Optimising the inactivation of grape juice spoilage organisms by pulse electric fields

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ABSTRACT

The effect of some pulsed electric field (PEF) processing parameters (electric field strength, pulse frequency and treatment time), on a mixture of microorganisms (Kloeckera apiculata, Saccharomyces cerevisiae, Lactobacillus plantarum, Lactobacillus hilgardii and Gluconobacter oxydans) typically present in grape juice and wine were evaluated. An experimental design based on response surface methodology (RSM) was used and results were also compared with those of a factorially designed experiment. The relationship between the levels of inactivation of microorganisms and the energy applied to the grape juice was analysed. Yeast and bacteria were inactivated by the PEF treatments, with reductions that ranged from 2.24 to 3.94 log units. All PEF parameters affected microbial inactivation. Optimal inactivation of the mixture of spoilage microorganisms was predicted by the RSM models at 35.0 kV cm−1 with 303 Hz pulse width for 1 ms. Inactivation was greater for yeasts than for bacteria, as was predicted by the RSM. The maximum efficacy of the PEF treatment for inactivation of microorganisms in grape juice was observed around 1500 MJ L−1 for all the microorganisms investigated. The RSM could be used in the fruit juice industry to optimise the inactivation of spoilage microorganisms by PEF.

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1. Introduction

The food industry aims to satisfy consumer expectations of products with unmodified natural taste and nutritional values that are microbiologically safe. New processing technologies such as high intensity pulsed electric fields (PEF) have been developed in response to these requirements. A lot of research has dealt with the effects of PEF on microbes in water, or models solutions with various physical, chemical and biochemical properties, resulting in information on the fundamentals of this technology (Sale and Hamilton, 1967; Huishgeier et al., 1983; Vega-Mercado et al., 1996). However, a necessary step to promote industrial application of this technology is experimentation on real foods such as fruit juices.

Studies of microbial responses to PEF treatments of juices have been carried out with various juices including orange juice (Elez-Martínez et al., 2004, 2005), apple juice (Gupta et al., 2003; Garcia et al., 2005), carrot–orange juice mix (Rodrigo et al., 2001; Selma et al., 2004) and berry juice (Gupta et al., 2005). In all the reported work the degree of inactivation microorganisms by PEF varied with the species and strain, the juice, the design of the PEF device and the processing parameters. However, there is little information about the effect of PEF treatments on microorganisms that spoil grape juice, although it is one of the most widely traded juice commodities. Jaya, Varadharaju and Kennedy (2004) and Wu, Mittal and Griffiths (2005) applied PEF to grape juice, but without reporting the species that were studied. Other studies investigated the effects of PEF treatments on oxidative enzymes of grape juice (Marsellés-Fontanet and Martín-Belloso, 2007), its nutritive components (Garde-Cerdán et al., 2007a), and on sensory characteristics of wine obtained from PEF processed grape juice (Garde-Cerdán et al., 2007b, 2008).

The aim of the study reported in this paper was to obtain reliable data about the behaviour of typical flora (yeasts and bacteria) under PEF processing conditions similar to those likely to be used in the industry. Microbial inactivation data were used to obtain a predictive model for each microorganism investigated, which after an optimisation process should allow the identification of the best PEF processing parameters to reduce the content of spoilage microorganisms in grape juice.

2. Materials and methods

2.1. Experimental designs

The response variable (S) in experimental designs was calculated according to the formula:

\[ S = - \log_{10} \left( \frac{N}{N_0} \right) \] (1)

where \( N \) and \( N_0 \) are the number of viable cells of each microorganism on treated and untreated juice samples, respectively. Therefore, all the results were positive numbers, and the higher the S-value the
more effective the PEF treatment because more microorganisms were inactivated.

2.1.1. Response surface design

A central composite design was used to evaluate the effect of each factor on the response analysed, and to develop a mathematical relationship between the response and the independent factors (Khuri and Cornell, 1996; Myers and Montgomery, 2002). This relationship allows estimation of the optimum response and the values of the factors needed to achieve such a response. Table 1 shows the experimental design with 20 experiments randomly distributed among factorial, star and central points.

Treatment time (t), electric field (E), and pulse frequency (F) were taken as the continuous independent factors to be optimised. The experimental design required three values of each factor under study so 0, 0.500 and 1.000 ms were selected as the treatment time values, 20.0, 27.5 and 35.0 kV cm⁻¹ were chosen for the electric field strength values, and 100, 200 and 600 Hz were selected as the pulse frequencies. The highest values correspond to the upper values for the operations conditions that could be achieved in previous studies (Garde-Cerdán et al., 2007a; Marsellés-Fontanet and Martín-Belloso, 2007). The minimum values were chosen to cover a wide range of experimental conditions. The central values were arbitrarily chosen to look for non-linear behaviour (Giesbrecht and Gumpertz, 2004).

2.1.2. Factorial design

The predictions obtained with RSM were compared with the results of a factorial set of experiments using electric field strengths of 20.0, 25.0, 27.5, 30.0 and 35.0 kV cm⁻¹ and pulse frequencies of 100, 200 and 600 Hz. Microbial populations were measured after 0, 0.125, 0.250, 0.500, 0.750 and 1.000 ms treatment times using the same equipment and analytical methods. Four grape juice batches were used and the microorganisms were inoculated from the same stock cultures as in the previous experimental design.

The same data allowed study of the responses of each microbial species subjected to PEF treatments. A possible relationship between the energy per volume unit delivered to grape juice and the inactivation of the microorganisms was also investigated.

Table 1
Response surface design and experimental results

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<th>Experiment</th>
<th>E (kV cm⁻¹)</th>
<th>F (Hz)</th>
<th>t (ms)</th>
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<td>3.00</td>
<td>2.68</td>
<td>1.55</td>
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</tbody>
</table>

a Average of three measurements.

b Electric field strength in kV cm⁻¹.

c Pulse frequency in Hz.

d Treatment time in ms.

e Inactivation level in log units.

2.2. Sample preparation

Pasteurised grape juice (Vitis vinifera L. variety Parellada) was supplied by a local wine maker (Baixas Lehnbeg SCP, Santes Creus, Tarragona, Spain). Microorganisms selected as type specimens of the most common yeast and bacterial species occurring in grape juice (ICMSF, 1998) were used to inoculate the juice. These organisms were Saccharomyces cerevisiae strain P29 (yeast wine collection, Instituto Catalán de la Viña y el Vino (INCAVI), Vilafranca del Penedès, Barcelona, Spain); Kloeckera apiculata (Hanseniaspora uvarum) CECT 11105 (Spanish Type Culture Collection, Universidad de Valencia, Valencia, Spain); Lactobacillus plantarum strain C11 (INCAVI); Lactobacillus hilgardii CECT 4786 (Universidad de Valen-
cia) and Gluconobacter oxydans LMG 1408 (Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium).

The initial culture of each microorganism was propagated in three steps. The stock culture of each species was inoculated in 10 mL of broth. When the microbial population reached the stationary phase, 10 mL of fresh broth were added. After again reaching the stationary phase, 80 mL of grape juice were added to obtain 100 mL of microcultural medium. Microbial growth was monitored through the absorbance measured at 600 nm. The five 100 mL inocula were mixed together into 500 mL of grape juice to obtain a final volume of 1 L. The numbers of each microorganism ranged between 10⁶ and 10⁹ colony forming units (cfu)·mL⁻¹. Finally, 50 mL aliquots were PEF treated according to the experimental designs. Microbial culture manipulations were performed under aseptic conditions.

G. oxydans was grown at 28 °C, under aerobic conditions, in a broth containing 0.5% yeast extract, 0.3% peptone and 2.5% mannitol. Lactic acid bacteria were grown at 30 °C, under an aerobic atmosphere containing 5% carbon dioxide, in Oenococcus oeni medium of pH=4.8, mixed with 30% tomato juice. K. apiculata and S. cerevisiae were grown at 30 °C, on an aerobic atmosphere with 5% carbon dioxide in a broth containing 1% yeast extract, 2% peptone and 2% glucose. Obtaining the inocula for the grape juice preparation took 7, 6, 5 and 3 days, respectively.

Yeast extract, mannitol and Oenococcus oeni medium were provided by Scharlau Chemie, S.A., Barcelona, Spain. Glucose and soy peptone were provided by Panreac Quimica, S.A., Barcelona, Spain. Tomato juice was obtained from tomatoes, which were purchased in a local market, chopped and pressed through a sieve of 1 mm diameter. Pressurised carbon dioxide was obtained from Abelló Linde, S.A., Barcelona, Spain.

2.3. Assessment of viable microbial populations

Serial decimal dilutions of the PEF treated and untreated samples were prepared with Ringer solution. Each dilution was filtered through nitrocellulose filters (Whatman International Ltd, Maidstone, England) of 0.45 pore diameter to collect the microorganisms, and the filters were placed on plates of the appropriate selective growing environment.

G. oxydans were incubated in an aerobic atmosphere at 28 °C for seven days, on glucose (5%), yeast extract (2%), nystatin (50 mg L⁻¹), penicillin G (3 unit mL⁻¹) agar of pH=4.5. Lactic acid bacteria were incubated under anaerobic atmosphere at 30 °C for five days, on a 70% O. oeni agar of pH=4.8 supplemented with 30% tomato juice, nystatin at 50 mg L⁻¹ and chloramphenicol at 500 mg L⁻¹. K. apiculata was grown in an aerobic atmosphere containing 5% carbon dioxide at 30 °C for four days on peptone (2%), glucose (2%), yeast extract (1%), cycloheximide (50 mg L⁻¹), chloramphenicol (500 mg L⁻¹) agar. S. cerevisiae was incubated in an aerobic atmosphere containing 5% carbon dioxide at 30 °C for seven days, on peptone (2%), glucose (2%), yeast extract (1%), chloramphenicol (500 mg L⁻¹), ethyl alcohol (13% v/v) agar.
Chloramphenicol, cycloheximide, nystatin and penicillin G were purchased from Sigma-Aldrich Inc. Stinheim, Germany. Ethanol was obtained from Panreac Quimica, SA.

2.4. Grape juice characterisation

Parellada grape juice was characterised by analysing the solubles solid content (degrees brix), density (g mL\(^{-1}\)), pH and total acidity (meqvl/1 of 0.1 N NaOH (Scharlau) required to reach pH 8.1 mL\(^{-1}\) of grape juice). The analyses were performed following the Spanish regulation about grape juice (BOE, 1988). A digital refractometer (Atago RX-1000, Atago Co, Ltd, Tokyo, Japan) with temperature correction and a precision of ±0.1% was used to measure the solubles solid content of grape juice. Density was assessed as the ratio between the weight (±0.1 mg) of 10 mL (±0.1 mL) of grape juice contained in a pycnometer (Afora, S.A., Barcelona, Spain). Total acidity and pH were measured using a pH-meter (Crison 2000, Crison, S.A., Barcelona, Spain) with a precision of ±0.02 pH units. The measurements of density, pH and acidity were carried out at 20 °C. In addition, electrical conductivity of grape juice at temperatures from 10 to 45 °C was determined with a conductivity meter (Testo 240, Testo AG, Lenzirch, Germany) with a precision of ±1 µS cm\(^{-1}\). A mathematical relationship between the electrical conductivity and the temperature was obtained using linear regression. For all the analyses, six samples of untreated juice were used and each measurement was made in triplicate.

2.5. PEF equipment and treatments

An OSU-4F bench-scale continuous unit manufactured by the Ohio State University (Columbus, OH, USA) was used to treat the grape juice samples (Marsellés-Fontanet and Martin-Belloso, 2007). Eight co-field chambers, each with a volume of 12 mm\(^3\) and a gap distance of 2.9 mm between electrodes were connected in series. Four cooling coils were connected before and after each pair of chambers and submerged in a water-ice mixture to maintain the grape juice temperature below 35 °C. The device was sterilised chambers and submerged in a water-ice mixture to maintain the

Temperatures were recorded by thermocouples (T type, ±0.1 °C) located before and after each pair of treatment chambers. Pulse waveform, voltage, intensity, pulse width and pulse frequency delivered in the treatment chambers were recorded using a digital oscilloscope (Tektronix T720A, Tektronix, OR, USA) to check the accuracy of the equipment with a precision of ±3% reading +0.1 div + 1 mV. Measured temperature and electrical values were recorded in a computer. The flow rate was set with a peristaltic pump (Millipore, Bedford, MA, USA) calibrated with grape juice before each use.

Grape juice was processed with pulses of 5 µs in bipolar mode, with the values of treatment time, electric field strength and pulse frequency of the experimental designs. Grape juice temperature at the inlet of the first chamber was kept at 15 °C. The maximum temperature of 30.4 °C was reached at the last pair of treatment chambers with the treatment that delivered the maximum electrical power to juice circulating at a flow rate of 3.33 mL s\(^{-1}\).

The PEF treatment time of grape juice samples (t, ms) were calculated as the product of the average pulse width (τ, ms) and the number of pulses (n) delivered in the treatment chambers. That is:

\[
t = \tau \cdot n
\]

(2)

The number of pulses can be obtained from the pulse frequency (F, Hz) and the average residence time (t\(_r\), s) of the sample in the treatment chambers as:

\[
n = F \cdot t_r
\]

(3)

and the residence time is calculated as the relationship between the volumetric capacity of the treatment chambers (V\(_c\), mL) and the average flow rate (q, mL s\(^{-1}\)):

\[
t_r = \frac{V_c}{q}
\]

(4)

The pulse voltage difference (V, kV) between electrodes divided by the electrode gap (d, cm) of the treatment chambers was taken as the electric field strength value (E, kV cm\(^{-1}\)):

\[
E = \frac{V}{d}
\]

(5)

The energy per volume unit, also known as the energy density (E\(_d\), MJ L\(^{-1}\)) applied to the grape juice was calculated using the equation (Abram et al., 2003; Sampedro et al., 2007):

\[
E_d = 10^{-6} \cdot E^2 \cdot \sigma \cdot t
\]

(6)

were E (V m\(^{-1}\)) is the electric field strength, \(\sigma\) (S m\(^{-1}\)) is the electrical conductivity of the sample and t (s) is the treatment time.

2.6. Data analysis

All data were organised using an electronic spreadsheet (OpenOffice.org Calc, OpenOffice 2.0, Sun Microsystems Inc. Santa Clara, CA, USA). All statistical assessments and tests with a 95% confidence level, numerical optimisations and figures and graphics were performed using a statistical software application (R Development Core Team, 2006).

2.6.1. Response surface design

A polynomial model was used to fit the experimental S-values of each microorganism according to the least squares method (Eq. (7)). The terms of the polynomial model come from a Taylor development up to second order of the investigated factors (Khuri and Cornell, 1996; Myers and Montgomery, 2002):

\[
S = k_0 + k_1 \cdot E + k_2 \cdot F + k_3 \cdot t + k_4 \cdot E^2 + k_5 \cdot F^2 + k_6 \cdot t^2 + k_7 \cdot E \cdot F + k_8 \cdot E \cdot t + k_9 \cdot F \cdot t + \varepsilon
\]

(7)

where \(k_i\) are the coefficients of the polynomial equation with the corresponding units to obtain a dimensionless value of the response (S); E, F and t are the continuous factors under study; and \(\varepsilon\) is the experimental error.

The least squares method provided the coefficients of the polynomial models and a F-test was used to discriminate the terms of the model that produced statistically significant changes of the response variables. The presence of outliers and influential data was checked for, by means of the studentised residuals and the Cook’s distance, respectively. The goodness of fit and linearity of the fitted model, the independence, normal distribution and constant variance of the residuals, which are the common assumptions in Normal distributions, were analysed using residual plots (Faraway, 2005).

The response surface equation for each microorganism was optimised to obtain the highest value of S. After this, the optimum treatment conditions for maximum inactivation of all the microorganisms in the mixture were identified.

2.6.2. Factorial design

The predictions of the equations obtained from the response surface analysis were compared with the results obtained from the factorial design experiments, to assess the accuracy of the predictions. This was performed for each microorganism calculating the linear correlation (r) between both data sets. Moreover, the factorial data set allowed study of the relationship between the S-values and the type
of microorganism, to confirm the S-values obtained with the response surface methodology. The statistical model used was:

\[ S = \mu + m + \epsilon, \]  

where \( \mu \) is the average S-value if the type of microorganism does not affect the microbial populations after PEF processing, \( m \) is the species of microorganism, and \( \epsilon \) is the random variation of the experimental S-values. Significant differences were assessed using the Tukey honest difference test.

Finally, a graphical distribution of the S-values from the factorial data set against the energy density delivered to the grape juice samples was proposed. The electric conductivity values of Eq. (6) were achieved using the experimental electrical conductivity values for the grape juice at the highest temperature reached during each experiment.

3. Results

The general characteristics of Parellada grape juice were refractive-index, 14.9 ± 0.3 brix; density, 1.060 ± 0.002 kg L\(^{-1}\); pH, 3.79 ± 0.06, and acidity, 3.7 ± 0.3 meq of NaOH·L\(^{-1}\). Its electrical conductivity (\( \sigma, \text{S m}^{-1}\)) was given by the expression:

\[ \sigma = (0.12 \pm 0.01) + (6.0 \pm 0.5) \cdot 10^{-3} \cdot T, \]  

where \( T \) is the grape juice temperature (°C). The positive slope value indicates a gradual increase of grape juice electric conductivity within the range of temperatures found in the study.

The highest S-value for each microorganism were 3.88, 3.94, 3.54 and 2.24 log reductions for \( K. \) apiculata, \( S. \) cerevisiae, the mixture of lactic acid bacteria and \( G. \) oxydans, respectively (Table 1). There were experiments that yielded a null response (\( S=0 \)) as a consequence of the definition of S [Eq. (1)]. The least squares method applied to the data of Table 2 provided estimates of the unknown parameters of the statistical models. Table 2 also shows which factors affected the measured response of each microorganism.

With \( K. \) apiculata the S-values were affected by the treatment time and the pulse frequency (\( t, F, T^2 \) and \( F^2 \)). The significance of the squared factors suggests that the trend of their influence was not linear. The response surface (Fig. 1) displays graphically the effects of these processing parameters. The p-values of the \( k_i \)-estimates of \( S. \) cerevisiae show that it was affected by only the treatment time (\( t \) and \( T^2 \)). Again, the statistical significance of the squared factor showed a non linear effect of the treatment time (Fig. 1).
Fig. 1. Response surface plot of (a) K. apiculata, (b) S. cerevisiae, (c) Lactic acid bacteria and (d) G. oxydans.

Fig. 2. Effect of PEF energy density on (a) K. apiculata, (b) S. cerevisiae, (c) Lactic acid bacteria and (d) G. oxydans.
units for *K. apiculata*, *S. cerevisiae*, lactic acid bacteria and *G. oxydans*, respectively.

Fig. 2 shows the S-values obtained for each microorganism versus the energy delivered to the grape juice. Inactivation of all the microorganisms is maximum around 1500 MJ L\(^{-1}\). Moreover, all inactivation patterns, except that for *G. oxydans*, seem to be similar with the degree of inactivation increasing rapidly with increasing energy density at first, then decreasing slowly as the energy density increases beyond the point of maximum inactivation.

4. Discussion

The reductions obtained in the current study are similar to those reported by other researchers who applied PEF to grape juice. In a study of the effect of PEF processing on the free amino acid and fatty acid contents of Parellada grape juice, *S. cerevisiae* was used as the target microorganism for confirmation of the effectiveness of the treatment (Garde-Cerdán et al., 2007a). Four log reductions for yeast populations were obtained. The treatment conditions and equipment were similar to those used in our study. Wu et al. (2005) achieved a reduction of spoilage flora of up to 4.2 logs using PEF at 50 °C, and they achieved higher reductions when they combined PEF with the addition of various antimicrobials. The same authors confirmed that below 44 °C there was no evidence of lethal thermal effects of PEF treatments on microorganisms. In all the experiments carried out in the current study the temperature was below 31 °C, so any lethal effects of heating during these treatments can be discounted. Jaya et al. (2004) reported final bacterial counts of 400 cfu mL\(^{-1}\) and complete inactivation of the yeasts in grape juice. The authors did not report the initial microbial load or the species present in the juice, so comparison of the findings of that and the current work is difficult.

Although there has been no report of the effects of PEF on specific bacteria in grape juice, information of that sort is available for other juices. Elez-Martínez et al. (2005) obtained a 5.8 log reduction of *Lactobacillus brevis* suspended in orange juice. Rodrigo et al. (2001) and Sampedro et al. (2007) obtained 2.5 log reductions of *L. plantarum* in an orange–carrot juice and an orange juice–milk mixture, respectively. Both used similar PEF conditions with electric field strengths around 35 kV cm\(^{-1}\) and pulses of between 2.5 and 5.0 µs. They concluded that electric field strength and treatment time were the most important parameters affecting the survival of the microorganisms. The disparity of data probably reflects the numerous differences between the conditions used in the various studies.

In our study, the electric field strength had no effect upon yeast populations, although all previous work on PEF indicates that is a key parameter of this technology (Cserhalmi et al., 2002). This discrepancy could be explained by the difference of PEF sensitivity of yeast and bacteria. The critical electric fields strength needed for any effect on bacteria is reported to be between 15 and 20 kV cm\(^{-1}\) (Heinz et al., 2002). Most of the experiments performed at this level in our study gave a null response for bacterial populations. However, the reported lethal threshold value for yeast, specifically for *S. cerevisiae*, is around 7.5 kV cm\(^{-1}\) (Zhang et al., 1994; Molinari et al., 2004). It is then possible that at 20 kV cm\(^{-1}\), which was the lowest level used in the current study, yeasts can be so severely damaged that any further increase of electric field strength would not enhance the lethal effects of PEF treatments.

Analysis of the results also gave information about how microorganism respond to pulse frequency. Some authors have claimed that frequency do not have any biological effects (Schirive et al., 2006). However, pulse frequency allows modulation of the way the electrical energy is applied to food being processed, so it could affect the efficacy of PEF treatments. In thermal processing, heat transfer into a medium depends only on the medium's heat transmission properties, whereas with PEF the rate at which energy is delivered to the product can be selected. The higher the pulse frequency, the higher the rate of energy input. Moreover, a high alternating pulse polarity might stress microbial membranes which would have to adapt to a continuously changing external electric field.

*K. apiculata* was maximally inactivated at intermediate pulse frequencies whereas low and high frequencies reduced the effectiveness of the treatments. Elez-Martínez et al. (2004) reported decreased inactivation of *S. cerevisiae* in orange juice as the pulse frequency of the applied PEF treatments increased. In our study, the pulse frequency of the treatments also affected the inactivation of *G. oxydans*. The effect on the lactic acid bacteria was not so evident and more experiments are needed to clarify the situation with these organisms. Elez-Martínez et al. (2005) reported a decrease of PEF effects on *L. brevis* as pulse frequency increased. More information is needed for explanation of these discrepancies.

The factorial data set showed that the mathematical models that were developed could be used for accurate prediction of yeast but not bacterial S-values. Consequently, the optimum treatments identified from the models would be more appropriate for yeast than for bacteria.

The species most commonly found in grape juice are *K. apiculata*, *S. cerevisiae*, lactic acid bacteria, that are mainly *L. plantarum* and *L. hilgardii*, and acetic acid bacteria that are mainly *G. oxydans*. Typical populations contain yeasts at 10⁶ to 10⁷ cfu mL\(^{-1}\), lactic acid bacteria at 10⁵ to 10⁶ cfu mL\(^{-1}\), and acetic acid bacteria at about 10⁴ cfu mL\(^{-1}\) (Hidalgo-Togores, 2002; Ribèreau-Gayon et al., 2003). When comparing these values with the log reductions of the model predictions, it is evident that the optimised PEF treatment of 35.0 kV cm\(^{-1}\) with pulses of 303 Hz for 1 ms can reduce microbial populations although not enough for adequate pasteurisation. In wine making, a PEF treatment of such characteristics could greatly reduce the natural flora without the adverse effects on juice quality of heating (Garde-Cerdán et al., 2007a). The treated juice could be then inoculated with a selected yeast strain to lead the fermenting process.

The optimisation process was aimed at identifying the best treatment for reducing microbial populations although it could be optimised to look for the efficiency of the process. In this context, the response of microbial populations to the energy density applied to grape juice seemed to have the same trend for all the microorganisms. Other authors (Sampedro et al., 2007) have studied the effects of energy density, but only at low energy ranges and with only single microorganisms, which precludes useful comparison of their findings with ours.

Evidently the natural flora of grape juice can be greatly reduced using PEF treatments. Increasing the treatment time increased the level of microbial inactivation; and the electric field strength and pulse frequency should be carefully selected for each microorganism. The response surface methodology seemed to be a suitable tool for defining optimum PEF treatment conditions for real food commodities.

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References


BOE. 1988. Orden de 27 de abril de 1988, métodos oficiales de análisis de zumos de uva. 7 de mayo, núm. 110, pp. 13905.
