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Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes

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ABSTRACT

The application of pulsed electric field (PEF) technology as a non-thermal cell membrane permeabilization treatment, was widely demonstrated widely to be effective in microbial inactivation studies, as well as to increase the rates of heat and mass transfer phenomena in food and biotechnological processes (drying, osmotic treatment, freezing, extraction, and diffusion). Nevertheless, most published papers on the topic do not provide enough information for other researchers to assess results properly. A general rule/guidance in reporting experimental data and most of all exposure conditions, would be to report details to the extent that other researchers will be able to repeat, judge and evaluate experiments and data obtained. This is what is described in the present recommendation paper. *Industrial relevance:* Pulsed electric field (PEF) treatment is a promising technology that has received considerable attention in food and biotechnology related applications food and biotechnology related applications of PEF include:

i) "cold" pasteurization of liquid foods and disinfection of wastewater by microbial inactivation
ii) PEF-assisted processing (drying, extraction or expression)

1. Introduction

Pulsed electric field (PEF) treatment is considered to be a promising technology that has in the last years received considerable attention in food and biotechnology related applications in the last years (Haberl, Miklavčič, Serša, Frey, & Rubinsky, 2013; Kotnik et al., 2015; Puértolas, Luengo, Álvarez, & Raso, 2012). The treatment bases on the application of electric pulses of high voltage and short duration (μs–ms) (Mahnič-Kalamiza, Vorobiev, & Miklavčic, 2014) to biomaterials of plant or animal origin, or suspensions of microorganisms placed between two electrodes. As a result, the biological material is exposed to an electric field whose intensity depends on the voltage across the electrodes, as well as on the geometry of both the electrodes and the interelectrode space containing the material to be treated. PEF impact causes membrane permeabilization, also synonymously termed as electroporation, leading to an increased permeability of the membrane to ions and molecules (Kotnik, Kramar, Pucihar, Miklavčič, & Tarek, 2012).

Depending on the intensity of the treatment applied (external electric field, single pulse duration, treatment time) and the cell characteristics (size, shape, orientation in the electric field), the viability of the electroporated cell can be preserved by recovering the membrane integrity, or the electroporation can permanently lead to cell death. Cell size differences between plant and microbial cells, give a wide range of treatment intensity: 0.5–1.5 kV/cm for induction of stress responses and reversible electroporation, 1.0–3.0 kV/cm for irreversible permeabilization in plant or animal tissues, and 15–40 kV/cm for microbial inactivation. Reversible "electroporation" is a procedure usually used in molecular biology and clinical biotechnological applications in vivo to gain access to the cytoplasm in order to introduce or deliver in vivo drugs, oligonucleotides, antibodies, plasmids, etc. (Miklavčič, Mali,

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Kos, Heller, & Serša, 2014; Yarmush, Golberg, Serša, Kotnik, & Miklavčič, 2014; Zorec, Préat, Miklavčič, & Pavšelj, 2013). However most of food and biotechnology related applications of PEF are based on irreversible permeabilization of the cell membranes and mainly include: i) "cold" pasteurization of liquid foods and disinfection of wastewater by microbial inactivation (Frey, Gusbeth, & Schwartz, 2013; Saldaña, Álvarez, Condón, & Raso, 2014); ii) PEF-assisted processing (drying, osmotic dehydration, freeze-drying, freezing, thawing, extraction or expression) for improving food quality, accelerating heat transfer processes, as well as enhancing the mass transfer efficiency of water, solutes (e.g., osmotic agents, cryoprotectants), juices, or high added value compounds from matrices of biological origin, such as plant tissues, suspension of microbial or algae cells, food waste and by-products generated during food processing, or agricultural and forestry residues (Donsì, Ferrari, & Pataro, 2010; Goettel, Eing, Gusbeth, Straessner, & Frey, 2013; Jalte, Lanoiselle, Lebovka, & Vorobiev, 2009; Mahnič-Kalamiza et al., 2014; Parniakov, Lebovka, Bals, & Vorobiev, 2015, 2016a,b; Phoon, Galindo, Vicente, & Dejmek, 2008; Puértolas et al., 2012; Sack et al., 2008; Wiktor et al., 2013).

Technical limitations impeded the exploitation of PEF at an industrial level during many years. The lack of reliable and viable industrial equipment was indeed a critical factor to support up-scaling of the volumes to be treated (from mL to m^3) (Sack et al., 2010; Toepfl, 2012). Large treatment volumes required a shift from the well-established batch methodologies used in basic science towards the flow processes, which is nowadays possible due to the recent developments in pulsed power generators. Other critical aspects that have contributed to limit the spread of PEF technology, are the poor description of the operating protocols, the control and monitoring of the pulse parameters, and the lack of a standardized way of reporting treatment conditions. As the first commercial applications of PEF technology are now available (Golberg et al., 2016), more details on the reports published on the new innovative research are required to improve the reproducibility of treatments when used at industrial level. Lack of such information is a barrier for the development and wide use of the technology.

Variability in results obtained in different laboratories on PEF research may be a consequence of a number of reasons including differences in PEF equipment and PEF treatment conditions. Additionally, in microbial inactivation works and in algae processing, the growth or cultivation conditions of the microorganisms, the treatment medium and the recovery conditions, can play an important role to the outcome of the process. Furthermore, pre and post-treatment conditions can considerably influence the efficiency of the PEF-assisted mass transfer processing.

Different aspects of experimental procedures (biological and engineering) must be described in sufficient details to allow the work to be reproduced in other laboratories. All data must be obtained by paying attention to statistical detail in the planning stage. If a sufficiently large number of replicates are not organized before the experiment is undertaken, biological variation is not eliminated satisfactorily. Replicate design has been recognized to be important to biological experiments for a considerable time (Dhand, 2014; McNutt, 2014).

This recommendation paper has been prepared based on initiative of the Steering Committee of the COST TD 1104 Action (www.electroporation.net), due to increased concern and awareness of low quality reporting practice. Specifically, it has been adopted by the committee within the Working group of Food and Biotechnology of electroporation, in COST action TD 1104 EP4Bio2Med (Miklavčič, 2012), and is in the series of publications that addresses the same topic in Preclinical research in electroporation as well as in the field of medical use of electroporation (Campana et al., 2016).

The objective of this paper is to provide recommendation guidelines on the key information that should be reported in studies regarding the application of PEF for microbial inactivation or PEF-assisted processing in food and biotechnological field. These guidelines are intended to facilitate the comparison of data, to create a reliable basis for a better understanding on the influence of different factors on the PEF efficacy, as well as on the involved mechanisms. It can also be expected that this report may help new researchers in the field to obtain data which are repeatable, reproducible and free from methodological errors.

2. Pulsed electric field (PEF) processing

2.1. Processing parameters

The most typical process parameters that characterize PEF technology are amplitude of electric pulses (U), electric field strength (E), treatment time (t), pulse shape, pulse width (τ), number of pulses (n), pulse specific energy (W) and pulse repetition frequency (f).

The electric field strength and the treatment time are the main process parameters that define the PEF treatment intensity.

Electric field strength refers to the field strength locally present in the treatment chamber during the sample treatment, and depends on the voltage applied between the electrodes, geometry of the treatment chamber, and the spatial distribution of dielectric properties of the material between the electrodes. For parallel plate electrode configuration of batch or continuous treatment chambers, apart from some edge effects (Donsì, Ferrari, & Pataro, 2007), the electric field is homogeneous within the interelectrode space (Fig. 1), and can be estimated by dividing the voltage (U) measured across the electrodes of the treatment chamber by the electrode distance (L):

$$E = \frac{U}{L} \tag{1}$$

In contrast, other chamber configurations, such as co-linear electrode configuration (Fig. 1), suffer from a non-uniform distribution of the electric field in the treatment zone, where the actual field strength is often lower than the estimation predicted by Eq. (1). Therefore, in such cases, several approaches based on numerical simulation procedures have been considered for obtaining a more accurate estimation of the actual field strength applied, such as those based on a graph showing the field strength distribution along the central axis of the treatment zone (Toepfl, Heinz, & Knorr, 2007), determination of the lowest electric field strength for the entire volume of the treatment zone (Meneses, Jaeger, Moritz, & Knorr, 2011) or considering different volume elements and calculation of an average field strength for the entire volume of the treatment zone (Gerlach et al., 2008).

Treatment time refers to the number of pulses applied multiplied by the pulse width (or pulse duration):

$$t = n \cdot \tau \tag{2}$$

where τ depends on the pulse shape. As it is shown in Fig. 2, the pulse shapes commonly used in PEF treatments are either exponential or square-wave pulses, unipolar or bipolar. Voltage and current waveforms of the electric pulses delivered in the treatment chamber, should be monitored continuously using high voltage and fast high current probes located as close as possible to the treatment chamber, in order to precisely define the treatment intensity. Generally, in fact, the voltage output from the pulse power is lower than the voltage measured in the treatment chamber, especially for chamber configuration characterized by a low intrinsic electrical resistance.

Thus, in order to accurately describe processing conditions, pulse characteristics, including peak voltage, pulse shape, pulse width, and pulse polarity, should be reported. To this purpose, a snapshot of the monitored pulses (voltage, current) delivered to the treatment chamber, should be provided, which implies that a digitized recording is included in the set-up of the PEF system.

Pulse duration, or *pulse width,* for a square pulse is the time that the voltage is kept at the maximum value (peak voltage) (Reberšek, Miklavčič, Bertacchini, & Sack, 2014). In the case of exponential decay

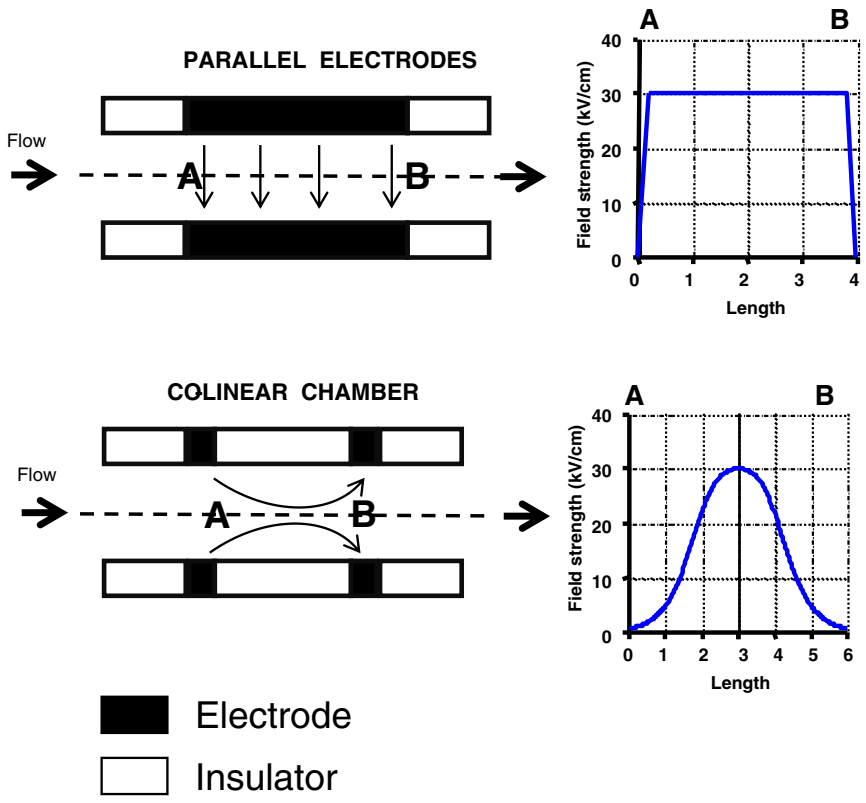

Fig. 1. Schematics of parallel plate and co-linear treatment chamber configuration with qualitative distribution of the electric field in the treatment zone.

pulses, the pulse width is defined as the time needed to decrease the voltage to 37% of its peak value (Fig. 2).

Frequency and protocol of application of the series of pulses should be also documented. Frequency indicates the number of pulses applied by unit of time, and it is reported in Hz (number of pulses/s). The specification of the pulse frequency is important, since it determines the amount of electrical energy delivered per unit of time on the product placed in the treatment chamber, which, in turn, affects the temperature increase of the processed product due to Joule effect. Moreover, pulse frequency has been proved to be, among others, a key factor affecting the extent of the unavoidable electrochemical reaction which occurs at the electrode-liquid interface of the treatment chamber, especially those involving the migration of metal from the electrodes into the biological matrices (Kotnik, Miklavčič, & Mir, 2001; Pataro, Barca, Donsì, & Ferrari, 2015a,b; Pataro, Falcone, Donsì, & Ferrari, 2014). This is a very important issue, since the metal released may affect microbial inactivation or may further react with the biomaterials present in the bulk, also after the application of the pulse treatment, as well as negatively affect the efficiency of the PEF treatment with time, and reduce the electrode lifetime (corrosion).

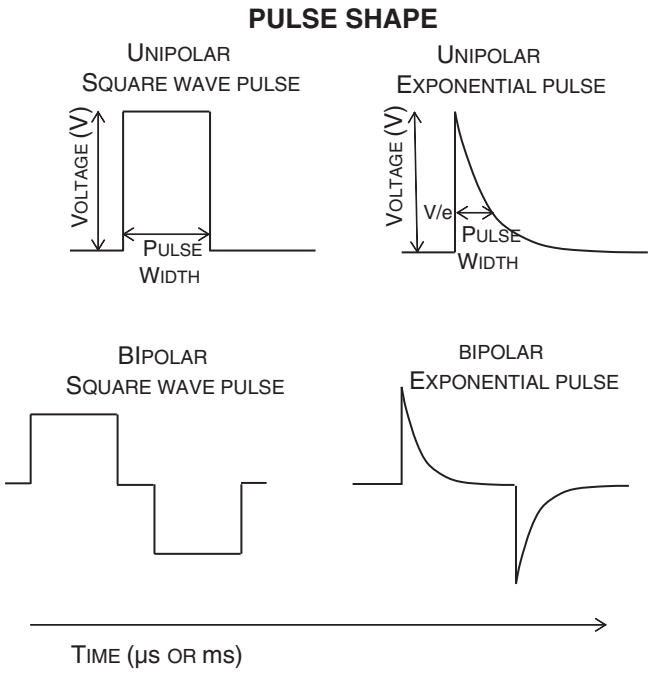

Fig. 2. Pulse shapes commonly used in PEF treatments.

In addition to pulse frequency, pulse protocol should be also described in detail. For batch treatment, number of pulses applied per each train, number of trains of pulses and time interval between two consecutive trains, should be reported. For continuous flow treatment, the number of recirculation of the treated product through the PEF chamber, should also be specified. Moreover, it is worth remembering that, in batch treatment the number of electric pulses to be applied is set directly by the user. In continuous flow process, instead, it is a function of the pulse frequency and residence time (t_r) of the product in the treatment chamber, which depends on the flow rate (F) and volume (v) of the treatment zone, according to the following equation:

$$n = t_r \cdot f = \frac{v}{F} \cdot f \tag{3}$$

The *energy density* or *specific energy per pulse* (W, in kJ/kg/pulse) is the electrical energy received by the treated product in the PEF chamber per each pulse. It depends on the electrical properties of the treated product and on the pulse shape (including peak voltage and pulse width). The electrical properties of treated product are changing —

conductivity is increasing for two reasons: membrane electroporation resulting in increased conductivity and due to diffusion of ions from cells to water/media, usually of low conductance at the beginning of the treatment. Due to the losses through the connections and the components of the discharge circuit, the value of W is generally different from the energy output from the pulse generator. Moreover, waveforms of voltage and current can considerably deviate from the ideal square or exponential shapes. Therefore, according to Eq. (4), the specific energy input per pulse W should be evaluated by the integral over time of the recorded waveforms of voltage and current at the treatment chamber.

$$W = \frac{1}{m}\int_0^{\infty}\frac{U(t)^2}{R}dt = \frac{1}{m}\int_0^{\infty}U(t)\cdot I(t)dt \quad (4)$$

where m is the mass of treated sample, $U(t)$ and $I(t)$ are, respectively, the voltage across and current through the treatment chamber load at time t. R (in Ω) is the electrical resistance of the treatment chamber, which can be calculated according to the following equation:

$$R = \frac{1}{\sigma}\frac{L}{A} \quad (5)$$

where σ is the electrical conductivity of the treated product (S/m) and A is the electrode area (m^2).

The *total specific energy input* (W_T, kJ/kg) can be calculated by multiplying W with the number of pulses applied:

$$W_T = W \cdot n \quad (6)$$

Electric field strength and total specific energy input (instead of treatment time) have been suggested as suitable parameters enabling to compare the data obtained under distinct conditions and equipments (Heinz, Alvarez, Angersbach, & Knorr, 2002). Particularly, total specific energy input should be preferred instead of treatment time especially when exponentially decay pulses are applied. Furthermore, the specification of the total energy input will also give an estimation of the energy consumption due to the PEF process.

Temperature is also a critical parameter that influences the efficacy of PEF treatment. Several reports described an enhanced microbial inactivation or cell degree permeabilization upon increasing the PEF treatment temperature (Lebovka, Praporscic, Ghnimi, & Vorobiev, 2005; Saldaña et al., 2010). This PEF-temperature synergy is likely due to the fact that membrane of biological cells become more fluid and their mechanical resistance decreases with increasing the processing temperature (Coster & Zimmermann, 1975), making the cell membrane more prone to electroporation.

On the other hand, the dissipation of the electrical energy delivered to the product during PEF treatment increases the temperature of the product, which in turn increases the electrical conductivity and may modify product viscosity. As a consequence, the increment of the product temperature may lower the resistance of the treatment chamber, leading to a decrease of the applied field strength, unless the external voltage is not increased accordingly. In addition, in continuous processes, flow rate and residence time of the processed product in the treatment chamber may also change as a result of the product temperature increase. Moreover, temperature increase may also lead to an overestimation of the effectiveness of the treatment due to the sensitizing effect of the temperature on the PEF resistance of biological cells (Heinz, Toepfl, & Knorr, 2003). It has been demonstrated that using static parallel electrode treatment chamber with temperature-controlled electrodes allows to obtain data on microbial inactivation at different temperatures at quasi-isothermal conditions preventing the artefacts caused by temperature increase when no temperature control of electrodes is used (Saldaña et al., 2010).

Temperature increase, as a consequence of Joule heating, is enhanced at higher electric fields, total specific energy, frequencies and pulse widths. Therefore, optimal processing conditions for studying the influence of these factors on the outcome of the PEF process should be chosen by minimizing the related heating effects, for instance by using treatment chambers in which it is possible to cool the electrodes (Saldaña et al., 2010). In any case, in batch treatments the initial temperature of the product as well as the final temperature after the application of the PEF treatment should be documented. In continuous flow processes, the temperature of the product entering the treatment chamber (inlet temperature) and that at the exit of the treatment chamber (outlet temperature) should be measured and reported (Meneses, Jaeger, & Knorr, 2011). An adequate description of the methods used for pre-heating the product before entering the PEF treatment chamber, as well as for cooling the treated product at the exit of the PEF chamber, should also be provided. Moreover, it is also necessary to specify the location of the temperature sensors in relation to the treatment chamber. When the experimental setup consists in several continuous flow treatment chambers connected in series, the temperature sensors should be located immediately before and after each treatment chamber, especially when the treated product is cooled in heat exchangers placed in between two consecutive treatment chambers. Temperature sensors whose measurement is not influenced by the electric field should be used. If a direct temperature measurement is not possible, the resulting temperature increase of the treated product can be estimated based on a calculation of the total specific energy and assuming adiabatic heating, i.e. all electrical energy is converted to heat.

2.2. PEF equipment

An appropriate description of the PEF generator and treatment chamber used to conduct the experiments should be provided. For commercial equipment, the name of the supplier company and the model should be specified. If the PEF generator is a laboratory prototype or specially fabricated unit, an adequate description of the components (power supply, capacitors, switches, transformers, etc), electrical configuration, measurement and data acquisition systems, and any other pertinent information that characterizes the equipment to reproduce exposure of sample to pulsed electric field should be provided. Laboratory studies on either microbial inactivation or improving mass transfer phenomena by PEF may be conducted in batch or in continuous flow treatment chambers that should be described in details.

The two most used treatment chamber designs considered for application of PEFs in continuous flow are parallel plate electrodes and co-linear configurations (Fig. 1). Parallel plate electrode configuration is the simplest in design and consists of a rectangular parallelepiped shape channel of insulating material with two electrodes on opposite sides. As previously reported, this configuration typically provides uniform electric field in the treatment zone, with the applied electric field being perpendicular to the product flow. However, because it is characterized by a large electrode surface and low intrinsic electrical resistance, it generally operates at high current, which also may facilitate the triggering of undesired electrochemical phenomena at the electrode-liquid food interface of the PEF chamber (electrode corrosion). In the co-linear configuration, couples of tubular electrodes are spaced with insulator spacer tubes. The product is treated as it flows from one electrode to the other, parallel to the electric field. Such configuration has advantageous fluid dynamics, highly desiderate for food processing and convenient for cleaning in place, as well as a high intrinsic resistance due to the low effective area of the cross section of the tubular electrodes. Thus, this device typically operates at lower current than the parallel plate configuration, which makes it suitable for limiting the occurrence of electrode reactions, as well as for the connection of multiple co-linear units in parallel from the electrical viewpoint. The main problem of this configuration is in-homogeneity in the electric field strength and temperature distribution in the treatment zone during PEF processing. Therefore, an adequate chamber design is required in order to ensure more uniform distribution of the electric field

(generally sufficiently high ratio between electrode gap and area, as well as rounded edges of the electrodes, are recommended).

A further cause of treatment inhomogeneity in both parallel plate electrode and co-linear treatment chamber, which is most important in microbial inactivation studies, is the existence of laminar flow into the treatment zone. This is because the higher flow rate required to promote turbulent flow conditions needs a higher pulse modulator power, as well as a higher commutation rate of the switching devices, in order to deliver the required amount of energy per volume element. Therefore, the use of the highest possible flow rate (Pataro, Senatore, Donsí, & Ferrari, 2011), as well as the generation of turbulent flow by modifying the treatment chamber geometry or by inserting a grid in front of the treatment zone have been suggested to improve the treatment uniformity (Meneses, Jaeger, Moritz and Knorr, 2011). Thus, in addition to the electrical parameters, also the flow conditions (rates, laminar or non-laminar) should be reported in studies regarding PEF applications (Jaeger, Meneses, & Knorr, 2009).

As already recommended for power supplies, the name of the supplier company and the model number should be specified for commercial treatment chambers. If the treatment chamber is a prototype or specially fabricated, an adequate description is required. A schematic drawing of the treatment chamber details, which describes the geometrical shape of insulator and electrode along the boundary to the material to be treated, should be provided. Additionally, the material of the electrodes and insulators, and the most relevant sizes such as electrode gap, surface area or dimensions of the electrodes (e.g., diameter of the tube for co-linear configurations), treatment volume, i.e. the volume where the specified electric field strength is present, should be reported.

PEF processing for industrial applications requires continuous flow processing, thus the results obtained in batch treatments need to be validated in a continuous flow installation before they can be successfully implemented on a large scale. Recently, there has been considerable progress in the development of both pulse generators and continuous flow treatment chambers design that are essential for scaling up the technology for industrial applications (Huang & Wang, 2009). In this frame, however, further studies based on the development and application of characteristic (dimensionless) numbers are necessary. This would lead to a more targeted approach for industrial scale-up and application of the PEF technology.

A detailed knowledge or a good estimation of the values assumed to be the critical process parameters inside the treatment chamber during processing of biological matrices is required. However, the small dimensions of the treatment chambers may make impossible to perform adequate measurements of the process parameters inside the treatment chamber with the corresponding probes without perturbation of the flow, temperature, and electric field distribution (Jaeger et al., 2009). Therefore, it is recommended to use numerical simulation techniques to provide information on the spatial and temporal distribution of the electric field strength, temperature, and flow velocity inside the treatment chambers. Moreover, it is worth noting that a numerical approach would also allow the use of local and time resolved information, which could help in obtaining insight in the mechanisms of action of the PEF technology, with respect to an analyses based only on integral values.

3. Microbial inactivation by PEF

Many studies on microbial inactivation by PEF have been conducted and reported in the literature. The technology of PEF follows general principles, however the numerous factors affecting microbial inactivation by PEF, the broad experimental conditions used by different research groups and the diversity of equipment available limits the comparison of results, the standardization of experimental procedures used in different laboratories and obtaining solid conclusions in this topic.

Due to the difficulty to standardize experimental procedures used in different laboratories, information that should be provide for researchers in any study of microbial inactivation by PEF are shown in Table 1.

3.1. Microorganism and culture conditions

It has been observed that there is a great variation in the sensitivity of different strains of the same species of bacteria to PEF treatments (Lado & Yousef, 2003; Saldaña et al., 2009). Therefore the strain of the

Table 1
Recommended information to be reported in studies on microbial inactivation by PEF.

Microorganism culture and recovery conditions	*Genus, species and strain of the microorganisms* *Culture conditions* Initial inoculum o Description of the procedure for microbial cultivation o Growth medium composition, growth temperature, incubation time and growth phase (exponential or stationary) *Recovery conditions* o Time and storage conditions between treatment and microbiological analysis o Description of the procedure for enumerating microorganisms o Composition of the recovery medium, incubation time and incubation temperature
PEF equipment	*PEF generator* o For commercial: equipment name of the supplier company and model o For prototypes: adequate description of the components, electrical configurations, electrical specifications *Treatment chamber* o For commercial: equipment name of the supplier company and model o For prototypes: adequate description (e.g., configuration of the electrodes, material of electrodes and insulators, dimensions, volume, gap) *Auxiliary devices* o Pump o Heat exchangers o Voltage and current o Temperature probe o Measurement/data acquisition system
Processing parameters	*Batch treatments* o Pulse amplitude (voltage and current), electric field strength, pulse energy, number of pulses, pulse shape, pulse width, pulse protocol treatment time, frequency, initial and final temperature *Continuous flow treatments* o Pulse amplitude (voltage and current), electric field strength, pulse energy, number of pulses for each treatment chamber, pulse shape, pulse width, pulse protocol treatment time, frequency, mass flow, residence time, inlet and outlet temperature for each treatment chamber
Treatment medium properties	o Composition o pH o a_w o Electrical conductivity

Table 2
Recommended information to be reported in studies of PEF-assisted processing for improving heat and mass transfer phenomena in food and biotechnological processes.

Raw material	*Origins, variety, maturation and storage conditions of plant matrices and cell (microbial, algae) suspensions* *Plant matrices* o Variety o Geographical origin o Degree of ripeness o Moisture content o Storage conditions (temperature, humidity, storage time) *Cells suspension* *Microbial cell* (see Table 1) *Algae cells* o Genus, species, strain, source of supply of the microalgae o Description of the bioreactor and cultivation procedure o Growth medium composition, growth temperature and time, growth phase (exponential or stationary) o Biomass concentration
Upstream process	*Equipment for raw material pre-treatments and characterization* o For commercial equipment: name of the supplier company and model o For prototypes: adequate description and operating mode *Characterization of pre-treated raw material* o Size, shape, particle size distribution (slicing/mechanical grinding) o Temperature, time (pre-heating) o Moisture content o Electrical conductivity of solid and liquid phase o Cell concentration or inoculum size (for microbial cells) o Biomass concentration (for algae cells) o Biomaterial (plant)/treatment medium ratio
PEF equipment	*PEF generator* o For commercial: equipment name of the supplier company and model o For prototypes: adequate description of the components, electrical configuration, electrical specifications *Treatment chamber* o For commercial: equipment name of the supplier company and model o For prototypes: adequate description (e.g., configuration of the electrodes, material of electrodes and insulators, dimensions, volume, gap) *Auxiliary devices* o Pump o Heat exchangers o Voltage and current probes o Temperature probe o Measurement/data acquisition system
Processing parameters	*Batch treatments* o Pulse amplitude (voltage and current), electric field strength, pulse energy, number of pulses, pulse shape, pulse width, pulse protocol treatment time, frequency, initial and final temperature *Continuous flow treatments* o Pulse amplitude (voltage and current), electric field strength, pulse energy, number of pulses for each treatment chamber, pulse shape, pulse width, pulse protocol treatment time, frequency, mass flow, residence time, inlet and outlet temperature for each treatment chamber
Treatment medium properties	o Composition o pH o a_w o Electrical conductivity
Downstream process	*Extraction by mechanical pressing* o Type of press (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode) o Pressing procedure (Pressure, time, pressing cycles) *Extraction with solvent* o Type of extractor (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode) o Type of Solvent (composition, pH) o Temperature and time o Solid/solvent ratio o Shaking conditions *Purification of the extracts* o Centrifugation (revolution per unit of time, time, temperature) o Filtration (type and size of filter) o Concentration (pressure, temperature) *Thermal drying* o Type of dryer (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode) o Initial temperature and moisture content of sample before drying o Hot air properties (e.g., temperature, humidity, velocity) o Drying time o Degree of dehydration *Osmotic dehydration* o Type of osmotic dryer (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode) o Type of osmotic solution o Concentration of the osmotic agent o Pressure o Dehydration temperature and time o Solid/liquid ratio o Shaking conditions o Degree of dehydration

(continued on next page)

Table 2 (*continued*)

Freeze-drying
o Type of freeze-dryer (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode)
o Initial temperature and moisture content of sample before freeze-drying
o Freeze-drying temperature, pressure, and time
o Degree of dehydration
Freezing
o Type of freezer (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode)
o Initial temperature and moisture content of sample before freezing
o Freezing temperature, pressure, and time
o Air velocity
o Type and concentration of cryoprotectant
Thawing
o Type of thawing chamber (for commercial equipment: name of the supplier company and model; for prototypes: adequate description and operating mode)
o Initial temperature of sample before thawing
o Thawing temperature and time
o Air velocity

microorganism used should be reported including the name with genus, species and strain number. It should be desirable that that the strain or strains used in the study should be available for other researchers.

The preparation of the microbial culture and the storage conditions can significantly affect the microbial sensitivity to PEF. Therefore, the cultivation of the microorganism should be standardized to minimize its influence on variability between repeated experiments either from day to day, or from test period to test period. Initial inoculum, growth medium composition, growth temperature, time of incubation and growth phase of the cells used for inactivation experiments should be reported.

Several studies indicate that microorganisms at the exponential phase of growth are more PEF sensitive than those at the stationary phase (Álvarez, Pagán, Raso, & Condón, 2002; Rodrigo, Ruíz, Barbosa-Cánovas, Martínez, & Rodrigo, 2003; Wouters, Dutreux, Smelt, & Lelieveld, 1999). This higher microbial sensitivity could be related to the larger size of cells in the exponential phase or to the manifestation of an alternative sigma factor when microorganisms enter in the stationary phase resulting in the expression of a number of genes that confer stress resistance, as well altered metabolism, structural and morphological changes (Somolinos, García, Mañas, Condón, & Pagán, 2008). On the other hand, reported data indicate that cells, grown at temperatures lower than the optimal one, are more PEF sensitive that those grown at the optimal temperature (Álvarez et al., 2002; Ohshima, Akuyama, & Sato, 2002). Lipid composition variations in the cytoplasmic membrane induced by modifications of the growth temperature have been suggested as the origin of the distinct PEF sensitivity. At lower growth temperatures the degree of fatty acid insaturations of the phospholipids of the cell membrane raises which could increase the fluidity of the bacterial cell membrane and increased its sensitivity to electroporation.

3.2. Treatment medium

The treatment medium used for the inactivation studies should be well defined to allow reproduction in other laboratories. Composition of the treatment medium should be reported and factors that may affect microbial inactivation such as pH, conductivity, activity of water (osmolarity), as well as presence of preservatives should be measured and reported.

3.3. Inactivation studies

For inactivation studies it has been recommended a minimum of three replicate sets per trial repeated on separated days in order to be able to measure the experimental error as well as differences in response due to biological variability has been suggested (Balasubramaniam, Ting, Stewart, & Robbins, 2004). For testing microbial resistance to a lethal treatment such as PEF, acquisition of multiple data points along the time for a given electric field strength is preferred because they give more information than end-point measurements based on the inactivation produced by a given treatment. The acquisition of multiple data points permits the elaboration of the survival curves in which the logarithmic of survivors is plotted against inactivation time for a given treatment intensity. Survival curves can be described by mathematical models (Dermol & Miklavčič, 2015). Modelling kinetics data obtained under different experimental conditions and developing of predictive models are very useful tools for quantifying the influence of different factors on microbial inactivation by PEF, as well as to define equivalent treatment conditions to achieve a given level of inactivation.

3.4. Recovery conditions

Quantification of the survivors after the treatment is one of the most important factors in estimating the efficacy of an inactivation technique such as PEF. It is important to use procedures that recover the greatest number of microorganisms. Recovery medium, incubation time and temperature during incubation should be reported because they have a significant effect on the number of microorganisms recovered after the PEF treatment. The time and storage conditions between treatment and microbiological analysis should also be reported.

Comparison of cell counts of PEF treated samples on selective and nonselective media is the most conventional technique to detect sublethal injury. Sublethally injured population fails to survive and multiply in harsh environments tolerant by untreated cells (Mackey, 2000). If the existence of sub-lethal injured microorganisms is detected by adding selective agents in the recovery medium it is necessary to establish previously the maximum concentration of the selective agent that has not inhibitory effect on untreated cells. The selective agent and the concentration used for detecting sub-lethal injured microorganisms need to be given. Generally longer incubation times are required when microorganism are plated on selective media because inactivation may be overestimated when the incubation time is the same in nonselective and selective media.

4. PEF-assisted processing for improving mass transfer phenomena in food and biotechnological processes

The application of PEF as a mild cell disintegration technique for improving food quality, accelerate heat transfer process, as well as mass transfer efficiency of target compounds from matrices of biological

origin, demonstrated its efficiency especially in extraction by solvent diffusion or pressing, as well as in drying, osmotic dehydration, freeze-drying, freezing, and freeze-thawing (Barba et al., 2015; Bobinaitė et al., 2014; Eing, Goettel, Straessner, Gusbeth, & Frey, 2013; Jalte et al., 2009; Parniakov et al., 2015, 2016a,b; Wiktor et al., 2013).

However, similarly to the application of PEF for microbial inactivation, it is difficult to compare between studies of different groups, due to the large number of parameters, which are interrelated, as well as the large variety of experimental conditions and equipment used by several researchers.

Moreover, no or very few systematic studies are available, to date, taking into account the entire production process including complex interactions between raw material properties, their changes after pre-treatment, cell disintegration by PEF, and downstream processes, which are all of relevance on the outcome of the entire process (Jaeger, Schulz, Lu, & Knorr, 2012).

For these reasons, in Table 2 we summarize the main information regarding raw material characteristics, the upstream processes (e.g., grinding, slicing, heating, concentration), the PEF process (e.g., electric field strength, energy input, pulse characteristics), as well as downstream processes (e.g., extraction, drying, freezing, purification) that should be reported in published papers.

This information is essential to allow standardization of experimental procedures and reproducibility of the experiments in view of the utilization of bench-scale data on PEF assisted processing to define processing conditions in commercial size equipment.

4.1. Raw material

Information on raw materials is very important since it can contribute to define the optimal processing conditions as well as the properties of the final product.

Therefore, for the case of raw material of plant origin, information such as geographical origin, variety, degree of ripeness, moisture content, as well as storage time and conditions (e.g., temperature, air humidity) before processing should be reported. Similarly, in the case of cell (microbial, algae) in suspension, detailed description of the genus, species, strain number and source of supply, as well as growth or cultivation conditions should be provided, as reported in detail in the Section 3.1 and Tables 1 and 2.

4.2. Upstream process

Raw materials are typically pre-treated before PEF-assisted processing with the aim of softening or hydrating biomaterial, reducing the particle size, increasing the surface–volume-ratio, or to induce mixture densification. For example, raw materials of plant origin are typically subjected to peeling, slicing, mechanical grinding or pre-heating. In the case of cell suspensions, a concentration step of the biomass could be required.

Therefore, detailed information on the features of the equipment used (name of the manufacturer and model) and processing conditions should be specified.

Moreover, when plant material is pre-treated, information on the textural properties, size, shape and particle size distribution of the mash after grinding, pre-heating time and temperature, moisture content of the plant tissue, electrical conductivity of solid and liquid phase, solid/liquid ratio, as well as any other physical and chemical-physical properties of relevance for the next processing steps, should be provided. In the case of cell suspensions, the type, pH and electrical conductivity of suspending medium, as well as the cell concentration (for studies on microbial cells), should be reported. For microalgae processing the component yield per kg of biomass (dry weight) in suspension is in focus. Thus, the content of biomass (dry weight) in the treated suspension is a mandatory value to be reported in experimental studies. Also component yields have to be related to the processed biomass dry weight, as usual in the microalgae processing community.

Finally, it is worth noting that raw material pre-treatment may also cause partial or total cell disintegration (Jaeger et al., 2012). Therefore, the impact of conventional pre-treatment on cell membrane disruption should be reported in order to be discriminated from that of the PEF treatment.

4.3. PEF equipment and processing conditions

As previously reported, an appropriate description of the PEF generator, treatment chamber and auxiliary devices (e.g., voltage, current and temperature probes, pump, heat exchangers) should be provided. In addition, in order to allow a proper comparison between data of different authors, electric field strength and total specific energy input W_T [kJ/kg] should be chosen as parameters to describe the treatment intensity. Moreover, also (initial) voltage applied, pulse shape, pulse width, number of pulses or treatment time, frequency, pulse protocol, initial and final temperature for batch processes, and mass flow, residence time, inlet and outlet temperature for continuous flow chamber, should be also specified.

4.4. Downstream process

The design and the operating mode of the equipment to be used for processing of the electroporated matrices, may play an important role for the exploitation of the potential benefits that may result from PEF pre-treatment. In addition, the results achieved from the PEF-assisted processing investigations that are typically used to compare data obtained from different studies, are generally collected after the characterization of the final product. It is, therefore, crucial to provide detailed information on the equipment (manufacturer, model), the experimental conditions, the protocol analysis and methods used in the downstream process (Table 2).

For example, in the case of the extraction processes by mechanical pressing, a detailed description of the type of press as well as the pressing conditions (e.g., pressure, pressing time, and number of pressing cycle) should be reported. When the extraction process following the PEF pre-treatment is carried out by using solvents, a detailed description of the type of extractor, as well as information on the type of solvent (e.g., composition, pH), the temperature and extraction time, the solid/solvent ratio, and shaking conditions, should be reported. If the extract solution requires a further purification stage before analyses, detailed information on the type of the devices and protocols of purification adopted, should be also reported.

In PEF assisted drying processes (thermal drying, osmotic dehydration, freeze-drying) information on the type of dryer, initial temperature and moisture content of the biomaterial, thermodynamic properties of hot air (e.g, temperature, humidity) and air velocity, type and concentration of osmotic agent, as well as temperature, pressure and time of drying, should be included.

Finally, when PEF is used to assist freezing/thawing processes, a detailed description of the type of freezing/thawing chamber, as well as initial temperature and moisture content of sample before freezing/thawing, processing conditions (temperature, pressure, time, air velocity), as well as type and concentration of cryoprotectant (if applicable), should be reported.

5. Conclusions

In this paper, basic principles of PEF technology and its application in food and biotechnological processes have been reviewed, and the main problems that a researcher may encounter when conducting experiments with the PEF technology, have been described. This paper provides recommendations for standardization of research methodology, as well as key information that should be reported in studies regarding

the application of PEF technology, in order to be able to compare data obtained in various laboratories. It is expected that this paper will contribute to improve the current state of knowledge on electroporation mechanisms, and to identify the critical factors affecting electroporation, with final objective of extending the commercial exploitation of PEF processing in the food and biotechnological industries.

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