbial inactivation mechanisms, providing us with leverage to engineer more efficient treatments.

The Future of Technology Transfer from Research to Industrial-Scale Processing

Final remarks and conclusions in relevant modern literature on industrial scale-up of electroporation-assisted food processing (see "Industrial Equipment Design, Implementation and Commercial Applications" section) almost invariably contain two bottom-line observations: primarily, that electroporation applications for food treatment, as evaluated based on research and pilot plant scale experimental setups, show promising prospects for application; and secondly, there are still major hurdles that need to be dealt with before electroporation will be accepted and firmly established in the food industry. Namely, highly resistant spores of many pathogenic microorganisms are problematic. Their inactivation calls for treatment parameters that considerably increase energy and equipment costs. The most commonly identified obstacles from the technical or economic point of view are the high initial cost of the systems that need to be introduced into an existing processing line; the availability of such systems, specifically in terms of commercial units available on the market; the lack of research on reliability and optimization of processing parameters in the industrial environment; and the lack of standards and regulative in this field of an emerging novel technology, which is related both with the industrial and consumer acceptance. On the other hand, the identified benefits of the technology that will help establish it in the industry are low energy requirements and processing costs; easy implementation and suitability for continuous operation; and the many advantages of electroporation in processing of high value-added foods (Sack et al. 2010b; Morales-de la Pena et al. 2011; Toepfl 2012). Structural changes of food material in particular (meat, fruit and vegetables, juice extraction, etc.) seem to have a bright future in the industry.

Concerning legislation and regulations, electroporation in food processing as a novel technology must be authorized by regulatory bodies, in order to certify the technology and its application to food materials as safe and effective. Two major concerns are raised in this respect: the efficacy of electroporation for food pasteurization and sterilization as compared to the traditional and well-established (e.g. thermal) treatment; and the possible interactions between the treated product and electroporation processing equipment with possible undesired effects and changes to the material (e.g. electrode erosion, electrochemical effects, etc.). In the U.S., the adoption of technology into the food processing industry is subject to the regulations of the Food and Drugs Administration (FDA), and in EU, it falls under the Novel Foods Regulation (EC) 258/97 (European Commission Regulation 258/97 1997; Góngora-Nieto et al. 2002; Jaeger et al. 2008).

Knowledge Transfer Within the Field of Electroporation Research and with the Industry

In July 2012, a European project COST[2] Action TD1104—*EP4Bio*^{2}*Med* was launched (Miklavcic 2012), bringing up until now together more than 500 researchers coming from 35 countries and over 180 institutions and enterprises throughout the world, with one common denominator—they are all working with or are interested in electroporation. The Action's purpose is to facilitate better exchange of knowledge and collaboration among researchers working with electroporation in different domains of its applications; this ranges from the biomedical field to food processing and environmental applications. The member institutions, individual researchers and industrial partners connected through their participation and involvement in the project, are divided into five working groups. One of these groups is dedicated to those primarily interested in basic mechanisms and the theory of electroporation, one each to the fields of biomedical, food processing and environmental applications, and the fifth to the aspects of technology development and transfer. The ultimate goal is to establish lines of communication and transfer of knowledge and people within and between these groups, so

[2] COST stands for European Cooperation in Science and Technology.

that every research field as well as the industry may benefit to a greater extent from the current state-of-the-art in the related, but up to now evolving virtually in parallel domains of research. One of the principal purposes of putting this Action into place is to prevent duplication of research efforts on the European and also global scale, so that the national and European research funds can be spent more efficiently and findings from, in example biomedicine, need not be arrived at independently also by those focused on food processing. The underlying premise is that, among these diverse fields where electroporation has found its place, the basic principles and mechanisms of its action on biological material remain similar, if not the same.

Within the scope of the TD1104 Action, the international School on Electroporation-based Technologies and Treatments or EBTT was and will be organized in the years 2012–2015 (for more information on the upcoming School, visit 2014.ebtt.org or simply ebtt.org). The School has been organized biennially since 2003 in Ljubljana, Slovenia and annually since 2011. The purpose of the EBTT School is to provide all those interested in basic principles and techniques in electroporation-related applications with a quick and intensive course, transmitting knowledge gained by the last 30 years or so of electroporation research by means of lectures given by faculty members and invited lecturers, supplemented by practical hands-on laboratory work and an e-learning session. Traditionally, it was focused towards electroporation in biomedicine, but has recently (since it is being supported by the Action) expanded in scope towards food processing applications, covering also some topics on the design of industrial equipment for electroporation.

In January 2014, the first international School on Applications of Pulsed Electric Fields for Food Processing (PEFSchool) was organized at the Faculty of Veterinary Medicine of University of Zaragoza in Zaragoza, Spain (Raso et al. 2014a, b). Co-organized by COST TD1104, it was dedicated to applications of electroporation in the food processing industry and research and aimed at everyone academically or professionally interested in basic concepts of using electroporation for food materials manipulation. The School is planned to become an annually organized event, with venues selected every year in a different Action member institutions, one that is capable of organizing and hosting the School. The next edition is scheduled to be held on February 8–12, 2015, organized jointly by the ProdAl S.c.a.r.l. consortium and the University of Salerno, Fisciano, Italy.

Conclusions

In this paper, we gave a quick introduction to important concepts in electroporation of biological cells and tissues, followed by a brief review of some of the well investigated and promising applications of electroporation in food processing. Among these, we find applications with many different objectives, such as extraction of juices and other valuable compounds, e.g. anthocyanins, proteins, lipids; tissue dehydration and impregnation (cryopreservation); microbial inhibition and inactivation for pasteurization and/or sterilization; enhancing organoleptic properties of food materials (e.g. winemaking); growth stimulation; and even "cooking" by electroporation. We conclude by a general overview of some important methods for assessing effects of electroporation in plant materials; of factors that influence the success of electric treatment of food materials; of contemporary approaches in the field using modelling techniques and of considerations in industrial-scale equipment design and implementation. The final section on perspectives for future research and development highlights more issues related with transferring the knowledge from research into the industrial environment; possible future applications where electroporation may prove to be the treatment of choice for effective and efficient harvesting of valuable compounds, thus solving some of the pressing economic and environmental problems, and a seemingly hidden caveat of applying the technology to treatment of microorganisms. This paper is a review in the broadest sense of the word, written with the purpose of orienting the interested newcomer to the field of electroporation applications in food technology towards the pertinent, highly relevant and more in-depth literature from the respective subdomains of electroporation research.

In conclusion, the field of research into applications and phenomena related with electroporation of food products and raw materials is well established and its growth is gaining momentum with alimentation and energy sources becoming ever greater global issues. With increasing market demands for calories and nutrients that are safe and of high quality motivated by the evolving educated and conscientious consumer on the one hand, and pressing demands by the food industry to cut on processing costs predominantly due to globalization of production on the other, electroporation might present a large step in arriving at the next optimal equilibrium state between these two competing forces.

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