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Defining winery processing conditions for the decontamination of must and wine spoilage microbiota by Pulsed Electric Fields (PEF)

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ABSTRACT

This study investigated the PEF-resistance of *Saccharomyces bayanus*, *Brettanomyces bruxellensis, Lactobacillus plantarum*, and *Oenococus oeni* in must or wine under continuous PEF processing. Results showed the capacity of PEF to achieve 3.0-$\log_{10}$-cycles (CFU/mL) of inactivation of all the microorganisms under moderate conditions (< 155 kJ/kg). Developed tertiary models accurately predicted the effect of PEF parameters on microbial inactivation, and Monte Carlo simulation considered the variability of factors and the maximum assumable microbial load in the final treated product. Results showed that PEF-treatments at 15 kV/cm and 129 or 153 kJ/kg would ensure the adequate decontamination (< 10 CFU/mL) of spoilage microorganism in must or wine, respectively.

Industrial relevance: PEF technology has been shown to achieve adequate levels of microbial inactivation (3-$\log_{10}$) in must and wine under industrial applicable processing parameters, making it a suitable alternative to SO_2 or sterilizing filtration for microbial control in winemaking. Reductions of 3-$\log_{10}$ CFU/mL of must and wine microbiota were found by continuous flow PEF-processing at 15 to 25 kV/cm and 175 to 148 kJ/kg, parameters applicable at industrial scale at 1 ton/h.

1. Introduction

Winemaking is a traditional process with thousands of years of history in which the apparently simple concept of transforming grape must into wine has undergone significant developments. Today, the procedure of obtaining a stable, defect-free wine can be facilitated by using coadjutants, enzymes, additives, or disruptive methodologies with technological benefits (Cosme, Filipe-Ribeiro, & Nunes, 2021; Pérez-Coello & Díaz-Maroto, 2009). Microorganisms play an essential role in the steps of the fermentation process, and the use of commercial starter cultures has improved yields and outcomes. However, the primary control strategy used against microbial spoilage in wineries still consists in applying one of the oldest preservatives: sulfur dioxide (SO_2). This can be explained by the fact that SO_2 not only has antiseptic but also antioxidant activity, which makes it a virtually irreplaceable preservative (Giacosa et al., 2019). In the last decades, however, concern has grown regarding the extensive use of SO_2 in wineries due to its toxic and allergenic nature, which can affect consumer health. It is nevertheless challenging to find a good alternative to replace SO_2 as an antimicrobial, considering its broad spectrum of activity. The main microbial groups of concern in wine spoilage are yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) by virtue of their high level of tolerance to ethanol and low pH. The most common spoilage yeasts are *Saccharomyces*, due to their capacity to re-ferment wines, the highly undesirable *Brettanomyces bruxellensis*, which produces a typical "horse sweat" taint, and other non-saccharomyces genera (*Zygosaccharomyces*, *Kloeckera/Hanseniaspora*, *Pichia*, and *Candida)*, which can generate turbidity, cause film layers, and/or produce undesired metabolites. The growth of LAB species, including *Lactobacillus*, *Pediococcus*, and certain *Oenococcus* species, is associated with undesirable aroma and flavor compounds that generate bitterness; moreover, all AAB species are regarded as spoilage bacteria capable of producing a vinegary aroma (Bartowsky, 2009). Consequently, in order to substitute SO_2 in wineries, any potential alternative method needs to be effective in controlling all the microbial groups enumerated above. Although several new chemicals (dimethyl dicarbonate, lysozyme, and sorbic acid) as well as a series of physical methods (high hydrostatic pressure, ultrasound, ultra-high pressure homogenization) have been proposed, their effectivity and real applicability on an industrial scale has been called into question (Lisanti, Blaiotta, Nioi, & Moio, 2019). Conversely, Pulsed Electric Fields (PEF)

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have the capacity to inactivate vegetative cells in continuous flow, and the procedure is easy to install in a wine production line. Moreover, flexible pulsed power devices that cover industrial processing volumes have recently been developed. For all these reasons, PEF has become a promising technology for microbial control in the wine industry.

In PEF processing, the delivery of microsecond pulses at high voltages generates an electric field strength (kV/cm) in the chamber, which is made up of two electrodes. When microbial cells are subjected to an external electric field of sufficient intensity, their cytoplasmic membrane becomes more permeable, causing a homeostasis imbalance that can lead to cell death. Furthermore, the lethality of PEF, combined with even moderate temperatures (< 60 °C), maintains the nutritional, organoleptic, and fresh-like characteristics of heat-sensitive food products (Timmermans, Nierop Groot, & Matser, 2022). Moreover, the application of PEF at these elevated temperatures has been demonstrated to enhance the electroporation rates and thus rising the lethality of the treatments by a synergetic effect (Saldaña et al., 2010; Timmermans et al., 2019). The application of PEF technology has been especially studied in the area of liquid foods: results are particularly encouraging when it is applied as a cold pasteurization method for acidic products such as fruit juices (Bevilacqua et al., 2018; Katiyo, Yang, & Zhao, 2017; Marsellés-Fontanet, Puig, Olmos, Mínguez-Sanz, & Martín-Belloso, 2009; Min, Jin, Min, Yeom, & Zhang, 2003; Raso & Álvarez, 2016). The use of PEF has also resulted in effective microbial inactivation of the typical spoilage microorganisms in wine under laboratory growth conditions (González-Arenzana et al., 2015; Puértolas, López, Condón, Raso, & Álvarez, 2009). However, most of those studies were conducted with non-scalable PEF parameters including batch conditions and intense electric field strengths (> 30 kV/cm), which would be difficult to apply with current commercial PEF devices at the processing capacity required in the industry (González-Arenzana et al., 2018; van Wyk, Farid, & Silva, 2018; van Wyk, Silva, & Farid, 2019). On the other hand, most studies have not considered the potential effect on microbial inactivation of temperature increase during PEF application in continuous flow configuration. Consequently, there is still a lack of fundamental, systematic research in this area; further investigations are needed in order to obtain a sufficient body of data to define PEF protocols that meet the microbial standards of wineries. Such an approach would require the development of predictive models and the identification of the limiting microorganisms, thereby allowing for the implementation of risk-based management strategies. Predictive microbiology has proven to be an essential tool capable of linking scientific development with practical application in the food industry (Membré & Lambert, 2008). Therefore, to determine whether PEF could serve as an effective preservation technology for microbial control in wineries, microbial resistance characterization needs to be assessed under industrially feasible PEF conditions. In this study, it was evaluated the inactivation of several spoilage microorganisms of concern (*Saccharomyces*, *B. bruxellensis*, *L. plantarum*, and *O. oeni*) in terms of the main PEF processing parameters in conjunction with controlled wine-intrinsic parameters (pH, ethanol content, sulfite concentration) that could be used in future predictive models. Although (Puértolas et al., 2009) already applied a similar approach, our study's significant contribution lies in the procurement of inactivation data under industrially scalable PEF parameters and continuous flow conditions. Furthermore, the developed models were validated on wines provided by a winery at two different steps of the winemaking process.

2. Materials and methods

2.1. Microorganisms and cultivation conditions

Table 1 details the conditions of culture media and treatment media used in this study for each microorganism. The strains used in this study, *Saccharomyces cerevisiae* var. *bayanus* (CECT 1969), *Brettanomyces bruxellensis* (CECT 11045), and *Lactobacillus plantarum* (CECT 220), were supplied by the Spanish Type Culture Collection (CECT); the commercial culture of *Oenococcus oeni* was purchased from Enoferm BETA (Lallemand, St. Simon, France). Strains were revitalized from freeze-dried samples and maintained on slants of the corresponding media (Table 1). A broth subculture was prepared from each slant by inoculating a single colony in a test tube of 10 mL of broth, followed by incubation as indicated. From subcultures, flasks with 50 mL of the corresponding culture media were inoculated to a final concentration of 10^4 for yeast and 10^5 CFU/mL for bacteria. To achieve alcoholic fermentation, flasks containing 3 L of a commercial red grape must (GREIP, PepsiCo, Vitoria, Spain) were inoculated with *S. bayanus*. Before inoculation the must was added with 70 g/L of sucrose (Oxoid) to achieve a final concentration of around 220 g/L, corresponding to a potential ethanol content of 12.9% (*v*/v). Microbial cells reached the stationary growth phase after the incubation time indicated in Table 1. Prior to PEF treatments, microbial cultures were centrifuged at 10,000*g* for 4 min and resuspended in a commercial red grape must (GREIP, PepsiCo, Vitoria, Spain) or in the wine after removing by centrifugation (10.000 $g \times 10$ min) the cells of *S. bayanus* involved in the fermentation. In the case of *S. bayanus*, the wine was directly treated with the suspended yeast cells at the end of the fermentation period. The use of a wine obtained under laboratory conditions ensured a lower degree of variability in terms of wine properties and allowed us to avert the potential influence of sulfites, which are abundantly present in commercial wines. The pH and electrical conductivity of the must were 3.2 and 1.4 mS/cm, and 3.1 and 1.3 mS/cm for the wine.

2.2. PEF treatments

Monopolar square waveform pulses were applied with a commercial PEF generator (Vitave, Prague, Czech Republic) capable of applying a maximum voltage of 20 kV and current up to 500 A. PEF treatments were applied to must and wine in a continuous flow system fixed at 5 L/h by means of a peristaltic pump (BVP, Ismatec, Wertheim, Germany) sent

Table 1
Culture media, temperatures, growth times, and treatment media of the strains used, at each stage of the investigation.

Microorganism	Slant	Preculture	Culture	Treatment medium	Initial microbial concentration $log_{10}N_0$ ±SD (CFU/mL)
S. bayanus (CECT 1969)	PDA	SD broth (25 °C, 24 h)	SD broth (25 °C, 48 h) Must + Sucrose (20 °C, 9 days)	Must	6.37 ± 0.10
				Wine	7.65 ± 0.04
B. bruxellensis (CECT 11045)	Brett. Agar	SD broth (25 °C, 24 h)	SD broth (25 °C, 5 days)	Must	6.26 ± 0.16
				Wine	6.45 ± 0.16
L. plantarum (CECT 220)	MRSA	MRS broth (37 °C, 24 h)	MRS broth (37 °C, 24 h)	Must	7.24 ± 0.14
				Wine	7.24 ± 0.28
O. oeni (Enoferm BETA)	MRSA	MRS broth (30 °C, 24 h)	MRS broth (30 °C, 24 h, anaerobic conditions)	Wine	7.85 ± 0.02

SB broth: Sabouraud Dextrose broth. PDA: Potato Dextrose Agar. MRS broth: Mann Rogosa Sharpe broth. MRS agar: Mann Rogosa Sharpe agar. (Oxoid, Basingtok, UK) Brett. Agar: Brettanomyces Agar (Scharlab, Sentmenat, Barcelona).

through a parallel titanium chamber of 0.4 cm gap (3 cm length, 0.5 cm width), generating a residence time of 0.43 s. Pulse waveform and treatment chamber configuration is shown in Fig. 1. Samples were pre-tempered at 20 °C by a coil heat exchanger before entering the PEF chamber. Upon leaving the chamber, samples were cooled down by a further cooling exchanger that reduced the product temperature below 15 °C in less than 5 s after the PEF treatment. Actual voltage during treatments was measured by a high-voltage probe (Tektronik, P6015A, Wilsonville, Oregon, USA) connected to an oscilloscope (Tektronik, TDS 220). Inlet and outlet temperatures were measured by a type-K thermocouple (Ahlborn, Holzkirchen, Germany) located in the circuit before (inlet temperature) and after (outlet temperature) the treatment chamber. Total specific energy (W) of treatments was estimated by calculating the temperature increase during pulses delivering presumable adiabatic conditions (Heinz, Alvarez, Angersbach, & Knorr, 2001), using the following equation:

$$W = (T_{outlet} - T_{inlet})^{*}Cp \quad (1)$$

where T_{outlet} is the temperature of the sample immediately after the PEF treatment, T_{inlet} is the temperature of the sample just before entering the treatment chamber (20 °C), and Cp is the specific heat capacity of must or wine according to the average values from Genc, Genc, and Goksungur (2017). Outlet temperatures were constant during experimental times of PEF processing and the thermal conduction of the electrodes was negligible.

To obtain a substantial amount of microbial inactivation data under industrially scalable conditions, different PEF parameters of electric field strength (10, 15, 20, and 25 kV/cm) and total specific energies (30 to 175 kJ/kg) were tested at a constant pulse width of 5 μs by modifying the pulse rate from 20 to 170 Hz. After treatments, *S. bayanus* and *B. bruxellensis* were recovered, respectively, by plating onto PDA and incubating 48 h at 25 °C, and onto Brettanomyces agar incubating for five days at 25 °C. *O. oeni* and L. *plantarum* were recovered onto MRS agar and incubated for 24 h at 30 °C and 37 °C, respectively, under anaerobic conditions (< 1% O_2) (MACS VA500 Microaerophilic Workstation, DW Scientific, UK). After the incubation times, the number of colonies counted corresponded with the number of viable microorganisms expressed as colony-forming units per milliliter (CFU/mL). The survival fraction was calculated by dividing the number of microorganisms that survived the treatment (N_t) by the initial number of viable cells (N_0) in the treatment media before PEF. Survival curves ($\log_{10}$ of the survival fraction vs. total specific energy) for each electric field strength and investigated medium were obtained.

2.3. Curve fitting

In order to describe the survival curves obtained for the different microorganisms and treatment media, a mathematical equation (primary model) based on Weibull distribution was used (primary model):

$$Log_{10}\frac{N_t}{N_0} = -\left(\frac{W}{\delta}\right)^{\rho} \quad (2)$$

where N_t is the number of microorganisms that survived the treatment, N_0 is the initial number of the microbial population, W is the specific energy, δ is the scale parameter, and ρ the shape parameters. The δ parameter represents the specific energy (kJ/kg) required to inactivate

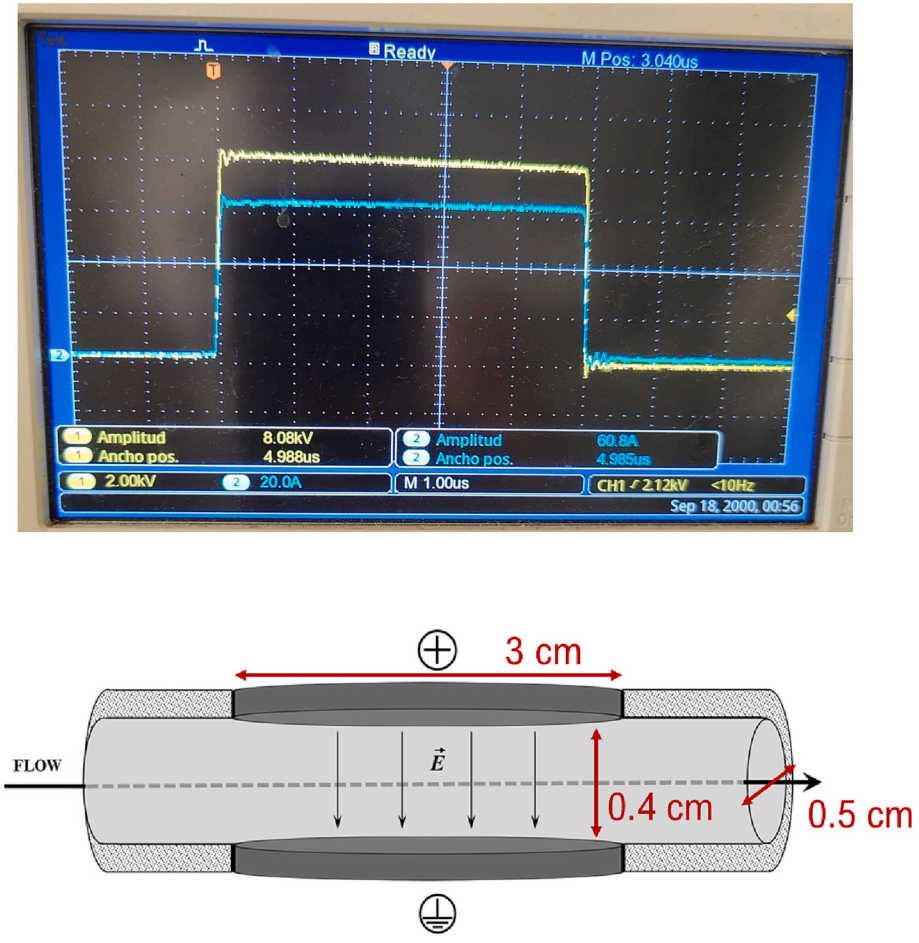


Fig. 1. Picture of the waveform of pulses in the oscilloscope (top) and scheme of the parallel electrode configuration of the treatment chamber and its dimensions (bottom).

the first $\log_{10}$ cycle of the microbial population. The ρ parameter accounts for upward concavity of a survival curve ($\rho < 1$), a linear survival curve ($\rho = 1$), and downward concavity ($\rho > 1$) (van Boekel, 2002).

The determination of the δ and ρ values was performed by fitting survival curves with the freeware model-fitting tool GInaFiT 1.7 (KU Leuven, Belgium) and the Solver function of the Excel 16.0 package (Microsoft, Seattle, WA, USA). The mathematical relationship (secondary model) between parameters δ and ρ with the electric field strength for each studied treatment medium and microorganism was defined by using GraphPad Prism (Graph- Pad Software, San Diego, California, USA) to obtain secondary models. Global equations were developed by substituting the secondary equations of δ and ρ in Eq. (2).

To establish the models' accuracy, R^2 (determination coefficient) and RMSE (root mean square error) were calculated based on Baranyi, Pin, and Ross (1999).

2.4. Experimental validation

For the validation of the models obtained under laboratory conditions, two red wines provided by a winery (San Juan Bautista cooperative, Fuendejalón, Spain) after alcoholic fermentation (AF) and malolactic fermentation (MLF) were used. *S. cerevisiae* var. *bayanus* (CHP, Levuline, OENOFRANCE, Magenta, France) and *O. oeni* (Viniferm OE AG-20, Agrovin, Ciudad Real, Spain) performed each fermentation process, respectively, in the winery facilities. At the end of each fermentation, a matrix of 15 to 20 different PEF treatments was applied to wines in the same ranges previously investigated (see Section 2.2). The pH, % of ethanol content, free SO_2, and electrical conductivity were, respectively, 3.8, 15.7%, 19 ppm, and 1.9 mS/cm for the wine that contained *S. bayanus* and 3.5, 14.3%, 2.0 mS/cm, and 10 ppm in the case of the wine containing *O. oeni* cells. Inactivation rates were assayed by plating on the corresponding media and incubating as indicated in Table 1.

2.5. Monte Carlo simulation

Response surface methodology (RSM) was employed using Design-Expert 13 software to establish a matrix of electric field strengths (ranging from 10 to 25 kV/cm) and specific energies (ranging from 15 to 155 kJ/kg) to develop the subsequent Monte Carlo simulation (MC). Rather than assigning discrete levels to the factors, a continuous range of experimental conditions was specified.

By means of the tertiary models developed for the microorganisms *S. bayanus* and L. *plantarum* in both must and wine, and along with the parameter deviation of each model, Monte Carlo simulation (MC) was conducted to assess the risk of obtaining more than 10 CFU/mL of *S. bayanus* and *L. plantarum* in must or wine after PEF processing, depending on process parameters. The initial microbial concentrations (N_0) taken into account for the MC simulation were based on experimental data obtained from previous studies performed in a winery. The function used to assess the risk was:

$$Risk(\%) = \frac{\#Runs\ with\ N_t < 10}{\#Runs} \times 100 \quad (3)$$

where N_t is the number of microorganisms that survived the treatment. Subsequently, a MC simulation was performed with the Monte Carlo Simulation App from OriginLab in the Origin Pro 2021 software (OriginLab, Northampton, Massachusetts, USA) with 9000 runs per point as previously established with Design-Expert. The percentage of Monte Carlo runs that achieved a remaining amount of microorganisms corresponding to a maximum of 10 CFU/mL were used as responses for the RSM. Finally, response surfaces were compared for both must and wine to establish treatment conditions that would achieve a risk lower than 10%. The response surface was then optimized to pinpoint the conditions that meet a criterion of a maximum 10% risk of the aforementioned microbial survival rate after PEF processing.

3. Results

Fig. 2 shows the inactivation of *S. bayanus*, *B. bruxellensis*, and L. *plantarum* obtained after PEF treatments in must (Fig. 2A) and of the same microorganisms and *O. oeni* in wine (Fig. 2B). Graphs shows the experimental data at different electric field strengths and the curves obtained after fitting data to Weibull distribution. Since the total specific energy was calculated based on the temperature increase due to the PEF treatment (Eq. (1)), the outlet temperature achieved immediately after the PEF treatments is also represented (secondary OX axis). For all the microorganisms and independently of the treatment medium, the inactivation rate increased with the total specific energy for all the electric fields. However, the inactivation kinetics were different depending on the treatment media and the type of microorganism. Yeast strains treated in must presented linear kinetics up to around 75 kJ/kg, after which the inactivation velocity slowed down, generating a concave upward shape. Meanwhile, the inactivation kinetics of L. *plantarum* in must and of all microorganisms treated in wine exhibited a downward concave shape. The shoulders observed in those survival curves were more or less pronounced depending on the microorganism. For example, for L. *plantarum* in wine, the shoulder extended up to 125 kJ/kg, whereas in the case of *B. bruxellensis*, it was around ten times lower. For all studied microorganisms and all treatment media, PEF treatments were able to attain between 2.0 and 3.0 $\log_{10}$ cycles of inactivation with total specific energies up to 136 kJ/kg and maximum temperatures of 55 °C. Nevertheless, PEF treatments of lower intensities (up to 97 kJ/kg; 45 °C) were capable of achieving the inactivation of yeasts and *O. oeni* in the same ranges (2.0 to 3.0 $\log_{10}$).

To determine the influence of PEF parameters and treatment media on the inactivation kinetics of the different microbial strains, survival curves were mathematically described by fitting the obtained survival curves to equations. Since survival curves did not follow a first-order inactivation kinetics, a mathematical model based on the Weibull distribution (Eq. (2)) was used which enabled to fit concave upward and downward curves (Mafart, Couvert, Gaillard, & Leguerinel, 2002). Table 2 shows the values of the model parameters δ (scale) and ρ (shape) determined for each microorganism, treatment medium, and electric field strength. As can be observed, the scale parameter (δ value) decreased when the electric field increased, and it was generally higher for treatments in wine than in must, as well as for both media containing L. *plantarum*. This relationship was not as evident in the case of the shape parameter (ρ value). It was <1 when survival curves presented an upward concave behavior (yeasts in must), but generally, it was >1, meaning downward concave shapes. The accuracy values of R^2 and RMSE ranged from 0.85 to 0.99 and from 0.07 to 0.56, respectively, indicating adequate goodness of fit.

Based on the obtained value of the primary model parameters, as shown in Table 2, the relationship between the calculated δ and ρ parameters and the applied electric field strength was carried out. The equations (secondary models) that describe the relationship between the model parameters and the treatment conditions (electric field strength and specific energy) for each microorganism and treatment medium are shown in Table 3. Fitting was performed according to the equations that better described each correlation among data. The associated standard deviations of each parameter, along with the RMSE and R^2 values, are also shown. Although in thermal treatments the logarithm value of δ has usually been found to be linearly dependent on the treatment temperature (van Boekel, 2002), our results show that this is not always the case in the relationship between the logarithm value of δ and, in this case, the electric field. In some of the data, the δ values followed a good linear relationship with the electric field (as was the case for *S. bayanus* and *B. bruxellensis* in wine) or were even independent of the field strength (as was the case for *O. oeni*). Similar relationships between shape parameter and electric field strength have been described in the

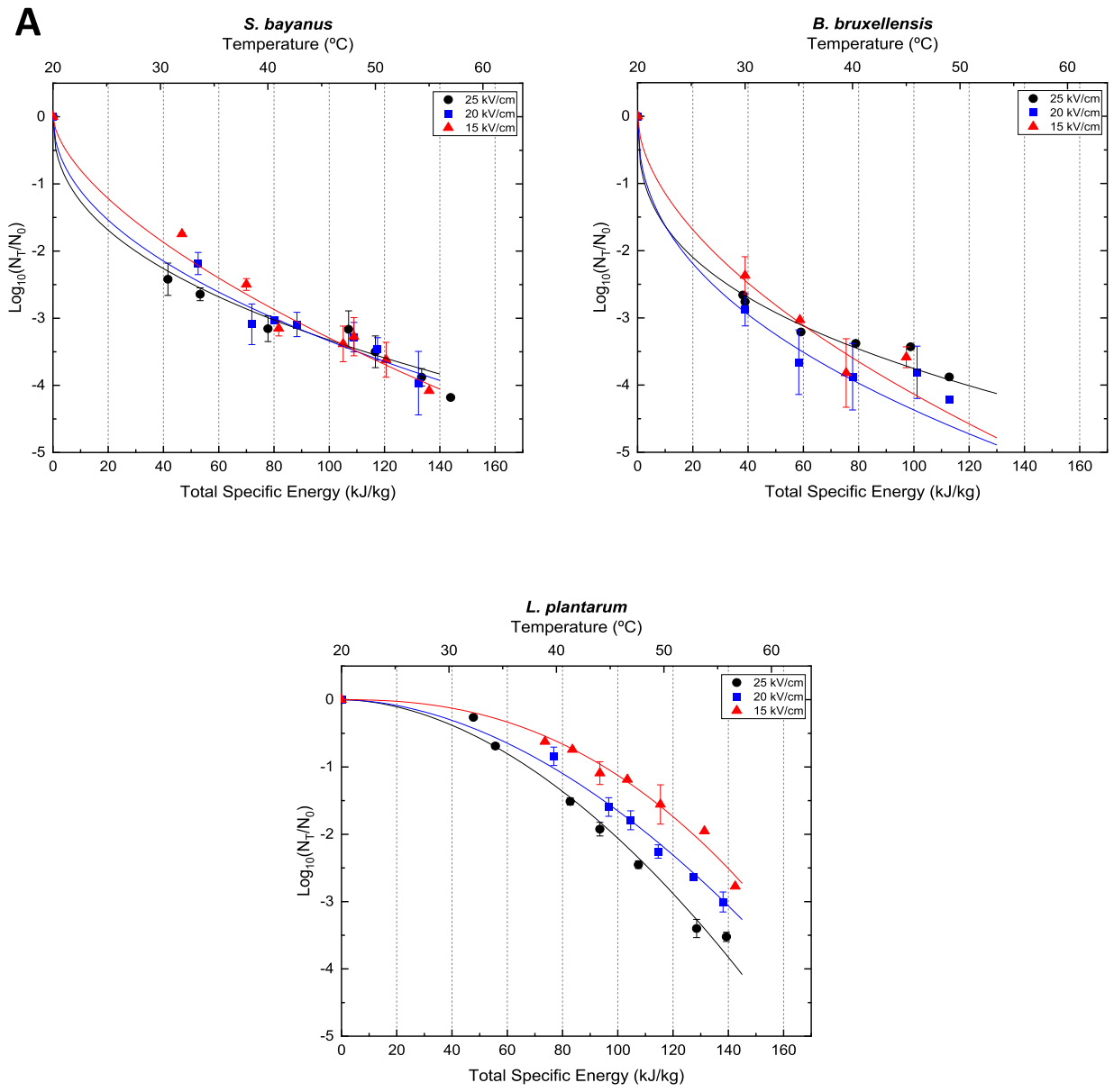


Fig. 2. Survival curves of *S. bayanus*, *B. bruxellensis, L. plantarum* and *O. oeni* at different electric field strengths: 10 kV/cm (◆),15 kV/cm (▲), 20 kV/cm (■), and 25 kV/cm (●) treated in must (A) and wine (B). Dots represent the experimental data and continue lines represents the obtained fitted curves to Weibull distribution.

literature, but mainly when static treatments were applied (Saldaña, Puértolas, Condón, Álvarez, & Raso, 2010).

Based on these equations (secondary models) (Buzrul, 2022) and including them in the Weibull model (primary model), equations (tertiary models) that permitted to estimate the rate of microbial inactivation for each microorganism and treatment medium under all types and combinations of PEF treatment conditions were obtained (tertiary models). Values predicted by the obtained equations versus experimental values corresponding to the inactivation of the different microorganisms in must and wine are shown in Fig. 3. RMSE and R^2 values reflect the good correlations between experimental values and those estimated by the global equations obtained independently of the microorganism or the treatment medium. In general, R^2 values were higher than 0.90, and RMSE lower than 0.16. Based on these results, the obtained equations allow for an accurate description of the lethality of PEF treatments against the investigated microorganisms suspended in must or wine in the range of electric field strengths and specific energies under investigation.

Based on those validated equations (tertiary models), the specific energy and electric field strength required to reach a certain level of inactivation (1.0 or 3.0 $\log_{10}$ cycles) for each microorganism studied in must and wine are shown in Fig. 4. As can be observed, the specific energy or the outlet temperature required to obtain a given inactivation in must or in wine was lower at higher electric field strengths, with the exception of *O. oeni* in wine, for which the achieved inactivation only depended on the total specific energy and was independent of the electric field strength. Generally, however, for all the investigated microorganisms, the decrease in total specific energy required to achieve a given level of inactivation occurring at higher electric field strength was very low. For example: in order to obtain 3.0 $\log_{10}$ cycles of inactivation of *S. bayanus* in wine, an increment in electric field strength from 10 to 25 kV/cm only reduced the total specific energy from 128 to 104 kJ/kg (e.g., a 19% reduction in energy requirement) or from 190 to 150 kJ/kg (21% reduction) for L. *plantarum*. On the other hand, Fig. 4 shows that L.

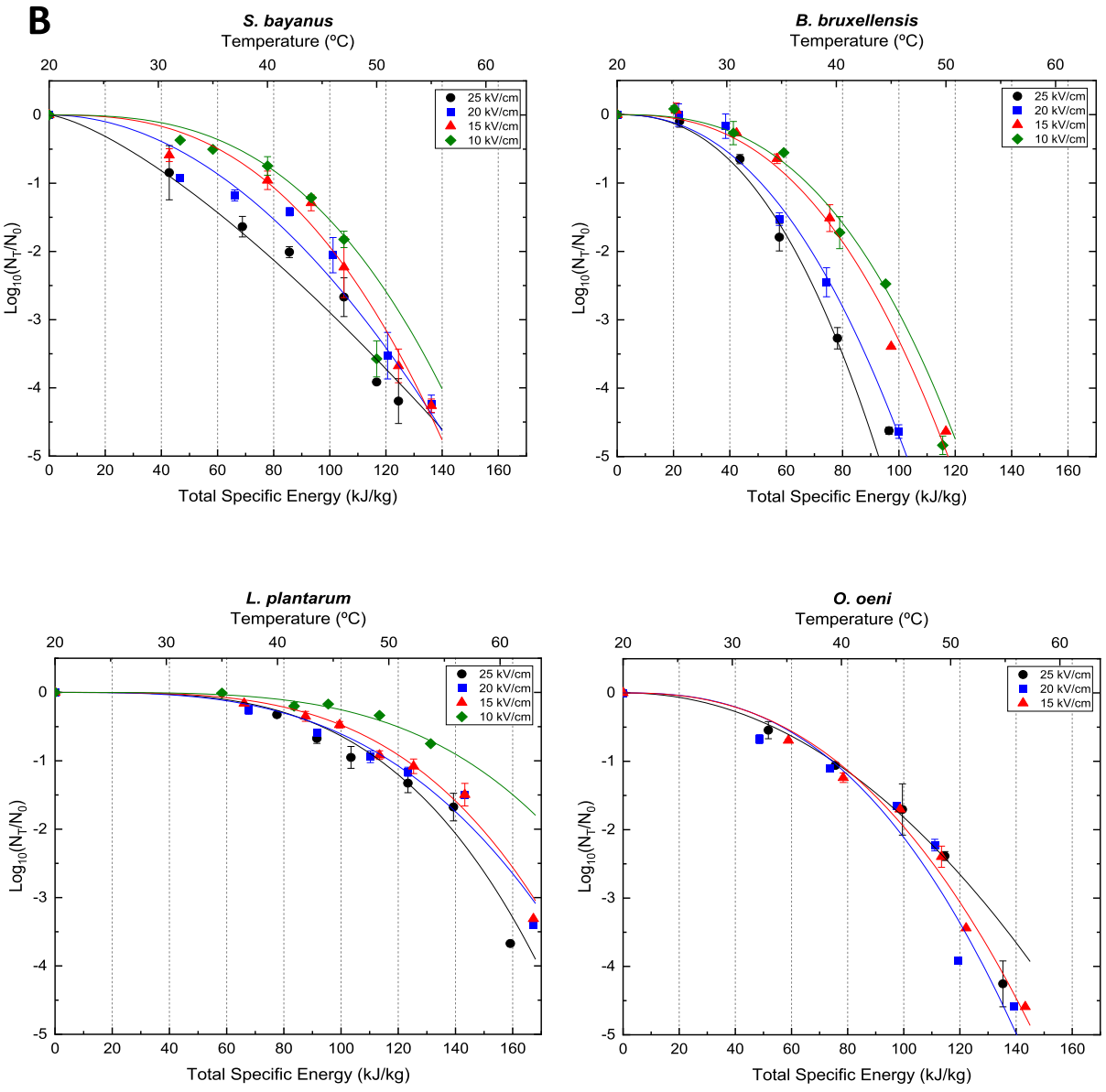


Fig. 2. (*continued*).

plantarum was the most resistant microorganism in both must and wine, followed by *O. oeni*, *S. bayanus*, and *B. bruxellensis*. Furthermore, microbial resistance to PEF was higher in wine than in must in all cases. Thus, for example, treatments of 15 kV/cm, 90 kJ/kg, and a final temperature of 42 °C would be sufficient to inactivate 3.0 $\log_{10}$ cycles of yeasts in the must; in wine, however, 125 kJ/kg (52 °C) at 15 kV/cm or 101 kJ/kg (46 °C) at 25 kV/cm would be necessary to achieve the same lethal outcome. Similarly, to achieve 3.0 $\log_{10}$-reduction of L. *plantarum*, the required total specific energy increased from 125 kJ/kg (52 °C) in must to 148 kJ/kg (58 °C) in wine at 25 kV/cm.

In order to validate the tertiary models with independent data, two red wines provided by a winery immediately after alcoholic and malolactic fermentations were subjected to a series of different PEF treatments. The inactivation results obtained from that independent data were compared with the predicted values estimated by the developed equations. Fig. 5 compares the experimental data with the data predicted by the equation developed for each microorganism. The equation obtained for the most resistant microorganism (*L. plantarum*) (Fig. 5A) underpredicts the observed rate of inactivation (RMSE = 2.76). Similarly, the equation that predicts the inactivation of *O. oeni* in wine also underpredicted the inactivation of *O. oeni* in the wine supplied by the winery immediately after malolactic fermentation (Fig. 5C). On the other hand, a perfect correspondence was observed between estimated inactivation data of *S. cerevisiae* in the wine supplied by the winery just after alcoholic fermentation and data corresponding to the inactivation predicted by the model developed for *S. bayanus* (RMSE = 0.137; R^2 = 0.914) (Fig. 5B). In any case, none of the equations overpredicted the inactivation ultimately obtained in the wines containing the microorganisms supplied by the winery after alcoholic or malolactic fermentation.

To investigate the effects of energy and electric field strength as continuous entry variables (factors) on the risk of microorganism prevalence, we carried out a response surface experimental design (see above, Section 2.5). An energy range of 15 to 155 kJ/kg was considered, while the electric field strength varied between 10 and 25 kV/cm. The experimental design and models used for the Monte Carlo simulation are summarized in Table 4. MC input parameters for the initial microbial concentration (N_0) were selected based on experimental data obtained in must and wine from wineries. Fig. 6 represents the conditions (continuous lines) of electric field strength and specific energy necessary

Table 2
δ and ρ values from the fitting of the mathematical model based on the Weibull distribution to the experimental data at different electric field strengths for each studied microorganism and treatment medium.

Microorganism	Treatment medium	Field strength (kV/cm)	δ value			ρ value			R^2	RMSE
S. bayanus	Must	15	14.53	±	4.15	0.62	±	0.08	0.96	0.29
		20	8.16	±	3.26	0.48	±	0.07	0.95	0.31
		25	5.76	±	2.51	0.42	±	0.06	0.97	0.27
	Wine	10	85.97	±	3.55	2.85	±	0.61	0.97	0.14
		15	78.19	±	5.26	2.68	±	0.35	0.97	0.29
		20	64.57	±	6.40	1.98	±	0.31	0.93	0.38
		25	46.29	±	5.60	1.38	±	0.17	0.95	0.30
B. bruxellensis	Must	15	7.82	±	12.72	0.56	±	0.23	0.85	0.71
		20	3.20	±	2.80	0.43	±	0.10	0.92	0.52
		25	2.57	±	7.29	0.36	±	0.13	0.95	0.37
	Wine	10	67.41	±	3.87	2.70	±	0.36	0.97	0.19
		15	62.87	±	3.33	2.57	±	0.20	0.99	0.21
		20	51.03	±	3.81	2.30	±	0.22	0.98	0.25
		25	47.34	±	3.32	2.39	±	0.28	0.98	0.20
L. plantarum	Must	15	95.18	±	7.20	2.39	±	0.41	0.95	0.22
		20	76.08	±	5.72	1.84	±	0.19	0.97	0.19
		25	67.60	±	8.37	1.84	±	0.19	0.95	0.30
	Wine	10	143.82	±	5.12	3.77	±	1.02	0.93	0.07
		15	123.04	±	3.63	3.58	±	0.44	0.95	0.18
		20	117.26	±	4.14	3.14	±	0.39	0.95	0.19
		25	113.77	±	4.33	3.49	±	0.58	0.94	0.24
O. oeni	Wine	15	75.98	±	4.03	2.45	±	0.27	0.99	0.15
		20	74.53	±	7.40	2.55	±	0.88	0.94	0.34
		25	74.88	±	8.43	2.07	±	0.47	0.95	0.37

Table 3
Equations used to describe the relationship between the δ and ρ values and the electric field strength in must and wine for each investigated microorganism along with their associated deviation and accuracy parameters.

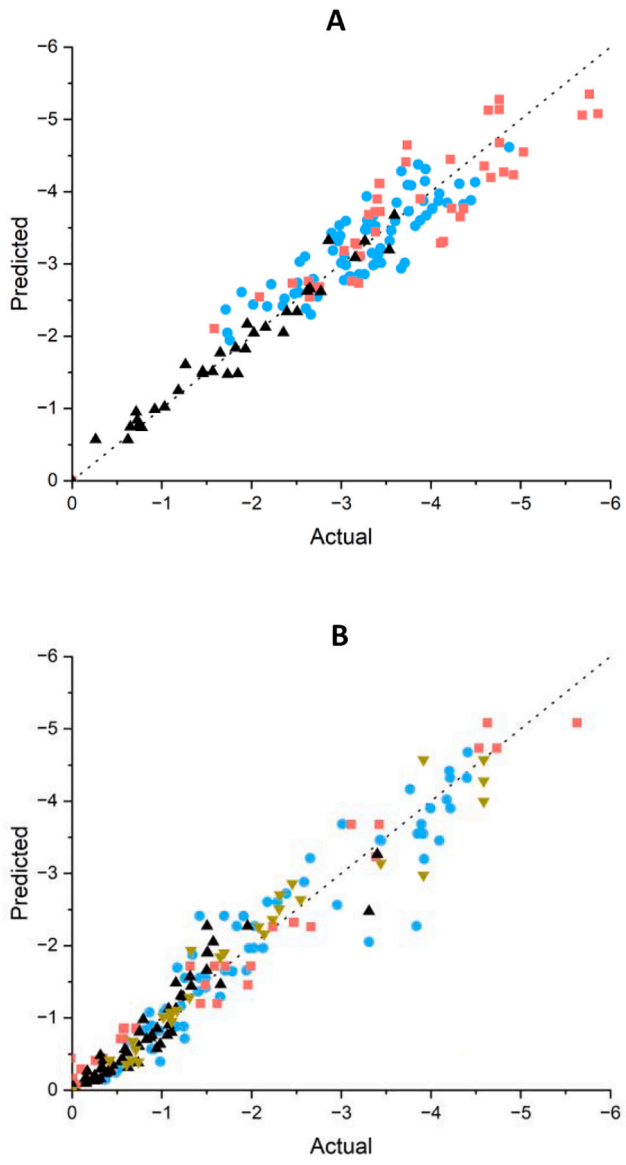
Microorganism		Secondary models	Associated std. deviation of each parameter			
			Slope	Y intercept	R^2	RMSE
S. bayanus	Must	$\log_{10}\delta = -0.04020\,\bar{E} + 1.749$	0.0138	0.2406	0.7687	1.8800
		$\log_{10}\rho = -0.01761\,\bar{E} + 0.051$	0.0048	0.0922	0.8465	0.0257
		$\delta = -2.653\,\bar{E} + 115.2$	0.2866	5.2660	0.9925	1.6550
	Wine	$\rho = -0.1022\bar{E} + 4.011$	0.0196	0.3597	0.8682	0.6259
B. bruxellensis		$\log_{10}\delta = -0.04795\,\bar{E} + 1.568$	0.0338	0.4319	0.9758	0.6901
	Must	$\rho = -0.01954\,\bar{E} + 0.8397$	0.0072	0.1331	0.9863	0.0145
		$\delta = -1.441\,\bar{E} + 82.38$	0.0016	0.0266	0.8087	4.5010
	Wine	$\rho = 2.4903$	0.0033	0.0542	–	0.3180
L. plantarum		$\log_{10}\delta = -0.01539\,\bar{E} + 2.204$	0.0032	0.0613	0.9720	2.3620
	Must	$\log_{10}\rho = -0.01237\,\bar{E} + 0.550$	0.0052	0.1023	0.7732	0.1608
		$\log_{10}\delta = -0.00688\,\bar{E} + 2.214$	0.0011	0.1089	0.9146	4.1650
	Wine	$\rho = 3.495$	–	0.1756	–	0.6084
O. oeni		$\delta = 75.131$	–	1.9980	–	5.9950
	Wine	$\rho = 2.355$	–	0.1876	–	0.5629

to guarantee that in 90% of samples the final microbial counts (Sb for *S. bayanus* and Lp for L. *plantarum*) lie below 10 CFU/mL. The fields marked in yellow represent the combinations of electric fields and specific energy that would ensure that less than 10% of must or wine samples would contain more than 10 CFU/mL of L. *plantarum* (the most resistant microorganism studied). According to Fig. 6, PEF treatments of total specific energies in the range of 113 or 141 kJ/kg at 25 kV/cm and 130 or 153 kJ/kg at 15 kV/cm would be necessary in must or wine, respectively, to achieve the defined requirements, assuming a 10% risk. In general, the required energy in both media could be reduced by increasing the electric field strength, but the impact would be moderate. In wine, for example, the energy could be reduced from 155 to 141 kJ/kg by increasing the electric field from 13 to 25 kV/cm, implying a reduction of less than 1.2 kJ/kg per unit increment of electric field.

According to our MC simulation (Table 4), treatments of 15 kV/cm and 153 kJ/kg in wine or 15 kV/cm and 129 kJ/kg in must would be sufficient to attain that risk (e.g., a < 10% probability of finding >10 CFU/mL in PEF-treated must or wine), compared to the 3-$\log_{10}$-reductions treatments previously defined based on a deterministic approach in wine (25 kV/cm;150 kJ/kg) and must (25 kV/cm;124 kJ/kg or 15 kV/cm;150 kJ/kg). On the other hand, according to the MC simulation, PEF treatments at 15 kV/cm of 68 or 145 kJ/kg of total specific energy would be required in must or wine, respectively, for effective decontamination of *S. bayanus* to be achieved.

4. Discussion

In the present study, we conducted a systematic investigation under continuous process conditions of the PEF resistance of different wine-associated microorganisms, including microorganisms involved in wine fermentation (*S. bayanus* and *O. oeni*) and spoilage microorganisms (*B. bruxellensis* and L. *plantarum*). In the course of the winemaking process, microorganisms involved in alcoholic and malolactic fermentation need to be controlled once fermentation has finished, in order to prevent further re-fermentation that can spoil the wine. In initial experiments, microbial suspensions of *S. bayanus*, *B. bruxellensis*, *L. plantarum*, and *O. oeni* were obtained under laboratory-controlled conditions and treated in commercial must that did not contain any preservative that might exert an influence on lethal outcome, as well as in wine obtained under laboratory conditions by must fermentation



	Must		Wine	
	RMSE	R^2	RMSE	R^2
S. bayanus	0.102	0.947	0.154	0.922
B. bruxellensis	0.155	0.926	0.110	0.977
L. plantarum	0.025	0.975	0.052	0.904
O. oeni	-	-	0.089	0.955

Fig. 3. Correlation between experimental and predicted data obtained with the tertiary models in must (A) or wine (B) for *S. bayanus* (●), *B. bruxellensis* (■), *L. plantarum* (▲) or *O. oeni* (▼).

without added SO_2, which would otherwise also have affected inactivation.

With the aim of obtaining a comprehensive overview of the PEF resistance of microorganisms involved in must and wine spoilage, we investigated the influence of electric field and the specific energy (Fig. 2A and B). Electric field strength and total specific energy (instead of treatment time) have been propounded as key factors for the definition of PEF treatments, as a manner of facilitating comparison among results obtained under varying experimental conditions and different types of PEF generators (Heinz et al., 2001; Raso et al., 2016). This approach is especially convenient when data are obtained under continuous processing conditions, where the total specific energy determines the temperature increment of the samples due to the Joule effect.

The survival curves obtained after PEF treatments showed two different inactivation kinetics. While the survival curves for yeast strains in must (*S. bayanus* and *B. bruxellensis*) had a concave upward shape, all the other survival curves presented a concave downward shape. The differences in inactivation kinetics of *S. bayanus* could be related to the composition of the media in which the yeast was grown. The inactivation of *S. bayanus* in wine was conducted on the same cells that had participated in the must fermentation. The presence of ethanol in growth media has been shown to modify the composition of the cytoplasmic membrane of yeasts by increasing the unsaturated fatty acid content (Huffer, Clark, Ning, Blanch, & Clark, 2011). These changes in the cytoplasmic membrane of *S. bayanus* during must fermentation might affect that microorganism's resistance to PEF and its inactivation kinetics observed in wine. In the case of *B. bruxellensis*, the change in inactivation kinetics in wine as compared with must could be related to changes in the size of cells grown in laboratory media when they are inoculated in wine, as an adaptive response to wine's low pH and to the presence of ethanol. Cell size is a parameter that plays an important role in terms of the amount of external electric field required to achieve electroporation (Kotnik, Pucihar, & Miklavčič, 2010).

Conversely, although the shape of the survival curves of L. *plantarum* did not drastically change when the treatment was applied in must, a noticeable increase could be observed in the δ parameter (first $\log_{10}$ decimal reduction) when PEF was applied in wine (Table 2). This increment in the δ parameter resulted in an increase of the total specific energy required to obtain a given level of inactivation in wine as compared with must. These findings run counter to results obtained by Heinz and Knorr (2000), who reported that the presence of ethanol (5%) increased the lethal effect of PEF on *Bacillus subtilis* when the pH of the treatment medium was reduced; however, at pH 7, the presence of ethanol led to a higher resistance to PEF. The increment in resistance of L. *plantarum* when transferred to a medium with low pH and a high concentration of ethanol – such as wine – could be caused by a rapid adaptive response of the cells to the new environment, as in the case of yeasts. Furthermore, acid-ethanol shock (pH 3.5 / 10% *v*/v) has been shown to produce a significant rigidification in the cytoplasmic membrane of *O. oeni* (lactic acid bacteria) within minutes (Chu-Ky, Tourdot-Marechal, Marechal, & Guzzo, 2005). A similar phenomenon presumably also occurred the in L. *plantarum* cells in our study. However, when attempting to develop a proper characterization of resistance, it is not possible to obtain high concentrations of L. *plantarum* cells adapted to wine conditions, as opposed to nutrient-fed cells grown in laboratory media. Our results therefore stem from conditions in which these microorganisms are more resilient. Nevertheless, it is highly important to evaluate the resistance of wine-related spoilage microorganisms to PEF if we want to estimate this technology's potential. The initial approach implemented in this study therefore provides a foundation that needs to be validated in real wines.

Most survival curves obtained in this investigation displayed a downward concave shape ($\rho > 1$), which indicates that microorganisms gradually became more susceptible to damage inflicted by PEF with higher specific energies (van Boekel, 2002). Survival curves of similar shape have been reported for the inactivation of several different microorganisms in continuous flow (Mendes-Oliveira, Jin, & Campanella, 2020; San Martín et al., 2007). Such behavior might be the outcome of the temperature increment that occurs in the treatment medium when total specific energy is increased. The lethal effect of the rise in temperature in the treatment chamber can be considered practically negligible in view of the sample's short residence time therein (<1 s) and its

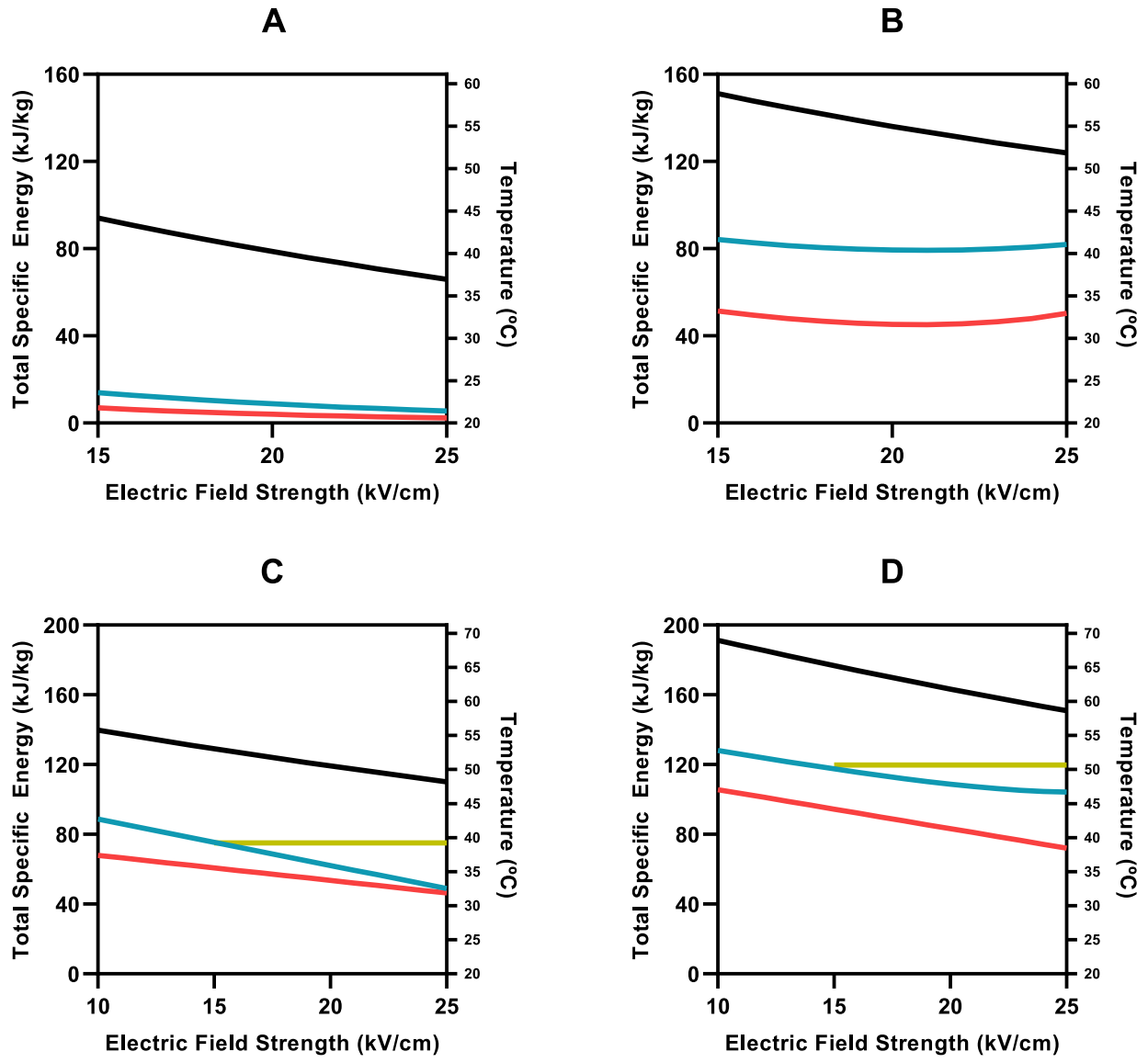


Fig. 4. Treatment conditions of electric field strength and total specific energy to achieved 1.0 $\log_{10}$ (A, C) or 3.0 $\log_{10}$ (B, D) cycles of inactivation in must (A, B) or in wine (C, D) of the different microorganisms. *S. bayanus* (■), *B. bruxellensis*(■), *L. plantarum* (—), *O. oeni* (■).

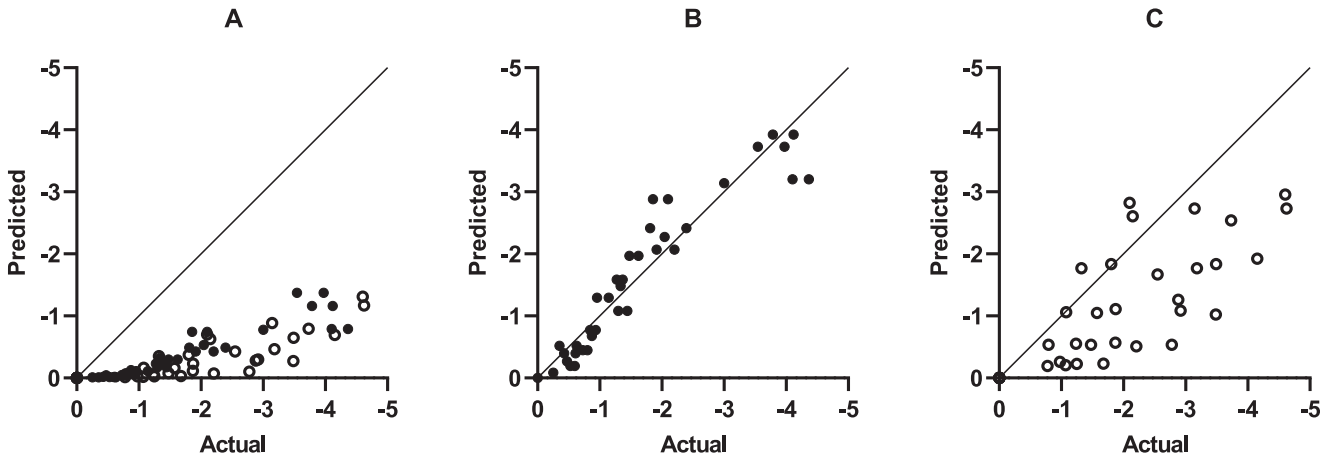


Fig. 5. Validation of tertiary models that describes inactivation in wine of L. *plantarum* (A), *S. bayanus* (B) and *O. oeni* (C) with independent inactivation data obtained after PEF treated wines from a winery after alcoholic (●) or malolactic fermentation (○) governed respectively by *S. cerevisiae* or *O. oeni*.

Table 4
Range, values, and characteristics of the input factors included in the Monte Carlo simulation and the simulation models obtained.

Experimental design				
Study type:	Randomized response surface – optimal – 37 experimental points			
Factor	Unit	Type	Minimum	Maximum
Energy	kJ/kg	Numeric/Continuous	15	155
Electric Field	kV/cm	Numeric/Continuous	10	25

Microorganism		Simulation models	$log_{10}N_0 \pm$SD (CFU/mL)
S. bayanus	Must	$N_t = 10^{log_{10}N_0 - \left(\frac{w}{10^{\delta_s \bar{E}+\delta_y}}\right)^{10^{\rho_s \bar{E}+\rho_y}}}$	3.4 ± 0.35
	Wine	$N_t = 10^{log_{10}N_0 - \left(\frac{w}{\delta_s \bar{E}+\delta_y}\right)^{\rho_s \bar{E}+\rho_y}}$	3.0 ± 0.50
L. plantarum	Must	$N_t = 10^{log_{10}N_0 - \left(\frac{w}{10^{\delta_s \bar{E}+\delta_y}}\right)^{10^{\rho_s \bar{E}+\rho_y}}}$	2.1 ± 0.20
	Wine	$N_t = 10^{log_{10}N_0 - \left(\frac{w}{10^{\delta_s \bar{E}+\delta_y}}\right)^{\rho_y}}$	2.0 ± 0.20

rapid cooling in the heat exchanger located after the treatment chamber. Consequently, the observed rapid increment in lethality of the PEF treatments by increasing total specific energy could be attributed to the high efficacy of PEF in the electroporation of cells at moderate temperatures. A significant rise in lethal outcome by applying PEF treatments at mild temperatures (30–50 °C) has been reported in different microorganisms (Saldaña, Monfort, Condón, Raso, & Álvarez, 2012; Timmermans et al., 2019). Changes in the phospholipids of the cytoplasmic membrane from gel to liquid-crystalline phase at higher temperature would make the membrane more vulnerable to electroporation (Liu, Zeng, Ngadi, & Han, 2017; Stanley & Parkin, 1991; Wang, Ou, Zeng, & Guo, 2019). Differences among microorganisms in terms of cytoplasmic membrane composition and intrinsic PEF-sensitivity would explain the observed differences among inactivation kinetics. Typically, fundamental studies on microbial inactivation by PEF conducted in batch tend to yield concave upward survival curves (Delso et al., 2022). Such kinetics would imply a limitation for the commercial exploitation of PEF as a food preservation technology, since treatments of a very high total specific energy would be required to obtain substantial microbial inactivation (Monfort, Gayán, Raso, Condón, & Álvarez, 2010; Puértolas et al., 2009; Qin et al., 2015; Walter, Knight, Ng, & Buckow, 2016). From a practical point of view, the use of PEF for wine decontamination in a winery requires continuous processing, in which an increment in temperature would contribute to obtain the required inactivation while applying moderate PEF treatment intensities.

As depicted in Fig. 3, the equations (tertiary models) based on the Weibull model developed demonstrated an overall high goodness of fit for the prediction of experimental data. These equations permitted to compare the investigated microorganisms' resistance to PEF under different levels of inactivation (Fig. 4). In must as well as in wine, the most sensitive strain was *B. bruxellensis*, while the most resistant one was L. *plantarum*. Similar findings were reported by Puértolas et al. (2009), who found that *Lactobacillus* strains were the most resistant ones in must and wine. It is widely reported that yeasts are more sensitive to PEF than bacteria due to their larger size, which makes them more susceptible to electroporation (Delso et al., 2022; Hülsheger, Potel, & Niemann, 1981; Sale & Hamilton, 1967; Wouters, Alvarez, & Raso, 2001). Further intrinsic microbial characteristics such as structure and composition of the cellular envelopes might exert an influence on microbial resistance to PEF, as previously indicated (Liu et al., 2017; Wang et al., 2019). In addition, as can be observed in Fig. 4, the influence of the electric field at equivalent specific energies was not very marked, while no impact on the inactivation of *O. oeni* was observed. In the best-case scenario, each unit increment in the electric field only allowed for a reduction of 2.5 kJ/kg of the total specific energy required to reach the target inactivation level. In contrast, when a similar evaluation was performed in static configuration (no temperature increase), up to 30 kJ/kg were reduced per unit of electric field increment (Puértolas et al., 2009). Furthermore, in that study, specific energies from 300 to 450 kJ/kg were reported to have reached similar lethal goals in must and wine at electric fields of 19–25 kV/cm, hence 2.5-fold the energies reported in our study. These observations reinforce the assumption that in continuous PEF processing, total specific energy is a crucial factor in describing PEF protocols

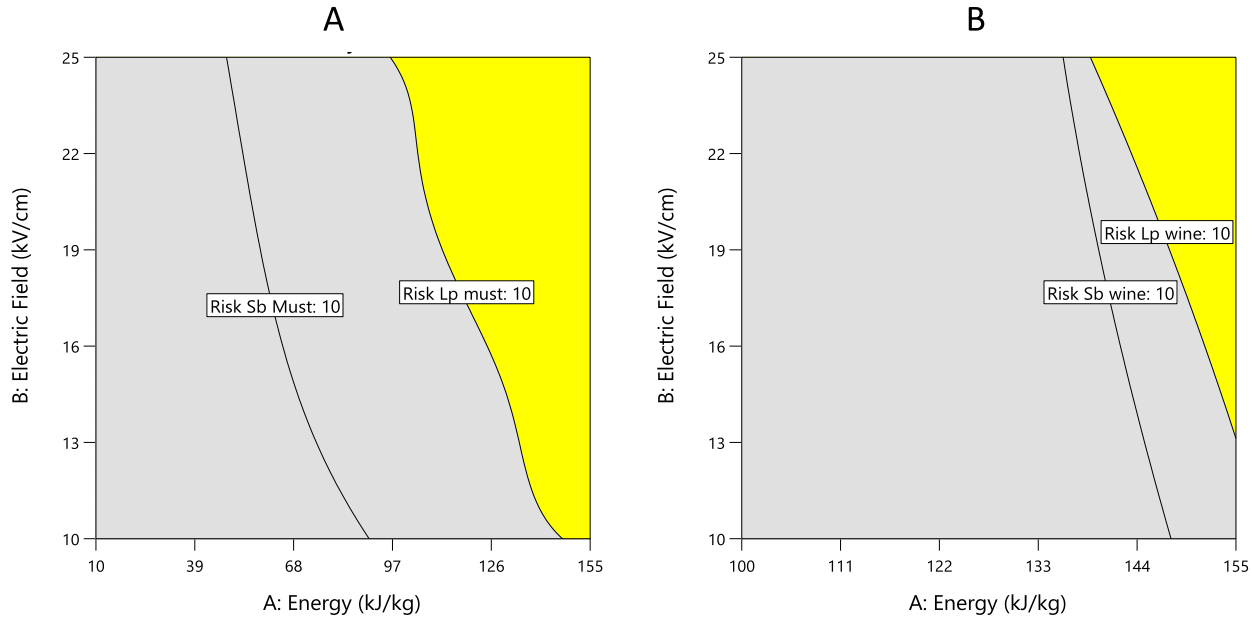


Fig. 6. Electric field strength and total specific energy treatment conditions to fulfill the requirement of MC simulation, ensuring a concentration lower than 10 CFU/mL assuming 10% of risk for *S. bayanus* (Sb) and L. *plantarum* (Lb) in must (A) and wine (B).

and seems to exert a greater impact than the electric field strength. The fact that even mild temperatures can provoke changes in the lipid conformation of the cytoplasmic membrane (thereby reducing the electroporation threshold and hence facilitating electroporation) would explain why electric field strength had less impact on lethality under continuous conditions compared to static ones. Consequently, continuous processing configuration would not only allow for a significant reduction of the total specific energy, but also of the electric field strength. The possibility of reducing electric field strength while maintaining high inactivation rates and moderate specific energies is of considerable importance for the application of PEF on an industrial scale. The lower the electric field required, the larger the PEF treatment chamber volume (distance between electrodes) and hence the lower risk of arching, the lower power requirements of PEF devices, and the greater the flow rates, thereby managing to meet the productive requirements and capacities of wineries.

Predictive microbiology is essential when evaluating the implementation of new decontamination technologies in the food industry. By assembling inactivation data and developing mathematical equations capable of describing a series of different scenarios, more far-reaching and efficient operational decisions can be made. Although the developed mathematical equations (tertiary models) had a good predictive capacity (Fig. 3), the conditions under which data are obtained to develop models do not necessarily represent real food conditions. We were nevertheless able to use the independent data obtained by inactivating microorganisms that had grown in wines from a winery in order to validate those tertiary models. *L. plantarum* proved to be the most PEF-resistant microorganism under the experimental conditions we investigated; it is thus the target microorganism when defining PEF processing conditions for microbial decontamination of must and wine. In view of the target microorganism's observed resistance to PEF, our model predictions underpredicted observations obtained independently of the group of microorganisms (yeasts or lactic acid bacteria) (Fig. 5A). Consequently, this validation demonstrated that by implementing the most preventive scenario, the required selected treatment conditions more than guarantee effective microbial decontamination of wine.

On the other hand, when lethal treatments are applied in the food industry, there is a huge variability in terms of efficacy and in terms of microbial load; additionally, at each stage of processing, there is a probability that the microbial load count can increase or decrease. Therefore, instead of defining PEF treatment conditions for a concrete inactivation value as indicated in Fig. 4, it may be of more interest to provide a probability or risk value of the presence of a certain level of microorganisms in the final product. Consequently, the microbial concentration of those microorganisms, combined with the risk boundaries, can be estimated by means of a probabilistic approach better suited to define PEF protocols, depending on the winemaking step and/or scenario. To achieve this, MC simulation was run in order to stablish with the purpose of establishing the PEF conditions required to accomplish a maximum assumable concentration load defined for *S. bayanus* and L. *plantarum* in must or wine, assuming a normal distribution of the initial microbial load (Table 4). Despite highly restrictive requirements (the most resistant microorganisms, a high initial microbial load, $<$10 CFU/mL of final concentration, and $\leq$ 10% of assumable risk), several combinations of moderate electric field strengths and energies could be selected that would ensure 90% probability of zero spoilage. In any case, the intensity of PEF treatments can vary, depending on the amount of risk that a winery is willing to assume. In comparison with the deterministic approach (Fig. 4), this probabilistic approach allowed to further optimize the PEF parameters by reducing the intensity requirements of PEF parameters in both must and wine. Consequently, total specific energy can be reduced by 10% and by 20% in must and wine, respectively, thereby allowing for the implementation of PEF treatments below 60 °C of maximum temperature, while maintaining low electric field strength (15 kV/cm) and fulfilling the restrictive MC input conditions. Furthermore, the initial count of L. *plantarum* used in the MC simulation was based on the experimental data obtained from the number of total lactic acid bacteria found in real wines from a winery: in other words, we deliberately overestimated the input value of the initial load of L. *plantarum*. As this limiting microorganism was considerably more resistant than the remaining ones under study, the defined protocols obtained by MC represented a "worst case scenario". Despite this, those protocols would be more than reasonably applicable on an industrial scale. Moreover, as mentioned above, we investigated L. *plantarum* cells under stress conditions that might trigger cross-resistance responses to PEF. This thus also suggests that even PEF processing conditions of lower intensity would be able to ensure the decontamination of L. *plantarum*, and thus of all the microorganisms under study. Nevertheless, a complete evaluation of the concentration probabilities of L. *plantarum* in all steps of the winemaking process would allow researchers to improve the risk assessment of PEF processing on an industrial scale.

5. Conclusions

As opposed to most previous modeling studies on PEF inactivation, which were performed in static processing, our research was carried out in continuous flow, thus allowing us to determine this technology's lethal effect in a more practical/realistic setting. Moderate PEF parameters of electric field strength (15 kV/cm) and total specific energy (129 or 153 kJ/kg) were shown to achieve adequate lethality levels for the decontamination of spoilage-related microorganisms in must or wine. These results underscore the promising potential of PEF technology in this area, particularly in view of these parameters' applicability using the current industrial-scale commercial PEF generator, while ensuring the fulfillment of winery production rates. Furthermore, the incorporation of probabilistic risk evaluation into PEF protocols is better adjusted to the standards and requirements of wineries, and will allow for more flexible processing strategies adapted to specific scenarios.

CRediT authorship contribution statement

Carlota Delso: Methodology, Investigation, Writing – original draft, Visualization. **Sebastián Ospina:** Data curation, Formal analysis, Software. **Alejandro Berzosa:** Validation, Visualization. **Javier Raso:** Supervision, Project administration, Conceptualization. **Ignacio Álvarez-Lanzarote:** Funding acquisition, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Baranyi, J., Pin, C., & Ross, T. (1999). Validating and comparing predictive models. *International Journal of Food Microbiology, 48*(3), 159–166. https://doi.org/10.1016/S0168-1605(99)00035-5

Bartowsky, E. J. (2009). Bacterial spoilage of wine and approaches to minimize it. *Letters in Applied Microbiology, 48*(2), 149–156. https://doi.org/10.1111/j.1472-765X.2008.02505.x

Bevilacqua, A., Petruzzi, L., Perricone, M., Speranza, B., Campaniello, D., Sinigaglia, M., & Corbo, M. R. (2018). Nonthermal technologies for fruit and vegetable juices and beverages: Overview and advances. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 2–62. https://doi.org/10.1111/1541-4337.12299

van Boekel, M. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology, 74*(1–2), 139–159. https://doi.org/10.1016/S0168-1605(01)00742-5

Buzrul, S. (2022). The Weibull model for microbial inactivation. *Food Engineering Reviews, 14*(1), 45–61. https://doi.org/10.1007/s12393-021-09291-y

Chu-Ky, S., Tourdot-Marechal, R., Marechal, P. A., & Guzzo, J. (2005). Combined cold, acid, ethanol shocks in *Oenococcus oeni*: Effects on membrane fluidity and cell viability. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1717*(2), 118–124. https://doi.org/10.1016/J.BBAMEM.2005.09.015

Cosme, F., Filipe-Ribeiro, L., & Nunes, M. F. (2021). Wine stabilisation: An overview of defects and treatments. In *Chemistry and biochemistry of winemaking, wine stabilization and aging* (pp. 225–240). IntechOpen. https://doi.org/10.5772/intechopen.95245.

Delso, C., Martínez, J. M., Cebrián, G., Condón, S., Raso, J., & Álvarez, I. (2022). Microbial inactivation by pulsed electric fields. In *Pulsed electric fields technology for the food industry* (pp. 169–207). https://doi.org/10.1007/978-3-030-70586-2_5

Genc, M., Genc, S., & Goksungur, Y. (2017). Exergy analysis of wine production: Red wine production process as a case study. *Applied Thermal Engineering, 117*, 511–521. https://doi.org/10.1016/J.APPLTHERMALENG.2017.02.009

Giacosa, S., Río Segade, S., Cagnasso, E., Caudana, A., Rolle, L., & Gerbi, V. (2019). SO2 in wines. In *Red wine technology* (pp. 309–321). Elsevier. https://doi.org/10.1016/B978-0-12-814399-5.00021-9.

González-Arenzana, L., López-Alfaro, I., Garde-Cerdán, T., Portu, J., López, R., & Santamaría, P. (2018). Microbial inactivation and MLF performances of Tempranillo Rioja wines treated with PEF after alcoholic fermentation. *International Journal of Food Microbiology, 269*, 19–26. https://doi.org/10.1016/j.ijfoodmicro.2018.01.008

González-Arenzana, L., Portu, J., López, R., López, N., Santamaría, P., Garde-Cerdán, T., & López-Alfaro, I. (2015). Inactivation of wine-associated microbiota by continuous pulsed electric field treatments. *Innovative Food Science & Emerging Technologies, 29*, 187–192. https://doi.org/10.1016/j.ifset.2015.03.009

Heinz, V., Alvarez, I., Angersbach, A., & Knorr, D. (2001). Preservation of liquid foods by high intensity pulsed electric fields—Basic concepts for process design. *Trends in Food Science & Technology, 12*(3–4), 103–111. https://doi.org/10.1016/S0924-2244(01)00064-4

Heinz, V., & Knorr, D. (2000). Effect of pH, ethanol addition and high hydrostatic pressure on the inactivation of *Bacillus subtilis* by pulsed electric fields. *Innovative Food Science & Emerging Technologies, 1*(2), 151–159. https://doi.org/10.1016/S1466-8564(00)00013-8

Huffer, S., Clark, M. E., Ning, J. C., Blanch, H. W., & Clark, D. S. (2011). Role of alcohols in growth, lipid composition, and membrane fluidity of yeasts, bacteria, and Archaea. *Applied and Environmental Microbiology, 77*(18), 6400–6408. https://doi.org/10.1128/AEM.00694-11

Hülsheger, H., Potel, J., & Niemann, E. G. (1981). Killing of bacteria with electric pulses of high field strength. *Radiation and Environmental Biophysics, 20*(1), 53–65. https://doi.org/10.1007/bf01323926

Katiyo, W., Yang, R., & Zhao, W. (2017). Effects of combined pulsed electric fields and mild temperature pasteurization on microbial inactivation and physicochemical properties of cloudy red apple juice (*Malus pumila* Niedzwetzkyana (Dieck)). *Journal of Food Safety, 37*(4), Article e12369. https://doi.org/10.1111/jfs.12369

Kotnik, T., Pucihar, G., & Miklavčič, D. (2010). Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *The Journal of Membrane Biology, 236*(1), 3–13. https://doi.org/10.1007/s00232-010-9279-9

Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative methods to SO 2 for microbiological stabilization of wine. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 455–479. https://doi.org/10.1111/1541-4337.12422

Liu, Z.-W., Zeng, X.-A., Ngadi, M., & Han, Z. (2017). Effect of cell membrane fatty acid composition of *Escherichia coli* on the resistance to pulsed electric field (PEF) treatment. *LWT - Food Science and Technology, 76*, 18–25. https://doi.org/10.1016/j.lwt.2016.10.019

Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in thermal preservation methods: Application of the Weibull frequency distribution model. *International Journal of Food Microbiology, 72*(1–2), 107–113. https://doi.org/10.1016/S0168-1605(01)00624-9

Marsellés-Fontanet, À. R., Puig, A., Olmos, P., Mínguez-Sanz, S., & Martín-Belloso, O. (2009). Optimising the inactivation of grape juice spoilage organisms by pulse electric fields. *International Journal of Food Microbiology, 130*(3), 159–165. https://doi.org/10.1016/j.ijfoodmicro.2008.12.034

Membré, J.-M., & Lambert, R. J. W. (2008). Application of predictive modelling techniques in industry: From food design up to risk assessment. *International Journal of Food Microbiology, 128*(1), 10–15. https://doi.org/10.1016/j.ijfoodmicro.2008.07.006

Mendes-Oliveira, G., Jin, T. Z., & Campanella, O. H. (2020). Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* in juices by pulsed electric fields: The role of the energy density. *Journal of Food Engineering, 282*, Article 110001. https://doi.org/10.1016/J.JFOODENG.2020.110001

Min, S., Jin, Z. T., Min, S. K., Yeom, H., & Zhang, Q. H. (2003). Commercial-scale pulsed electric field processing of orange juice. *Journal of Food Science, 68*(4), 1265–1271. https://doi.org/10.1111/j.1365-2621.2003.tb09637.x

Monfort, S., Gayán, E., Raso, J., Condón, S., & Álvarez, I. (2010). Evaluation of pulsed electric fields technology for liquid whole egg pasteurization. *Food Microbiology, 27*(7), 845–852. https://doi.org/10.1016/j.fm.2010.05.011

Pérez-Coello, M. S., & Díaz-Maroto, M. C. (2009). Volatile compounds and wine aging. In *Wine chemistry and biochemistry* (pp. 295–311). New York: Springer. https://doi.org/10.1007/978-0-387-74118-5_16.

Puértolas, E., López, N., Condón, S., Raso, J., & Álvarez, I. (2009). Pulsed electric fields inactivation of wine spoilage yeast and bacteria. *International Journal of Food Microbiology, 130*(1), 49–55. https://doi.org/10.1016/j.ijfoodmicro.2008.12.035

Qin, S., Timoshkin, I. V., Maclean, M., Wilson, M. P., Given, M. J., Wang, T., … Macgregor, S. J. (2015). Pulsed electric field treatment of *saccharomyces cerevisiae* using different waveforms. *IEEE Transactions on Dielectrics and Electrical Insulation, 22*(4), 1841–1848. https://doi.org/10.1109/TDEI.2015.005152

Raso, J., & Álvarez, I. (2016). Pulsed electric field processing: Cold pasteurization. In *Reference module in food science* (pp. 1–8). Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.03103-6.

Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (2016). Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative Food Science & Emerging Technologies, 37*, 312–321. https://doi.org/10.1016/J.IFSET.2016.08.003

Saldaña, G., Monfort, S., Condón, S., Raso, J., & Álvarez, I. (2012). Effect of temperature, pH and presence of nisin on inactivation of *Salmonella Typhimurium* and *Escherichia coli* O157:H7 by pulsed electric fields. *Food Research International, 45*(2), 1080–1086. https://doi.org/10.1016/j.foodres.2011.03.059

Saldaña, G., Puértolas, E., Álvarez, I., Meneses, N., Knorr, D., & Raso, J. (2010). Evaluation of a static treatment chamber to investigate kinetics of microbial inactivation by pulsed electric fields at different temperatures at quasi-isothermal conditions. *Journal of Food Engineering, 100*(2), 349–356. https://doi.org/10.1016/j.jfoodeng.2010.04.021

Saldaña, G., Puértolas, E., Condón, S., Álvarez, I., & Raso, J. (2010). Modeling inactivation kinetics and occurrence of sublethal injury of a pulsed electric field-resistant strain of *Escherichia coli* and *Salmonella Typhimurium* in media of different pH. *Innovative Food Science and Emerging Technologies, 11*(2), 290–298. https://doi.org/10.1016/j.ifset.2010.01.003

Sale, A., & Hamilton, W. (1967). Effects of high electric fields on microorganismsI. Killing of bacteria and yeasts. *Biochimica et Biophysica Acta (BBA) - General Subjects, 148*(3), 781–788. https://doi.org/10.1016/0304-4165(67)90052-9

San Martín, M. F., Sepúlveda, D. R., Altunakar, B., Góngora-Nieto, M. M., Swanson, B. G., & Barbosa-Cánovas, G. V. (2007). Evaluation of selected mathematical models to predict the inactivation of *Listeria innocua* by pulsed electric fields. *LWT - Food Science and Technology, 40*(7), 1271–1279. https://doi.org/10.1016/J.LWT.2006.08.011

Stanley, D. W., & Parkin, K. L. (1991). Biological membrane deterioration and associated quality losses in food tissues. *Critical Reviews in Food Science and Nutrition, 30*(5), 487–553. https://doi.org/10.1080/10408399109527554

Timmermans, R., Nierop Groot, M., & Matser, A. (2022). Liquid food pasteurization by pulsed electric fields. In J. Raso, V. Heinz, I. Alvarez, & S. Toepfl (Eds.), *Pulsed electric fields technology for the food industry* (pp. 299–323). Springer International Publishing. https://doi.org/10.1007/978-3-030-70586-2_8.

Timmermans, R. A. H., Mastwijk, H. C., Berendsen, L. B. J. M., Nederhoff, A. L., Matser, A. M., van Boekel, M. A. J. S., & Nierop Groot, M. N. (2019). Moderate intensity pulsed electric fields (PEF) as alternative mild preservation technology for fruit juice. *International Journal of Food Microbiology, 298*, 63–73. https://doi.org/10.1016/j.ijfoodmicro.2019.02.015

Walter, L., Knight, G., Ng, S. Y., & Buckow, R. (2016). Kinetic models for pulsed electric field and thermal inactivation of *Escherichia coli* and *Pseudomonas fluorescens* in whole milk. *International Dairy Journal, 57*, 7–14. https://doi.org/10.1016/j.idairyj.2016.01.027

Wang, R., Ou, Y., Zeng, X., & Guo, C. (2019). Membrane fatty acids composition and fluidity modification in *Salmonella Typhimurium* by culture temperature and resistance under pulsed electric fields. *International Journal of Food Science & Technology, 54*(6), 2236–2245. https://doi.org/10.1111/ijfs.14137

Wouters, P. C., Alvarez, I., & Raso, J. (2001). Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends in Food Science & Technology, 12*(3–4), 112–121. https://doi.org/10.1016/S0924-2244(01)00067-X

van Wyk, S., Farid, M. M., & Silva, F. V. M. (2018). SO2, high pressure processing and pulsed electric field treatments of red wine: Effect on sensory, *Brettanomyces* inactivation and other quality parameters during one year storage. *Innovative Food Science & Emerging Technologies, 48*, 204–211. https://doi.org/10.1016/J.IFSET.2018.06.016

van Wyk, S., Silva, F. V. M., & Farid, M. M. (2019). Pulsed electric field treatment of red wine: Inactivation of *Brettanomyces* and potential hazard caused by metal ion dissolution. *Innovative Food Science & Emerging Technologies, 52*, 57–65. https://doi.org/10.1016/j.ifset.2018.11.001