Chapter

Microbial Decontamination by Pulsed Electric Fields (PEF) in Winemaking

Carlota Delso, Alejandro Berzosa, Jorge Sanz, Ignacio Álvarez and Javier Raso

Abstract

Pulsed Electric Fields (PEF) is a non-thermal technique that causes electroporation of cell membranes by applying very short pulses (μs) of a high-intensity electric field (kV/cm). Irreversible electroporation leads to the formation of permanent conductive channels in the cytoplasmic membrane of cells, resulting in the loss of cell viability. This effect is achieved with low energy requirements and minimal deterioration of quality. This chapter reviews the studies hitherto conducted to evaluate the potential of PEF as a technology for microbial decontamination in the winemaking process for reducing or replacing the use of SO_2, for guaranteeing reproducible fermentations or for wine stabilization.

Keywords: PEF, SO_2, electroporation, microbial inactivation, wine, pulsed electric fields

1. Introduction

Winemaking is a complex process that extends from grape cultivation and harvesting to wine consumption. In the course of this process, many different chemical, physical, microbiological, and sensory reactions are involved. Microorganisms play an essential role, since alcoholic fermentation and frequently also malolactic fermentation are fundamental steps in winemaking. During these fermentation steps, the evolution of certain chemical compounds depends directly on their interaction with microorganisms, thereby resulting in many of the characteristic and desirable flavors in wine [1]. Conversely, microorganisms can also contaminate and spoil the wine in several steps of the winemaking procedure, causing re-fermentation, off-flavors, volatile acidity, and bottle explosion. Moreover, microorganisms can produce compounds that are hazardous for human health, such as biogenic amines [2]. The ultimate quality of wines and their commercial value are therefore directly associated with those microflora which are beneficial; nevertheless, microbial spoilage of wine can lead to a number of drawbacks and economic losses for the wine industry. It is thus essential to monitor the entire winemaking process in the endeavor to avoid contamination caused by microorganisms. This can be achieved using chemical preservatives and/or certain physical treatments designed to inactivate microorganisms, inhibit their growth, or directly separate them physically from wine.

The main yeasts regarded as true spoilage strains in wine are *Brettanomyces bruxellensis*, *Zygosaccharomyces bailii*, and *Saccharomyces cerevisiae*. *B. bruxellensis* is one of the most undesirable strains in wineries, as even at very low concentrations it can produce the typical "horse sweat" taint, and early detection is difficult [3]. Because of its tolerance to high sugar and sulfur dioxide concentrations, *Zygosaccharomyces* may cause turbidity, produce CO_2, and even re-ferment sweet wines and grape juices [4]. *S. cerevisiae*, although involved in the alcoholic fermentation process, can be responsible for wine spoilage when a nutritional imbalance in the grape juice triggers off-flavor production. Other species of the genera *Kloeckera/Hanseniaspora*, *Pichia*, and *Candida* can also produce film layers and undesired metabolites [5].

Lactic Acid Bacteria (LAB) are responsible for malolactic fermentation (MLF), but can also negatively affect the quality of wines as spoilage microorganisms when they proliferate at the incorrect time during winemaking [6]. Wine-associated microbial LAB genera are *Lactobacillus, Leuconostoc, Oenococcus*, and *Pediococcus*. LAB growth in wine can imply the production of undesirable aroma and flavor compounds, biogenic amines, acrolein, and ethyl carbamate, or can cause a slimy appearance. In the category of Acetic Acid Bacteria (AAB), the three main associated genera considered as spoilage bacteria in wines are *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter*. Their principal effect on wines is the production of acetic acid, acetaldehyde, and ethyl acetate, which confer sour, nutty, and solvent-like flavors, respectively. All these groups of spoilage microorganisms in wine have in common their ethanol tolerance, their ability to grow at low pH (< 4.0), and, in some cases, a high tolerance to SO_2. In order to establish a methodology for must or wine decontamination and stabilization, it would be necessary to establish which are the target microorganisms in the different steps of winemaking, and to study their tolerance/resistance to the chosen lethal agent.

2. Current innovative strategies for microbial decontamination in winemaking

At present, the main strategy applied to control spoilage microorganisms along the winemaking process is the addition of sulfur dioxide (SO_2), a compound which is able to ensure antioxidant protection and microbiological stability. Although SO_2 is a highly effective and inexpensive preservative widely used in the wine industry, concerns have been raised regarding its potentially adverse effects on human health. The general trend in the wine industry is thus currently to reduce SO_2 content, or even to eliminate it altogether [7].

Dimethyl dicarbonate (DMDC), lysozyme, and sorbic acid are chemical compounds proposed as alternatives to SO_2, and they are already allowed as antimicrobials in winemaking by the OIV. Although they have proven effective against certain wine spoilage microorganisms, at their maximum permitted doses none of them is sufficiently effective against the entire range of microorganisms of concern [7].

Microfiltration, on the other hand, is a common physical procedure applied in winemaking for purposes of microbial stabilization. However, this technique is only applied before bottling and has some drawbacks due to its potentially deleterious effects on flavor and color properties of wines, depending on filter media and intrinsic wine characteristics. Sterile filtration presents further practical problems associated with frequent fouling, the high cost of filters, their management, and the possible recontamination of wines during bottling [8]. Heat treatments, despite their well-known high efficacy in terms of microbial inactivation, are not commonly used in wineries due to the negative effects of high temperature on the valuable sensory properties of wine [9]. Generally, thermal pasteurization is only applied to low-medium quality wines prior to bottling.

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Similarly, emerging preservation techniques have been proposed for the microbial stabilization of wines. High hydrostatic pressure (HHP) is one of the most widely studied methods, and it has proven effective against most of the target microorganisms in wine [10]. However, due to the necessity of treating bottled wine and the possible acceleration of unwanted chemical reactions, along with the high cost and small flexibility of HPP devices, is ultimately not the most feasible technique for wineries [11]. Ultrasound, ultraviolet light, ionizing radiation, ultra-high pressure homogenization (UHPH), and microwaves have been also investigated for wine, for must, and even for barrel sterilization [12–17]. The main recent studies have focused on these techniques' lethal efficacy, but it is still necessary to obtain further knowledge about their effects on sensory quality and their actual feasibility at an industrial scale. Moreover, none of these innovative physical technologies is yet approved for wine stabilization by the OIV, except for HHP and UHPH.

In order to meet consumer demands, the wine industry is thus attempting to find new strategies to reduce or eliminate the use of SO_2. However, the chosen alternative technique should ensure that the levels of inactivation required for stabilization are achieved in each step of the winemaking process, without any detectable effect on sensorial and physicochemical properties of wine.

3. Fundamentals of pulsed electric fields technology

During processing with pulsed electric fields (PEF), products are subjected to very short pulses (μs) of high voltage (kV). The applied external voltage generates an electric field which, if intense enough, causes an electrical breakdown of the cell's cytoplasmic membrane. This phenomenon, referred to as electroporation, may cause the inactivation of vegetative cells of microorganisms, among other effects. The capability of PEF to inactivate microorganisms at temperatures that do not affect the flavor, color, or nutrient value of foods is highly attractive for the food industry.

3.1 Principles of PEF processing

PEF processing involves the intermittent application of direct-current voltage pulses (kV) for very short periods through a material placed between two electrodes. A typical PEF setup for food processing therefore includes a charging unit, an energy storage unit, and a switching unit that triggers pulse formation and releases the electrical pulses in the treatment chamber (**Figure 1**) [18]. According to the triggering system used for discharging the stored energy, the shape of the pulses delivered in the treatment chamber is either exponential or square. A PEF treatment chamber is composed of two electrodes held in position by insulating material, which forms an enclosure to contain the product to be treated. Parallel electrode and collinear configuration are the two proposed designs for the microbial decontamination of liquid foods by PEF [19]. Parallel electrode configuration hinders the formation of a uniform electric field in the treatment zone, whereas in a collinear treatment chamber the distribution of the electric field in the treatment zone is inhomogeneous. Nevertheless, the collinear chamber's higher load resistance, the configuration's overall lower energy requirements, and the circular section similar to the pipes used in food processing plants are nevertheless the reasons why collinear chambers are the ones currently used in industrial applications.

The effectiveness of PEF processing depends on several parameters, among which the ones most often used to describe the intensity of an applied PEF treatment are: electric field strength, processing time, total specific energy input, and

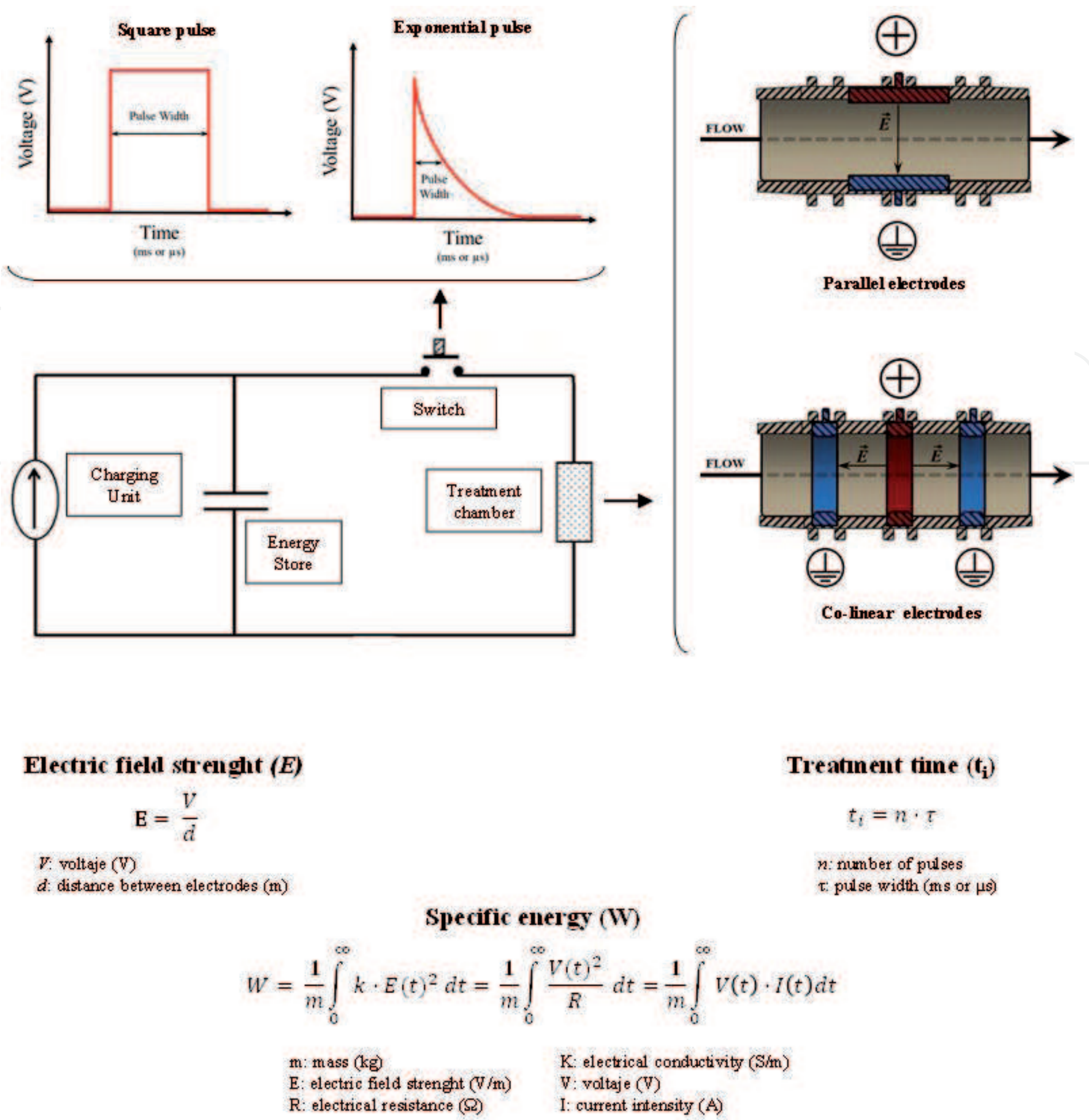

Figure 1.
Simplified diagram of an electrical circuit of a PEF generator. The different pulse shapes (exponential or square) and chamber geometries (parallel and collinear electrodes) used for the application of PEF treatments in continuous conditions are plotted. The main processing parameters of PEF technology are shown below.

temperature (**Figure 1**). Electric field strength depends on the external voltage applied, as well as on the distance between the electrodes. Treatment time represents the product's exposure time to the electric field, and depends on the number of applied pulses as well as on the pulse width. The treatment's specific energy (energy applied per mass unit) is dependent on the applied voltage, the pulse width, the number of pulses and the treatment chamber's resistance. Treatment chamber resistance varies according to its geometry and the product's conductivity. Finally, temperature is the other parameter to be considered in the evaluation of the efficiency of PEF processing in microbial inactivation. Inactivation usually increases at a higher temperature of the treatment medium – even within temperature ranges that are not otherwise lethal for microorganisms [20].

3.2 Effects of an external electric field on microorganisms

After the application of a PEF treatment, the presence of nucleic acid, proteins, and other components of the microbial cytoplasm such as adenosine triphosphate (ATP) has been observed in the medium surrounding the microorganisms. These observations suggest that PEF causes the formation of local defects or pores (electroporation), thereby leading to an increment of cell membrane permeability. Depending on the intensity of the treatment applied (electric field strength, processing time, specific energy) and cell characteristics (size, shape, orientation within the electric field), the electroporation of the cytoplasmic membrane can be either reversible or irreversible. It is reversible if the bilayer returns spontaneously to its initial state by recovering membrane integrity. If structural changes in the lipid bilayer due to PEF treatment are permanent, electroporation is irreversible. Permanent electroporation causes uncontrolled molecular transport across the membrane, hinders the cells' homeostatic capacity, and eventually leads to microbial death.

The electroporation of the cytoplasmic membrane caused by PEF indicates that this technology could be an effective procedure the inactivation of vegetative bacteria cells. But bacterial spores, which are a resting stage of some bacteria such as *Bacillus* and *Clostridium*, are resistant to these treatments. The low water content and unique cellular structure of bacterial spores, consisting of several layers surrounding the core, seem to provide resistance to the effect of the external high-intensity electric field generated during PEF processing.

4. Application of PEF for microbial decontamination in wineries

PEF treatments have been shown to cause microbial inactivation of vegetative cells of bacteria, yeast, and molds. Bacterial spores are resistant to PEF; nevertheless, since spores are not able to proliferate under acidic conditions, PEF represents a worthwhile alternative for the stabilization of acidic food such as must and wine. To implement PEF technology as a preservation method in wineries, it would be essential to determine the target microorganisms in every step of its application, and to conduct studies to prove that it ensures the level of microbial decontamination required to avoid spoilage. Finally, optimized PEF conditions should be applicable at an industrial scale without any negative effect on the appreciated quality properties of wine.

Several studies have demonstrated the potential of PEF for the inactivation of bacteria and yeast in must and wine. **Figure 2** shows the different winemaking steps in which the effectiveness of PEF for microbial decontamination and/or control of the microbial population in must or wine has been investigated. The main results obtained in those studies are described below.

4.1 Application of PEF for decontamination of must

PEF has proven highly effective in the inactivation of diverse microorganisms present in several kinds of fruit juice, including grape juice [21–23]. Reduction rates ranging from 2.0 to 4.0 log cycles were obtained by PEF (35 kV/cm, 1 ms) in must contaminated by a mixture of spoilage yeast and bacteria, such as *Saccharomyces cerevisiae, Kloeckera apiculata*, *Lactobacillus plantarum*, *Lactobacillus hilgardii*, and *Gluconobacter oxydans* [24]. In that study, the lethality of PEF was higher for yeast than for bacteria. Wu et al. achieved 4.0 log cycles of reduction in the natural spoilage flora of grape juice by applying a more intense PEF treatment (80 kV/cm, 40 μs) at 50°C that did not affect the juice's vitamin C content [25]. Further inactivation rates (up to 5.0 log cycles) were obtained when PEF was combined with certain antimicrobials such as lysozyme and nisin. Puértolas et al. established an optimum treatment of 186 kJ/kg at 29 kV/cm, reducing 99.9% of the spoilage flora of artificially contaminated must [26]. Moreover, PEF treatments have been shown

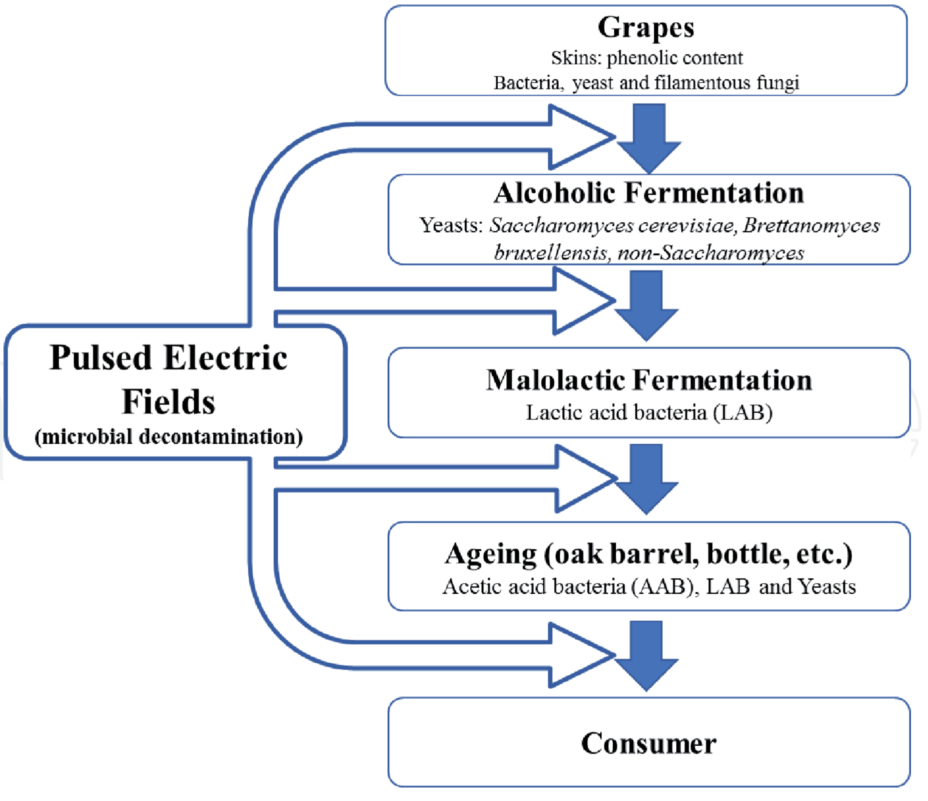

Figure 2.
Steps of winemaking in which pulsed electric fields have potential application for microbial control and decontamination.

to cause no significant changes in the physicochemical and nutritional properties of must, even when they are combined with mild temperatures (<50°C) [27, 28].

Studies in near-actual winemaking conditions have been conducted to evaluate the potential of PEF for replacing SO_2 prior to alcoholic fermentation, with the objective of stabilizing the must and thus facilitating the growth of the culture starters. PEF treatments in must at 35 kV/cm for 1 ms was shown to be effective for controlling the microbial population before the inoculation of the yeast strains selected for alcoholic fermentation. The wines obtained after the alcoholic fermentation of PEF-treated must do not show any change in terms of their volatile profile, nor any modification of their characteristics after subsequent aging in bottles in comparison to wines added with SO_2 [29, 30]. Alternatively, the use of non-*Saccharomyces* strains for alcoholic fermentation and for the improvement of the sensorial profile of neutral varieties is becoming a new trend in winemaking. Certain studies have confirmed that non-*Saccharomyces* yeasts implant themselves better in PEF-treated must [31, 32]. Consequently, higher levels of several specific metabolites of interest produced by non-*Saccharomyces* yeasts have been detected in wines obtained from PEF-treated musts.

Therefore, must stabilization by PEF is proving to be a good alternative for the reduction or elimination of the SO_2 dose, thereby facilitating the implementation of selected *Saccharomyces* and non-*Saccharomyces* yeast starters for purposes of alcoholic fermentation.

4.2 Application of PEF for wine decontamination after alcoholic fermentation

Although *S. cerevisiae* strains are predominant in wine after alcoholic fermentation (AF), certain other non-*Saccharomyces* yeasts may persist due to their ethanol tolerance. Not only yeasts, but also LAB and AAB from grapes and even other microbes present in winery facilities or in the environment can contaminate the wine. Some wines are subjected to malolactic fermentation (MLF) after AF. Generally, starter cultures of LAB are added to the freshly fermented wine to ensure good implantation and prevent the proliferation of undesirable bacteria. The usual addition of SO_2 prior to MLF can limit or hamper the implantation of the selected starters. PEF has thus been studied as a viable decontamination technique capable of reducing the competitive pressure exerted on MLF culture starters in freshly fermented wine.

González-Arenzana et al. tested the efficacy of PEF treatments in Tempranillo red wine at 17, 21 and 23 kV/cm (from 60 to 95 kJ/kg) in the inactivation of 25 different species of wine-associated microbiota [33]. Inactivation levels ranged from 1.70 to 3.04 log units for yeasts, from 1.01 to 4.16 for LAB, and from 0.64 to 4.94 for AAB. Similarly, Abca & Evrendilek investigated the effectivity of PEF treatments against a series of microbial strains suspended in red wine [34]. A PEF treatment at 31 kV/cm caused a reduction of more than 5.0 log cycles in the yeast population of *Saccharomyces cerevisiae*, *Hansenula anomala (Pichia anomala)*, and *Candida lipolytica*. Levels of inactivation of *Escherichia coli* and *Lactobacillus bulgaricus* with the same PEF treatment were 3.6 and 4.0 log cycles, respectively.

The application of PEF as an alternative to the addition of SO_2 in sweet wines to prevent re-fermentation was investigated by Delsart et al. [35]. A PEF treatment (20 kV/cm, 320 kJ/kg) inactivated 3.0 and 4.0 log cycles for *Saccharomyces* and *non-Saccharomyces* strains, respectively. Although the addition of SO_2 (250 mg/L) or the application of high-voltage electrical discharges (HVEDs) had a slightly greater lethal effect, PEF treatments caused less browning in the treated wines.

Attending to new consumer trends toward overall reduction of alcohol intake, wineries are producing low-alcohol wines [36]. Lower alcohol concentration might nevertheless lead to a higher risk of proliferation of spoiling or undesirable microorganisms in wine. PEF treatments (40 kV/cm, 250 μ) achieved inactivation levels up to 1.5 and 2.0 log cycles of LAB and yeasts in wines which had only 8.5% alcohol content [37].

Furthermore, a PEF treatment of 158 kJ/kg (33 kV/cm) has been validated as an improvement of the implementation of MLF starters in the production of four Tempranillo Rioja wines. The PEF-treated wines that were subjected to MLF preserved all their sensorial properties, as determined by sensory analysis through an expert panel [38].

Brettanomyces spp. is regarded as one of the most damaging and undesirable microorganisms in the wine industry due to its the high negative impact on the sensory properties of wines, even at very low concentrations. The capacity of PEF for the reduction of the population of this microorganism has been investigated by different authors. It has been observed that the lethal effect of PEF depends on the processing conditions, but differences in terms of PEF resistance among different strains have likewise been ascertained. Similar inactivation was achieved through a series of different combinations of electric field intensity and total specific energy in treatments applied under batch conditions. Inactivation of up to 4.0 log cycles was reported by applying 31 kV/cm and 150 kJ/kg [26] or 20 kV/cm and 320 kJ/kg [35]. Inactivation in the range of 2.5 to 3.0 log cycles was reported when the treatments were applied in continuous flow [33, 39].

4.3 Application of PEF for wine decontamination after malolactic fermentation

PEF inactivation of LAB strains involved in the MLF of wine has been studied by different authors. Among the microorganisms investigated, Puértolas et al. found that *Lactobacillus plantarum* and *hilgardii* displayed the highest resistance to PEF [26]. Similarly, out of a total of 25 different wine-related microorganisms, *Oenococcus oeni* O46 and *Pediococcus pentosaceus* were found to be the ones most resistant to a PEF treatment (23 kV/cm, 95 kJ/kg, 49°C) [33]. PEF treatments of 20 kV/cm and 320 kJ/kg were capable of inactivating up to 5.0 log cycles of *O. oeni* with a temperature remaining below 15°C [40].

Few studies have been conducted on the inactivation of microorganisms after malolactic fermentation. González-Arenzana et al. observed that after the MLF of three wines, the application of a PEF treatment (95 kJ/kg, 23 kV/cm) in combination with a low SO_2 concentration (15 mg/L) had similar or even greater effectivity than an increased dose of SO_2 (30 mg/L) in the microbial stabilization of wine [41]. PEF treatments alone, or combined with SO_2, allowed for a significant reduction in the overall population of the main microbial strains of yeasts, LAB, and ABB. Moreover, stabilization by PEF treatments was effective in inhibiting microbial growth after six months of storage, with no changes in physicochemical and sensory properties in comparison to wines stabilized by SO_2.

4.4 Application of PEF for wine decontamination before aging in barrels

Aging in oak barrels is one of the key steps in the production of high-quality wine, due to its gradual development in terms of aroma, color, and stability [42]. Oak wood is a porous material that is necessary for air exchange and for the maintenance of low oxidation conditions in wine during the aging process, but oak wood barrels are extremely difficult to clean and sanitize. They therefore present an ideal niche for microbial proliferation, and can be a source of contamination for subsequent batches of wine [43]. This is a great concern in wineries – especially in the case of *Brettanomyces* colonization, due to that yeast's negative impact on wine quality, along with the difficulty of early identification and the considerable economic losses associated with its proliferation. Many other microbial strains can colonize the oak barrels and become a source of contamination and wine spoilage. Any strategy for the microbial decontamination of aged wine in barrels should nevertheless preserve all the quality parameters acquired during this long and expensive process.

Aged preservative-free wine in oak barrels was successfully treated by PEF, with a high-level reduction in the population of the main naturally present strains [44]. However, the recovery of some of the main microorganisms involved in aging was observed in control and PEF-treated wines after 5–9 months of storage. Therefore, different PEF parameters should be tested in order to optimize PEF conditions in this scantly investigated step of winemaking. Further studies regarding the effect of PEF treatments on valuable aging characteristics prior to bottling should be carried out, as well as on the evolution of the microbial population during these long storage periods.

5. PEF treatment effects on the physicochemical and sensory properties of wine

One of the main concerns regarding the use of preservation techniques in the wine industry lies in their potentially negative effects on the quality characteristics of wines. As a non-thermal technology, Pulsed Electric Fields presents the advantage of having great effectivity in terms of microbial decontamination with minimum alteration of the physicochemical and nutritional properties of foods [45]. A series of studies have reported that PEF has no significant effects on the main physicochemical and sensorial quality parameters of must and wine, immediately after treatment or after a period of storage [24]. What is more, some of these studies have reported better sensory attributes for PEF-decontaminated wines in comparison with untreated wines or wines treated with SO_2.

After six months of storage, the physicochemical composition of three PEF-treated wines showed no differences in pH, total acidity, anthocyanin content, or total polyphenol index, but they displayed better quality in terms of volatile acidity and color intensity [41]. Moreover, sensorial analysis indicated that the organoleptic properties of the wines treated with PEF combined with SO_2 (15 mg/L) had the highest scoring values in comparison with wines treated only with PEF or treated only with SO_2 (30 mg/L). In white wines, intense PEF treatments of 20 kV/cm and 6 ms had an effect similar to the addition of sulfur dioxide (250 mg/L), but with a notable decrease of the browning effect [35].

Moreover, the application of PEF treatments combined with mild temperatures has been proven to significantly increase microbial inactivation levels [22, 23]. In this context, Abca & Evrendilek studied changes in the attributes of wine treated by PEF combined with different temperatures for purposes of microbial inactivation [34]. For all the strains studied (*E. coli, L. bulgaricus, C. lipolytica, S. cerevisiae*, *and H. anomala*), an increment of the treatment temperature from 10 to 30°C improved the lethal effect by at least 1.5 log cycles. Even the most intense treatment (31 kV/cm, 30°C) did not show any significant changes in pH level, °Brix, titratable acidity, color, anthocyanin, antioxidant capacity, total polyphenolic content, and sensorial properties.

Until now, no study has shown any significant negative effects on the sensory properties of wine treated by PEF. Further research should nevertheless be carried out with optimized PEF-parameters for microbial stabilization in the different steps of winemaking, and featuring different grape/wine varieties.

Among potentially negative effects of PEF, another important concern is the possible migration of ion metals from the electrodes to the food matrix. Although certain authors have reported the release of ion metals, this phenomenon seems to be thoroughly dependent on electrode material and geometry, as well as on processing parameters (conductivity, electric field strength, total specific energy, pulse width) [46, 47]. In wine, the increase of certain metal ions (e.g. arsenic, calcium, mercury, iron, copper, magnesium, and selenium, among others) can cause turbidity and a metallic taste; it can even represent a health risk for consumers. In red wine, Abca & Evrendilek did not observe significant differences in the concentration of 13 different metal ions between PEF and control wines, even at highest-intensity PEF conditions (31 kV/cm, 30°C) [34]. Similarly, no differences in iron and chromium concentration were detected in Cabernet Sauvignon red wine subjected to 34 and 53 kV/cm (50us) treatments [39]. Although those treatments slightly increased the concentration of nickel in the PEF-treated wines the levels reached were below the maximum limits permitted in food products.

6. Conclusions and future perspectives

Microorganisms in winemaking are as necessary as they are undesirable, depending on the strain and/or the time it proliferates. The growth of spoilage microorganisms in must and wine not only exerts a considerable influence on consumer acceptance, but can also lead to uncountable economic losses. Currently, the spoilage of wine by microorganisms is mainly controlled by applying SO_2. However, due to the current global concern about the negative effects of SO_2 on human health, the wine industry is facing the challenge of attempting to reduce or eliminate its use. Proposed chemical or physical alternatives are insufficient or/and non-feasible for implementation as microbial stabilization procedures in the wine industry.

Pulsed Electric Fields emerge as a thoroughly suitable alternative technique for the stabilization of must and wine, or as a technique combined with low doses of SO_2 to ensure antioxidant protection. PEF efficacy has been studied against the main wine-related spoilage microorganisms along the different winemaking stages, but mostly under lab-scale or pilot plant conditions. Best results have been obtained when PEF was combined with mild treatment temperatures and/or with low concentrations of SO_2, or with other preservatives. Furthermore, several studies have reported to have found no negative effects or changes in the sensory quality of wines treated with PEF.

PEF technology is currently being applied in a number of industrial food processing applications. Thus, the development and optimization of PEF devices and chambers is accelerating in order to adapt them to current demands while facilitating the industrial implementation of such new techniques to food. The devices' flexibility has been highly improved, along with different types of treatment chambers, depending on the type of matrix and on treatment conditions.

The International Organization of Vine and Wine (OIV) recently approved the application of PEF to grapes in order to enhance and reduce maceration time in winemaking [48]. The technology is currently being evaluated as a microbial stabilization and decontamination process. The OIV resolutions thus suggest that PEF is a gentle technology without negative consequences for must or wine, but offering interesting improvements in terms of their quality. The applicability of PEF in several other winemaking steps such as maceration or aging-on-lees, along with the current feasibility of scale-up potential, makes this procedure thoroughly attractive for future implementation as a highly versatile technology in the wine industry. Furthermore, the energetic requirements for must/wine PEF-optimized pasteurization can ranged from 20 to 200 kJ/kg. Thus, the power consumptions imply very low costs in comparison with the traditional techniques and the innovative ones suggested.

Generally, however, the ranges of PEF parameters studied for purposes of microbial decontamination (at laboratory scale), are still very intense in comparison with the ones used in grape electroporation (high-intensity voltages or long treatment times). Such intense conditions have certain drawbacks for implementation in wineries due to the power limitation of current PEF devices. This implies that PEF should be applied at very low flow rates, which are not feasible in winemaking on an industrial scale. The current challenge lies therefore in studying low and mild PEF conditions not investigated so far in-depth: PEF alone, or in combination with other methods. One of the most promising combinations is the application of PEF treatments in association with mild temperatures or/and with reduced doses of SO_2. A reduced amount of studies have already proven the synergetic effect that emerges between these methods when applied in combination. Thus, in order to successfully implement PEF technology in wineries for purposes of microbial decontamination, it will be necessary to define the lowest-intensity PEF parameters which, combined with mild temperatures and reduced-SO_2, have the highest synergetic effect. This would allow for a considerable increase in the processing capacity of PEF units, thereby facilitating this technique's industrial application in the wine industry without affecting sensory properties, while attending to widespread demands for the reduction of SO_2.

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