Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Impact of pulsed-electric field and high-voltage electrical discharges on red wine microbial stabilization and quality characteristics

C. Delsart[1,2], N. Grimi[3], N. Boussetta[3], C. Miot Sertier[1,2], R. Ghidossi[1,2], E. Vorobiev[3] and M. Mietton Peuchot[1,2]

1 USC 1366 Œnologie, ISVV, INRA, Villenave d'Ornon Cedex, France
2 Unité de recherche Œnologie, EA 4577, ISVV, Université de Bordeaux, Villenave d'Ornon, France
3 Equipe Technologies Agro-industrielles, Centre de Recherche de Royallieu, Transformation Intégrée de la Matière Renouvelable (TIMR, EA 4297, UTC/ESCOM), Université de Technologie de Compiègne, Sorbonne Universités, Compiègne, France

Keywords
bacteria, high-voltage electrical discharge, pulsed-electric field, stabilization, wine, yeast.

Correspondence
Nabil Grimi, Equipe Technologies Agro-industrielles, Centre de Recherche de Royallieu, Transformation Intégrée de la Matière Renouvelable (TIMR, EA 4297, UTC/ESCOM), Université de Technologie de Compiègne, Sorbonne Universités, B.P. 20529, 60205 Compiègne, France.
E-mail: nabil.grimi@utc.fr

2015/1287: received 27 June 2015, revised 17 September 2015 and accepted 25 September 2015

doi:10.1111/jam.12981

Abstract

Aims: In this study, pulsed-electric fields (PEF) and high-voltage electrical discharges (HVED) are proposed as new techniques for the microbial stabilization of red wines before bottling. The efficiency of the treatment was then evaluated.

Methods and Results: PEF and HVED-treatments have been applied to wine for the inactivation of *Oenococcus oeni* CRBO 9304, *O. oeni* CRBO 0608, *Pediococcus parvulus* CRBO 2.6 and *Brettanomyces bruxellensis* CB28. Different treatment times (1, 2, 4, 6, 8 and 10 ms) were used at 20 kV cm^{-1} for the PEF treatments and at 40 kV for the HVED treatments, which correspond to applied energies from 80 to 800 kJ l^{-1}. The effects of the treatments on the microbial inactivation rate and on various characteristics of red wines (phenolic composition, chromatic characteristics and physico-chemical parameters) were measured.

Conclusions: The application of PEF or HVED treatments on red wine allowed the inactivation of alteration yeasts (*B. bruxellensis* CB28) and bacteria (*O. oeni* CRBO 9304, *O. oeni* CRBO 0608 and *P. parvulus* CRBO 2.6). The electric discharges at 40 kV were less effective than the PEF even after 10 ms of treatments. Indeed, 4 ms of PEF treatment at 20 kV cm^{-1} were sufficient to inactivate all micro-organisms present in the wines. Also, the use of PEF had no negative impact on the composition of wines compared to the HVED treatments. Contrary to PEF, the phenolics compounds were degraded after the HVED treatment and the physico-chemical composition of wine were modified with HVED.

Significance and Impact of the Study: PEF technology seems to be an interesting alternative to stabilize microbiologically wines before bottling and without modifying their composition. This process offers many advantages for winemakers: no chemical inputs, low energy consumption (320 kJ l^{-1}), fast (treatment time of 4 ms) and athermal ($\Delta T \approx 10°C$).

Introduction

Some strains of micro-organisms, such as *Brettanomyces bruxellensis* (*B. bruxellensis*) (ethylphenol producers) (Chatonnet *et al.* 1992), *Oenococcus oeni* (*O. oeni*) (biogenic amine producers) (Lonvaud-Funel 2001), or even some strains of *Pediococcus parvulus* (*P. parvulus*) (polysaccharide producers) (Dols-Lafargue *et al.* 2008) which can alter the red wine quality must be eliminated in order to preserve it. The microbiological stabilization of red wines

before bottling is usually obtained by the addition of sulphur dioxide (Jackson 2008). The authorized concentration of sulphur dioxide is regularly reduced by the European Union due to the allergenic effect of the molecule and other harmful effects on human health (Puértolas *et al.* 2009). Actual researches are focused into new chemical products and physical processes that could complement or replace sulphur dioxide (Guerrero and Cantos-Villar 2015).

Studies have been carried out on additives such as sorbic acid, lysozyme, octanoic and decanoic acids, as well as numerous antibiotics and antiseptics (5-nitrofurylacrylic acid, pimaricine, dimethyldicarbonate, etc.). Sorbic acid is known to have a fungicidal action by disrupting homeostasis pH of cells (Plumridge *et al.* 2004) but it does not affect bacteria that degrade the sorbic acid into 2-ethoxy-3,5-hexandiene which gives off a geranium-like off-odour (Crowell and Guymon 1975). Saturated short-chain fatty acids such as octanoic and decanoic acids possess significant fungicidal properties (Geneix *et al.* 1983) but they have no effect on bacteria. Dimethyldicarbonate acts on yeasts by inhibiting certain glycolytic enzymes but below authorized maximal concentration it is not an effective preservative against lactic acid and acetic acid bacteria (Costa *et al.* 2008). Other alternatives have been introduced based on 'natural antimicrobial agents', of which the use of lysozyme is especially important, as well as some antimicrobial peptides or bacteriocins (Navarro *et al.* 2000). As lysozyme can cause human IgE-mediated immune reactions (Mine and Zhang 2002), its presence in food products, including wine, can cause some concerns. Nisin is a bacteriocin exhibiting antimicrobial activities towards a wide range of Gram-positive bacteria by forming pores in the cytoplasmic membrane but some strains of bacteria and many yeasts tolerate nisin via a nisinase activity (Rojo-Bezares *et al.* 2007).

Different physical processes aimed at the partial or total elimination of micro-organisms, such as thermal treatment, high pressures, filtration, racking and centrifugation, have also been studied. The thermal treatment of foods (pasteurization or sterilization) is remarkably widespread, and has proved its efficiency in the inactivation of spoilage and pathogenic micro-organisms. In parallel to this effectiveness, thermal treatments induce well-documented modifications in colour and flavour or even significant nutritional losses. The practical conditions vary according to the micro-organism, alcohol and sugar content, pH, and the phase of cell multiplication (Couto *et al.* 2005). Sterile microfiltration is effective for removing any type of micro-organism in wine, but the fouling of the membrane for some wines rich in suspended particles and in colloids such as polysaccharides, polyphenols and proteins, is still a problem. Indeed, this phenomenon may affect wine quality by the retention of some compounds and may also reduce permeation rates, which will have a negative effect on the economic viability of the process (El Rayess *et al.* 2011). High pressures (400–600 MPa) at chilled or mild process temperatures (<45°C) allow foods to be preserved with minimal effects on taste, texture, appearance, or nutritional value (Balasubramaniam *et al.* 2008). Similar conclusions were arrived at in the various studies that have been carried out on wine (Buzrul 2012). The limiting factor in using high-pressure treatments is the cost of the equipment.

Therefore, the wine sector needs to develop alternative or complementary techniques that are efficient, easy to implement, which consume little energy, and which will lead to a reduction in the use of the sulphur dioxide. Pulsed-electric fields (PEF) and high-voltage electrical discharges (HVED) fit these criteria. PEF and HVED methods require short times of treatment (a few ms) and low energy consumption depending on the treated product and the required application. Depending on the PEF generator characteristics, the energy cost of a PEF treatment (taking into account investment (85 k€) and operation costs) is about 0·12 € kWh^{-1} (El Crack generator (5 kW), Toepfl *et al.* 2006). When comparing PEF and high-pressure treatment, the estimated total costs for liquid processing reaches 200–700 € per ton for high-pressure treatment but only 10–80 € per ton for PEF. The biological cell subjected to PEF sees its transmembrane potential rise. This transient or permanent permeabilization of the cell membrane allows the transfer of intracellular material into the extracellular medium (Zimmermann 1986). Several studies have been carried out relating to PEF treatment on different products (Grimi *et al.* 2007) for various applications such as the destruction of micro-organisms at low temperatures (Delsart *et al.* 2015), changes in enzyme activity (Giner-Segui *et al.* 2009), or the extraction of plant cellular components (Corrales *et al.* 2008; Donsì *et al.* 2010; Delsart *et al.* 2012; Garde-Cerdán *et al.* 2013). This technology is athermal process, does not require or create chemical inputs (enzymes, dioxide...), and implies a low energy cost (a few hundred kJ l^{-1} for sterilization) (Marsellés-Fontanet *et al.* 2009). Furthermore, this technique is easy to integrate into an existing process and has already been implemented on an industrial scale for the production of apple juice at a rate of 20 000 kg h^{-1} (Heinz *et al.* 2003). High-Voltage Electric Discharges (HVED) create electric arcs by the application of electric fields between two pointed or flat electrodes. The intensity of the electric fields is higher than that used in PEF treatments and causes the fragmentation of solid particles. Different applications of electrical discharges have been studied:

degradation of organic compounds in an aqueous solution (Surgiarto and Sato 2001), inactivation of micro-organisms (Delsart *et al.* 2015), improvement of the extraction of high-value compounds from biological products such as polyphenols from grape pomaces (Boussetta *et al.* 2011).

The objective of this study is to determine the optimum parameters of PEF or HVED (variation in the duration and/or the electric field strength) in order to reduce the number of micro-organisms in the treated red wine. The inactivation of three lactic acid bacteria (*O. oeni* strain CRBO 9304, *O. oeni* strain CRBO 0608, *P. parvulus* strain CRBO 2.6) and one species of yeast (*B. bruxellensis* CB28) was studied. The consequences of PEF and HVED on the polyphenols, colour and the physico-chemical composition of the treated red wine were also analysed.

Materials and methods

Red wine samples preparation

A commercial red wine (Côtes de Bordeaux, 2010) was prepared prior to microbial inoculation. Free SO_2 was eliminated from the wine using hydrogen peroxide at 35%. The wine was subjected to a first clarifying plate filtration (SEITZ K200; Pall Corporation, New York, NY) and a second sterilizing plate filtration (SEITZ KS80; Pall Corporation), and then transferred to four autoclaved (sterilized) 10-l tanks in the aim to inoculate separately four different micro-organisms.

Micro-organisms and cultivation/inoculation conditions

Four micro-organisms were selected according to their representativeness and dangerousness in red wines. These organisms were three lactic acid bacteria (*O. oeni* strain CRBO 9304, *O. oeni* strain CRBO 0608, *P. parvulus* strain CRBO 2.6) and one species of yeast (*B. bruxellensis* CB28). The three strains of lactic acid bacteria were grown in an MRS (Man, Rogosa, Sharpe) liquid culture medium while *B. bruxellensis* was grown in YPG (Yeast, Peptone, Glucose) liquid culture medium. These laboratory strains were gradually adapted to media resembling more and more to the composition of wine in order to facilitate their implantation into the wine by avoiding cell stress (Renouf 2006). For the bacteria, the initial medium used was the ELB (Enrichment Lactic Bacteria) medium described by Renouf (2006). As for the *B. bruxellensis* yeast, the medium used was the EBB (Enrichment *B. bruxellensis*) medium described by Renouf and Lonvaud-Funel (2007). When the microbial population reached the end of the exponential phase in these media, a similar medium was prepared and supplemented with ethanol (6% v/v) to receive the previous microbial culture (inoculated at 10% v/v). Following that a new medium was prepared with ethanol (10% v/v) and inoculated via the 2nd medium at 10% v/v. Each strain contained in these media was assessed by a direct epifluorescence method (Divol and Lonvaud-Funel 2005). Depending on the microbial concentration a certain volume of these inocula was mixed into 8–8·5 l of sterilized red wine. These volumes represent less than 50 ml. Inoculations were performed 24 h before electric treatments. The four batches of wine were then kept at room temperature.

PEF and HVED equipment

The laboratory PEF and HVED batch treatment system (Fig. 1) consisted of a 1 l cylindrical polyethylene treatment chamber comprising two electrodes (anode and cathode), connected to a high-voltage pulse generator (Tomsk Polytechnic University, Tomsk, Russia). To obtain a pulsed-electric field, two parallel stainless steel plate-electrodes (diameter = 100 mm) were used with a distance (d, mm) between electrodes of 20 mm. For the creation of electrical discharges, the plate electrode (diameter = 30 mm) was used in conjunction but with a stainless steel rod-electrode (diameter = 10 mm) with a fixed inter-electrode distance (d, mm) of 5 mm (Fig. 1).

The generator of voltage $U = 40$ kV and intensity $I = 10$ kA could provide peak power up to $4{\cdot}10^5$ kW. Pulses were generated at frequency, $f = 0{\cdot}5$ Hz for PEF treatment and $f = 0{\cdot}33$ Hz for HVED treatment, by the discharge of two capacitors in parallel. The pulse shape was exponential decay for the PEF treatment and oscillatory decay for the HVED treatment. Pulse width t_i was approx. 10 μs. A pause Δt_t of 60 s was made every 50

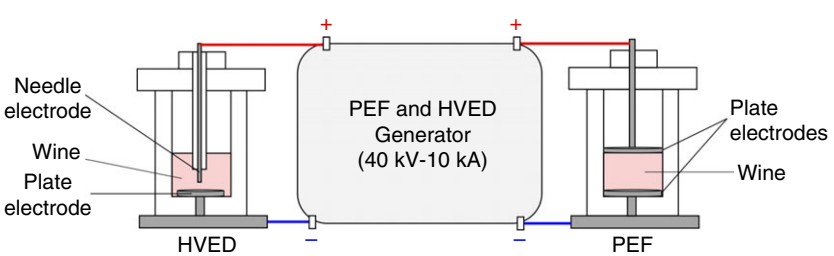

Figure 1 Experimental design with the electrodes configuration of chambers used for pulsed-electric fields and high-voltage electrical discharges treatments.

pulses. The PEF- or HVED-treatment time of samples (t_{PEF} or t_{HVED}, ms) were calculated as the product of average pulse width (t_i, μs) and the number of pulses (n) delivered in the treatment chambers. The total specific energy applied (W, kJ l^{-1}) was determined by the following formula: $W = (n \times P_i)/V$, where V is the volume (l) of the treated wine and P_i is the energy of one electric pulse (J) calculated from the following formula: $P_i = (C \times U^2)/2$ with C, the capacity of the capacitor (200 nF) and U, the voltage of the generator (40 kV).

Microbial inactivation experiments

For the determination of optimum parameters, the 200 ml aliquots were PEF or HVED treated according to the experimental designs. The control samples were not treated. Different treatment times (1, 2, 4, 6, 8 and 10 ms) were used at 20 kV cm^{-1} for the PEF treatments and at 40 kV for the HVED treatments. Energy consumption during the treatments ranged from 80 to 800 kJ l^{-1} depending on the different treatment modes. The two batches of wine that contained separately *O. oeni* CRBO 0608 and *P. parvulus* were not subjected to HVED treatments.

The treatments were performed under aseptic conditions. The cell was flamed with ethanol (96% v/v) and rinsed with sterilized distilled water between each treatment. Before and after treatments, the temperature (T, °C) of the wine in the treatment chamber was measured by means of a teflon-coated K-type thermocouple (±0·1°C) connected to the data logger thermometer Center 305/306 (JDC Electronic SA, Yverdon-Les-Bains, Switzerland). Then, the samples were placed in sterile containers and stored at 10°C, without light.

Determination of viable count

Immediately after the treatments, aliquots were taken in order to determine the number of micro-organisms as per the methods described by the O. I. V. (2013). The yeast and bacteria were recovered onto YPG or onto MRS respectively. The compositions of YPG and MRS media are detailed by the O. I. V. (2013). Pimaricine was added into the MRS media at a concentration of 100 mg l^{-1} in order to inhibit the growth of yeasts and moulds. Biphenyl (150 mg l^{-1}), chloramphenicol (100 mg l^{-1}) and cycloheximide (1 mg l^{-1}) were incorporated into the YPG media for the inactivation of moulds, bacteria and *Saccharomyces* yeast. Three consecutive decimal dilutions and an undiluted sample were taken. This operation was repeated in triplicate. Plates were incubated for 10 days at 30°C for *B. bruxellensis* in an aerobic atmosphere and 10 days at 25°C for *O. oeni* and *P. parvulus* in an anaerobic atmosphere. After incubation, the colony forming units (CFU) were counted.

Analyses of the wine

The physico-chemical parameters (pH, total and volatile acidity, concentration of glucose-fructose, residual reducing sugar, malic acid, lactic acid and tartaric acid) of wine samples were measured 8 days after electric treatments by means of a near infra-red spectrophotometric analyser (FOSS WineScan™ Flex 79000, FOSS, Hillerød, Denmark). Colour intensity (CI) was calculated as the sum of absorbance at 420, 520, and 620 nm of the wine using a spectrophotometer (Lambda 25 UV/Vis Spectrometer, PerkinElmer, Waltham, MA) with a 1 mm path-length quartz cuvette (O. I. V. 2013). The measurements of CI were carried out 8 days after electric treatments. Total polyphenol index (TPI), tannin content, and anthocyanin content were determined using the same spectrophotometer. TPI was measured 8 days after electric treatments by direct reading of the absorbance at 280 nm of wine diluted at 1/100 (v/v) (O. I. V. 2013). Tannin content was measured 16 days after electric treatments, comparing the absorbances at 550 nm of an acidified sample and a sample that was both acidified and heated at 100°C for 30 min (Ribéreau-Gayon and Stonestreet 1964). Anthocyanin analysis was based on a method using differences in pH and decoloration with sulphur dioxide (Ribéreau-Gayon and Stonestreet 1965) and was carried out 16 days after the treatments.

Statistical analysis

Each experiment was repeated at least three times. The data were collected via MICROSOFT® EXCEL® 2011 software (ver. 14.2.2) (Redmond, WA). The data were subjected to analysis of variance ($P \leq 0{\cdot}05$) to determine the significant differences between means using XLSTAT (ver. 2012) (Addinsoft, Paris, France). A Fisher test, at a significance level of $P = 0{\cdot}05$, was conducted for the separation of means. Groups detected as significantly different were marked in the table with different letters. Values were reported in the table as mean ± standard deviation. The error bars in all figures correspond to standard errors.

Results

Temperature variation

The temperature increase ΔT of the wine in treatment chamber measured before and after PEF or HVED treatments is presented in Fig. 2.

The higher the treatment energy, the greater was the resulting temperature increase (ΔT = 23°C after PEF treatment at 800 kJ l^{-1} and ΔT = 11·3°C for the same sample treated with PEF treatment at 80 kJ l^{-1} (wine inoculated with *P. parvulus*)). The temperature increase was greater after PEF treatments as compared to the HVED treatments (ΔT = 16·9°C after PEF treatment (W = 800 kJ l^{-1}) and ΔT = 11·4°C after HVED treatment (W = 800 kJ l^{-1}) for *B. bruxellensis*). Although a pause Δt_t of 60 s was made after each train of 50 pulses or 50 discharges in order to protect the thermosensitive molecules of the wine, maximum temperatures measured further to PEF and HVED treatment were 42·5°C (ΔT = 23°C, *P. parvulus*) and 34·4°C (ΔT = 14·4°C, *O. oeni* 9304) respectively.

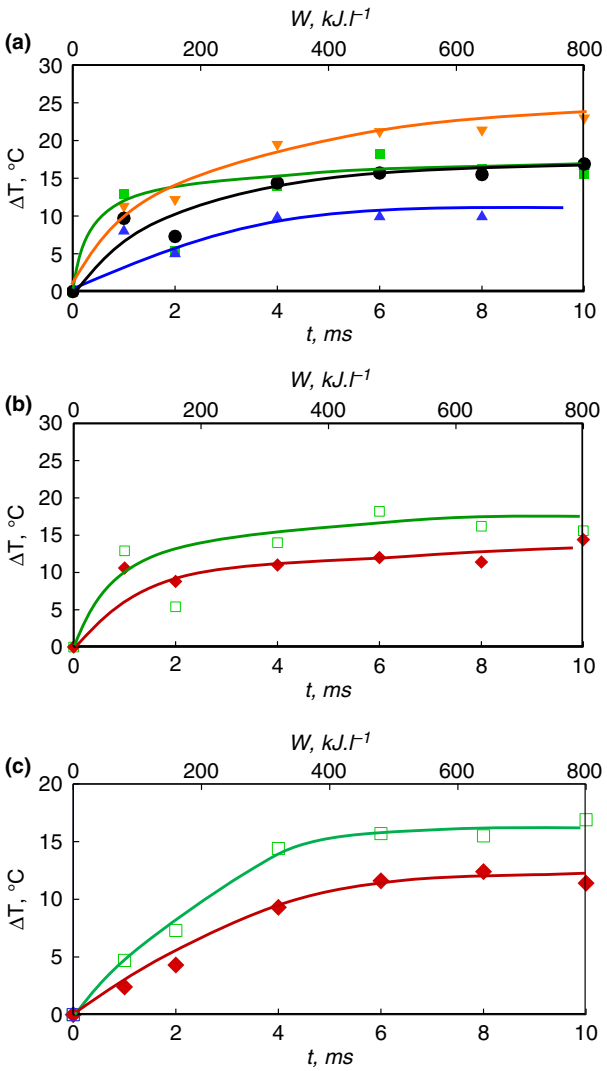

Figure 2 Temperature variations ΔT of red wines inoculated with different micro-organisms separately (*Pediococcus parvulus* (▼), *Oenococcus oeni* 9304 (■), *Brettanomyces bruxellensis* (●) and *O. oeni* 0608 (▲)) for pulsed-electric fields (PEF) at 20 kV cm^{-1} in function of energy inputs and treatment times (a). Temperature variations ΔT on wine inoculated with *O. oeni* 9304 (b) or *B. bruxellensis* (c) after PEF (□) or high-voltage electrical discharges (◆) treatments with different energy inputs and treatment times.

Bacteria and yeast inactivation in red wines

The results of counts for *P. parvulus* CRBO 2.6, *B. bruxellensis* CB 28, and *O. oeni* CRBO 9304 and CRBO 0608 in the wine samples were plotted (Fig. 3) against specific energy.

The initial concentrations of the different micro-organisms were 2·09 × 10^5, 9·56 × 10^1, 1·11 × 10^1 and 5·90 × 10^4 CFU ml^{-1} for *O. oeni* CRBO 9304, *O. oeni*

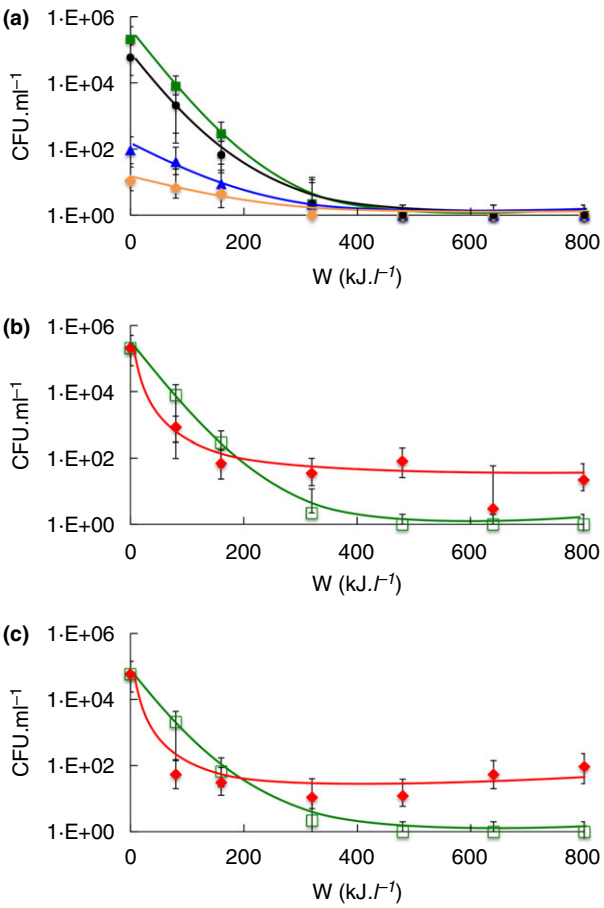

Figure 3 Counting of different micro-organisms inoculated in red wine (*Pediococcus parvulus* (◆), *Oenococcus oeni* 9304 (■), *Brettanomyces bruxellensis* (●) and *O. oeni* 0608 (▲)) for pulsed-electric fields (PEF) at 20 kV cm^{-1} in function of energy inputs (a). Counting of *O. oeni* 9304 (b) and *B. bruxellensis* (c) inoculated separately in red wine after PEF (□) or high-voltage electrical discharges (◆) treatments with different energy inputs.

CRBO 0608, *P. parvulus* and *B. bruxellensis* respectively. The inactivation of bacteria and yeasts in wine by electrical treatments increased with the augmentation of energy input. Contrary to the HVED treatment, total inactivation of these bacteria and yeasts by the PEF was observed. For example, for the same duration and energy of electric treatment (t = 4 ms, W = 320 kJ l^{-1}), the PEF treatment inactivated all the micro-organisms (Fig. 3a), whereas after the HVED treatment, the wine still contained 1 log cycle of *O. oeni* CRBO 9304 (Fig. 3b) and *B. bruxellensis* (Fig. 3c). PEF treatment at 20 kV cm^{-1} for 4 ms (W = 320 kJ l^{-1}) seems to provide a satisfactory degree of inactivation of the four micro-organisms under study.

The physico-chemical parameters of red wines

Different physico-chemical parameters of wines (pH, total and volatile acidity (TA and VA), concentration of glucose-fructose (GF), residual reducing sugar (RRS), malic acid (Mal), lactic acid (Lac) and tartaric acid (Tar)) were measured in wines (Table 1).

Two measurements were made to determine the quantity of sugars in the wines: glucose-fructose and residual reducing sugar concentrations. Following the HVED treatments, the glucose–fructose and residual reducing sugar concentrations increased by up to 123 and 111% respectively in the wines inoculated with *B. bruxellensis*. The PEF treatments on the wines also resulted in an increase in glucose-fructose (up to 50% for wines inoculated with *B. bruxellensis*) and residual reducing sugar concentrations (up to 56% for wines inoculated with *B. bruxellensis*) except for the wines inoculated with *O. oeni* 0608 (−3 and −6% for the glucose-fructose and residual reducing sugar concentrations respectively).

The pH was only slightly modified by the electric treatments. Following the PEF or HVED treatment (10 ms), the pH increased to a maximum of 1% (*B. bruxellensis*) and 4% (*B. bruxellensis* and *O. oeni* 9304) respectively. Inversely, the total acidity of the treated wines actually did not change (*P. parvulus* wines) or decreased compared to the untreated wines except for the wines inoculated with *O. oeni* 0608 (+4%). This decrease was greater in wines treated with electric discharges (−15% with *B. bruxellensis* and *O. oeni* 9304) than in wines treated with PEF (−5% with *B. bruxellensis*). As for the concentrations of organic acids, malic acid was not present in any of the wines while the tartaric acid concentrations were higher than those of lactic acid. Further to the electric treatments, the concentrations of lactic acid decreased while those of tartaric acid increased with the exception of wines inoculated with *O. oeni* 0608. These variations were more pronounced in the wines treated with HVED (the maximum increase in tartaric acid was 15% for

Table 1 Physico-chemical parameters of controls and wines treated by pulsed-electric fields or high-voltage electrical discharges

	Oenococcus oeni 9304			*Oenococcus oeni* 0608			*Pediococcus parvulus*			*Brettanomyces bruxellensis* CB28		
	Control	20 kV cm^{-1}, 10 ms	40 kV, 10 ms	Control	20 kV cm^{-1}, 10 ms	40 kV, 10 ms	Control	20 kV cm^{-1}, 10 ms	40 kV, 10 ms	Control	20 kV cm^{-1}, 10 ms	40 kV, 10 ms
GF, g l^{-1}	1·60 ± 0·00a	1·90 ± 0·14b	1·95 ± 0·07b	1·50 ± 0·00a	1·45 ± 0·07a		1·60 ± 0·00a	1·85 ± 0·07b		1·30 ± 0·00a	1·95 ± 0·07b	2·90 ± 0·14c
RSS, g l^{-1}	2·05 ± 0·07a	2·50 ± 0·14b	2·95 ± 0·07c	1·70 ± 0·00a	1·60 ± 0·00b		2·00 ± 0·00a	2·40 ± 0·00b		1·80 ± 0·00a	2·80 ± 0·00b	3·80 ± 0·00c
pH	3·65 ± 0·00a	3·66 ± 0·00a	3·80 ± 0·04b	3·63 ± 0·00a	3·66 ± 0·03a		3·64 ± 0·00a	3·67 ± 0·01b		3·63 ± 0·00a	3·67 ± 0·00b	3·79 ± 0·00c
TA, gH_2SO_4 l^{-1}	2·99 ± 0·00a	2·96 ± 0·00a	2·53 ± 0·00b	2·86 ± 0·00a	2·98 ± 0·01b		3·00 ± 0·01a	3·03 ± 0·01a		3·02 ± 0·01a	2·88 ± 0·01b	2·58 ± 0·01c
VA, gCH_3COOH l^{-1}	0·39 ± 0·00ab	0·38 ± 0·01a	0·46 ± 0·04b	0·39 ± 0·00a	0·33 ± 0·01b		0·39 ± 0·01a	0·35 ± 0·01b		0·42 ± 0·01a	0·37 ± 0·01b	0·44 ± 0·01c
Mal, g l^{-1}	0·00 ± 0·01a	0·00 ± 0·00a	0·00 ± 0·11a	0·00 ± 0·01a	0·00 ± 0·03a		0·00 ± 0·02a	0·00 ± 0·01a		0·00 ± 0·04a	0·00 ± 0·01a	0·00 ± 0·01a
Lac, g l^{-1}	0·98 ± 0·02a	0·92 ± 0·02a	0·76 ± 0·07b	0·93 ± 0·03a	1·08 ± 0·01b		0·97 ± 0·01a	0·97 ± 0·00a		0·97 ± 0·02a	0·86 ± 0·02b	0·72 ± 0·01c
Tar, g l^{-1}	2·16 ± 0·01a	2·36 ± 0·01ab	2·46 ± 0·11b	1·98 ± 0·01a	1·97 ± 0·13a		2·14 ± 0·01a	2·35 ± 0·00b		2·17 ± 0·01a	2·52 ± 0·01b	2·50 ± 0·00b

Different letters correspond to the groups detected as significantly different in Fisher test (α = 5%).

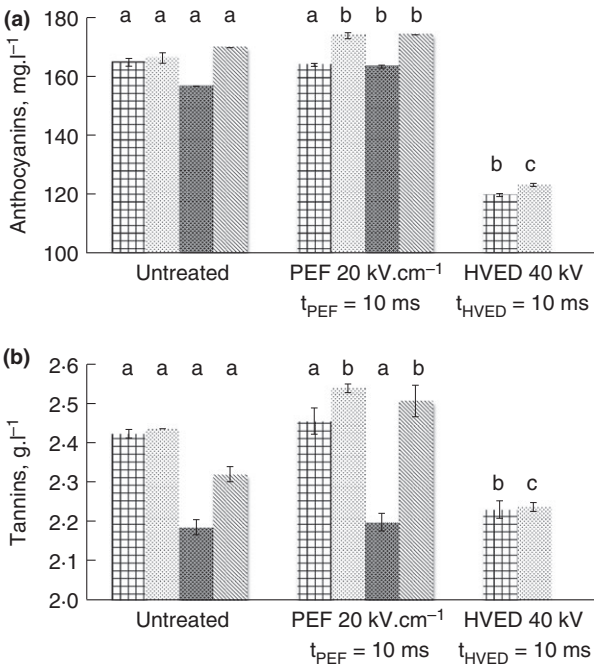

Figure 4 Influence of pulsed-electric fields or high-voltage electrical discharges treatments on anthocyanin (a) and tannin concentrations (b) in wine inoculated with *B. bruxellensis* (▦), *Oenococcus oeni* 9304 (▨), *O. oeni* 0608 (▩), *or Pediococcus parvulus* 2.6 (▧). (Different letters correspond to the groups detected as significantly different in Fisher test (α = 5%)).

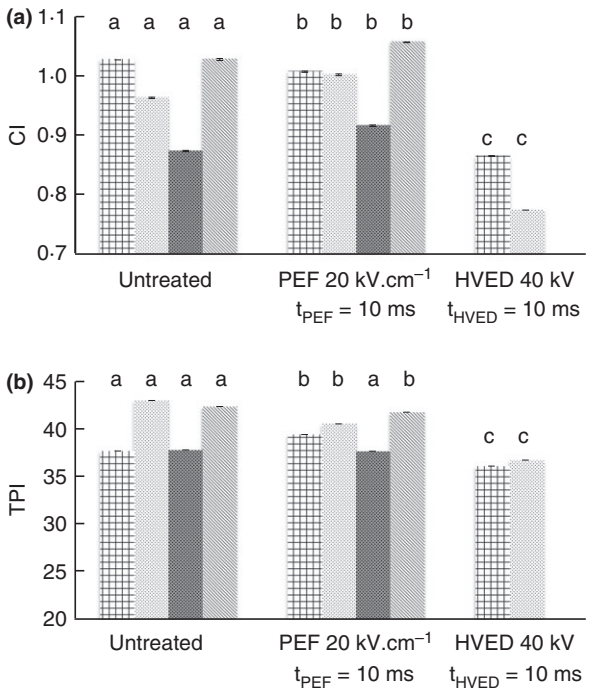

Figure 5 Influence of pulsed-electric fields and high-voltage electrical discharges treatments on colour intensity (a) and on total polyphenol index (b) of wine inoculated with *Brettanomyces bruxellensis* (▦), *Oenococcus oeni* 9304 (▨), *O. oeni* 0608 (▩) *or Pediococcus parvulus* 2.6 (▧). (Different letters correspond to the groups detected as significantly different in Fisher test (α = 5%)).

B. bruxellensis wines and O. oeni 9304 *wines*, and the maximum decrease in lactic acid was 26% for *B. bruxellensis wines*) as compared with wines subjected to PEF treatments (the maximum increase in tartaric acid was 16% and the maximum decrease in lactic acid was 11% for *B. bruxellensis wines*).

Following PEF treatments, the volatile acidity in the wines decreased by as much as 17% (wines inoculated with *O. oeni* 0608), whereas the volatile acidity in the wines treated with HVED increased up to 17% (wines inoculated with *O. oeni* CRBO 9304). The decrease in volatile acidity due to the PEF treatments was generally more pronounced as compared to the increase in this analytic parameter due to the HVED treatments.

The phenolic composition and chromatic characteristics of red wines

The consequences of treatments (PEF at 20 kV cm^{-1}, 10 ms; HVED at 40 kV, 10 ms) on wine colour were studied by measuring the concentrations of anthocyanins and tannins, the Colour Intensity (CI), and the Total Polyphenol Index (TPI) (Figs 4 and 5).

The concentrations of anthocyanins were similar or superior (by up to 5% for *O. oeni* 9304 wines) in the PEF-treated wines as compared to the control. The concentrations decreased (by up to 27% for *B. bruxellensis* wines) with the HVED treatments as compared to the control wines (Fig. 4a).

The concentrations of tannins in wines treated with HVED decreased (by up to 8% for *B. bruxellensis* and *O. oeni* 9304) while the PEF treatments resulted in a slight increase (up to 4 and 8% for *O. oeni* 9304 and *P. parvulus* respectively) (Fig. 4b).

The measurements for colour intensity in the wine before and after treatments are given in Fig. 5a. The CI of wines treated with PEF decreased (by up to 2% for *B. bruxellensis wines*) or increased (by up to 5% for *O. oeni* 0608 wines) slightly, whereas the HVED treatments resulted in a decrease (up to 20% for *O. oeni* 9304 wines) of the CI.

The determinations of total polyphenol index in the wine before and after treatments are given in Fig. 5b. Further to PEF treatment, the TPI of the wines decreased (by up to 6% for *O. oeni* 9304 wines) or increased (by up to 5% for *B. bruxellensis wines*) as compared to control wines. The TPI of the wines treated by HVED decreased (by up to 15% for *O. oeni* 9304 wines).

Discussions

Temperature variation following PEF or HVED treatment

Even if PEF or HVED are considered as nonthermal technologies, an increase in temperature was observed due to the electric current flowing through the wine (ohmic heating). Like observed in the Fig. 2, the temperature of electrically treated solution increases with the energy input. The temperature augmentation was greater after PEF treatments as compared to the HVED treatments. The different temperature increases can be explained by the frequency ($f = 0{\cdot}5$ Hz for PEF treatment and $f = 0{\cdot}33$ Hz for HVED treatment) and the shape of pulses (exponential decay for PEF and oscillatory decay). In both cases (HVED and PEF), the initial energy delivered by the generator is totally transferred into the treatment cell. In the case of PEF, this energy dissipates by heat conduction (Joule effect). In the case of HVED, the initial energy is also converted into mechanical energy (shock wave), into chemical energy (active species in the arc channel), into light. As a result, there is less energy converted into heat. Once the arc disappears, there is a rapid voltage decrease; the electric field around the arc also decreases and consequently the heat conduction is reduced.

Note that the heat generated during the treatment was not sufficient to directly inactivate micro-organisms (Zimmermann *et al.* 1974). Indeed, the higher the temperature, the greater was the conductivity of the treatment medium. A higher temperature increases cell membrane fluidity (Stanley 1991) and facilitates mass transfer from cells of certain compounds such as organic acids, which leads to an increased inactivation rate (Liu *et al.* 1997). In order to improve the inactivation effect without damaging the compounds of interest in the product, such as pigments and flavours, some researchers even recommend that PEF should be used at moderate temperatures, i.e. in the range of 45–55°C (Dunn and Pearlman 1987). However, as these temperatures are not reached, the inactivation of micro-organisms will not be a direct function of this parameter.

Bacteria and yeast inactivation in red wines following PEF or HVED exposure

The only study relating experimental results about the inactivation by PEF of yeasts and bacteria in wines shows that the population of *Dekkera bruxellensis*, *Dekkera anomala*, *Saccharomyces bayanus*, *Lactobacillus hilgardii* and *Lactobacillus plantarum* can be reduced by 5 log cycle after the application of a field strength of 31 kV cm^{-1} and an energy input superior to 300 kJ kg^{-1} (Puértolas *et al.* 2009). In our case with others strains of micro-organisms, the total inactivation (until 5 log cycle) has been accomplished following a PEF treatment of 20 kV cm^{-1} and an energy input of 320 kJ kg^{-1}. After application of HVED at 40 kV with different treatment times on red wines, bacteria and yeasts were not totally inactivated contrary to the PEF treatments. The main reasons for the lower efficiency of HVED compared to PEF are the temperature increase, the cell configuration and the treatment conditions. The temperature increase after PEF treatment seems to be more important than after HVED treatment and can to be a first explanation of this difference of efficiency between both techniques. In the case of batch processes, the main process parameters that affect the inactivation of micro-organisms are: electric field strength, intensity, number of pulses, frequency, pulse shape, specific energy, temperature and configuration of the treatment chamber (volume, gap) (Wouters *et al.* 2001). Under similar treatment conditions, different inactivation levels of microbial flora will be obtained according to the characteristics of the micro-organisms. The strength of the electric fields required to induce membrane permeabilization is related to the geometry and the size of the cell but also membrane constitution. As the size of the cells increases, the critical electric field decreases (Grahl and Märkl 1996). Thus, yeasts are more sensitive to electric field pulses than bacteria due to their larger size. In the present study, the *B. bruxellensis* yeasts did not seem to be more sensitive than the bacteria. Under stressful conditions, a certain part of the *B. bruxellensis* yeasts can take a resistant form ($<0{\cdot}45$ μm) known as viable but nonculturable (Millet and Lonvaud-Funel 2000), which could explain this resistance to electric fields. Moreover, variations in cell shape influence the inactivation rates induced by electric treatments. Heinz *et al.* (2002) demonstrated that, in order to achieve the same effect on a rod-shaped cell, there is a need of an electrical field that is five times stronger than for a spherical cell of similar dimension. This is explained by the fact that rod-shaped cell take up a position parallel to the direction of the electric field, which induces a lesser degree of electroporation (Eynard *et al.* 1997). *Oenococcus oeni* and *P. parvulus* cells have a spherical shape and may therefore be more sensitive to the electric field pulses than *B. bruxellensis* cells, which have a more or less elongated oval shape (Van der Walt 1970). The initial concentration of micro-organisms varied from $1{\cdot}11 \times 10^1$ to $2{\cdot}09 \times 10^5$ CFU ml^{-1} for *P. parvulus* and *O. oeni* CRBO 9304 respectively, and this variation can affect treatment efficiency. Indeed, the greater the size of the inoculum, the higher the rate of the inactivation because at high microbial concentrations the probability

of the formation of 'pearl chains' rises and these 'equivalent cells' of larger volume, equivalent to the sum of the individual cell volumes, increase PEF treatment efficiency (Molinari *et al.* 2004). *O. oeni* can appear as single cells, in pairs, or in chains of different sizes. *P. parvulus* cells are nonmobile. Yeasts tend to combine to form pairs or small chains (Smith and Grinsven 1984). Thus, the high resistance of *P. parvulus* may be due to its relatively low concentration in the wine. The same thing is observed for the different degrees of sensitivity of the two strains of *O. oeni*, which were present at two different concentrations.

Effects of PEF or HVED treatments on the physico-chemical parameters of red wines

Numerous parameters of wines (pH, total and volatile acidity, concentration of glucose-fructose, residual reducing sugar, malic acid, lactic acid and tartaric acid) influence the stability and/or the organoleptic characteristics of wines during the ageing process.

The concentrations of carbohydrates affect the organoleptic properties of wine. Indeed, they contribute to the sweetness on the palate, and the production of products such as glycerol, which gives body and roundness to wine. Glucose and fructose are the principal fermentable sugars. Residual sugar refers to all of the sugars that remain in the wine after fermentation, including the nonfermentable sugars. After the application of HVED, the glucose-fructose and residual reducing sugar concentrations increased in red wines. The same phenomena have been observed after PEF treatments on the wines but with a lower intensity except for the wines inoculated with *O. oeni* 0608 where the concentrations of glucose-fructose did not change while the concentrations of residual reducing sugar decreased slightly. The walls of Gram-positive bacteria are rich in polysaccharides, the release of which would explain the increase in sugar content. Yeast cell walls are composed of mannoproteins, glucans and chitins, which are rich in sugar. Their enzymatic degradation may explain the rise in the sugar content in the wine after the electroporation by electric treatments. The pulsed-electric treatments provoked cellular disorganization (cell wall, membranes, organelles) and the electroporation of cell membranes (Aronsson *et al.* 2001). Numerous enzymes are released and can also enhance the electroporation of cell walls and membranes. For example, autolysins of bacteria, such as β-1,4-*N*-acetylmuramidases, β-1,4-*N*-acetylglucosaminidases, *N*-acetylmuramyl-L-alanine amidases and peptidases are enzymes which hydrolyse various specific bonds in cell-wall peptidoglycan (Crouigneau *et al.* 2000). The high chemical reactivity of carbohydrates with acids, bases and proteins in aqueous matrices and different biochemical reactions where the action of different enzymes (pectinases, cellulases, hemicellulases, glycosidases...) is involved, must have been greater in treated wines than in control wines. These modifications of the sugar content by the PEF or HVED treatments of wines could have organoleptic consequences.

Acid levels directly affect colour, the stability of tartrates and proteins, and the sensory perception of wine. The measurements of pH, total acidity and the concentrations of malic acid, lactic acid and tartaric acid in the wines brought to light the impact of electric treatments on these parameters. Acids can disassociate and release hydrogen ions, which are measured by the pH. Total acidity is the sum of all organic acids, including their salts. The electric treatments modified only slightly pH of wines, while the total acidity of the treated wines actually decreased compared to the untreated wine except for the wines inoculated with *O. oeni* 0608 where the opposite was observed. These modifications were lower in wines treated with PEF than in wines treated with HVED. The wine had undergone malolactic fermentation so malic acid was replaced by the smoother tasting monocarboxylic lactic acid and was not detected in wines. Concentrations of lactic acid and tartaric acid have been changed following the electric treatments. Acidity is detected on the palate as a sharp, lively, tingling sensation. Moreover, medium and high levels of acidity provoke salivation. Thus, variations in the acidity of wines further to treatment can have organoleptic consequences. Like the modifications have been more important after the application of electric discharges, the sensorial impact should be stronger for the HVED treated wines than for PEF-treated wines.

Volatile acidity consists of volatile fatty acids, primarily acetic acid, and, to a much lesser degree, formic, propionic and butyric acids. Accounting for more that 90% of total wine volatile acidity, acetic acid plays the most important role in wine quality (Eglinton and Henschke 1999). The flavour threshold for acetic acid ranges from 0·4 to 1·1 g l^{-1} (Dubois 1994). At threshold concentration, it provides warmth to the palate and, as the concentration increases, it imparts a sourness/sharpness to the palate, with a vinegary odour at higher concentrations. All volatile carboxylic acids have marked odours: propionic acid is characterized as fatty, butyric acid resembles rancid butter, and C_6 to C_{10} carboxylic acids possess a goaty odour. Acetic acid occurs in wine primarily as a by-product of yeast and bacterial metabolism. Acetic acid can be produced by the metabolism of citric, malic, tartaric, and gluconic acids, as well as by that of hexoses, pentoses and glycerol. The production of acetic acid is rare under strictly anaerobic conditions (in the absence

of suitable reducible substances) (Jackson 2008). As HVED treatments produce reactive oxygen species and did not inactivate all the micro-organisms, this could explain the high level of volatile acidity in the wines treated with HVED.

All these measurements show that electric treatments (10 ms) can have significant organoleptic repercussions on wine quality. The consequences were more significant following HVED treatments than following PEF treatments. Microbial inactivation in the red wine by a PEF treatment of 20 kV cm^{-1} was obtained after a treatment time of 4–6 ms. Therefore, this duration of PEF treatment ought to be less destructive of the wine matrix.

Effects of PEF and HVED treatments on the phenolic composition and chromatic characteristics of red wines

The anthocyanins are the pigments responsible for the colour of red wine. Following HVED treatments, their concentrations in wines were decreased as compared to the control, while the concentration of anthocyanins in the PEF-treated wines was similar or superior. The stability of anthocyanins is influenced by many parameters such as pH, storage temperature, the presence of enzymes, oxygen, type of metals, light and processing conditions (Rein and Heinonen 2004). As for the HVED treatments, no study exists on the effects of this process on anthocyanin stability. As the temperature increases during the PEF treatment were higher (42·5°C) than those measured in wines treated with the HVED (34·4°C), it is apparent that the increase in temperature during the treatment is not the cause of the decrease in anthocyanins due to the HVED treatments. The variations in pH following HVED treatments were insufficient to explain the decrease in anthocyanin concentrations. However, the production of shockwaves, acoustic cavitation, free radicals and intense pulses of light with frequencies in the ultraviolet and the visible spectra by the HVED treatments (Morgan *et al.* 1988; Loske *et al.* 2002) could explain the degradation of anthocyanins in these wines. As certain metals affect the stability of anthocyanins in wines and the electrodes erosion can liberate metals by electrochemical reaction during the treatments (Morren *et al.* 2003), it would be interesting to identify and measure these metals.

Tannins are responsible for the sensation of astringency and bitterness in wines and are also involved in stabilizing the colour by association with the anthocyanins. Tannins can be hydrolysed, oxidized or precipitated by polymerization with proteins or polysaccharides via chemical or biochemical reactions. The inactivation of some enzymes by PEF treatments can counter theses phenomena and explain the slightly increased prevalence of tannins in PEF-treated wines. In fact, the high enzymatic inactivation rates that are provoked by PEF treatment have been demonstrated for many enzymes and many media, including polyphenoloxidase of grape juice (Huang *et al.* 2012). This increase in deactivation rates can also be linked to a higher degree of tannin depolymerization by PEF treatments (Delsart *et al.* 2013). On the other hand, HVED treatments caused increased tannin oxidation.

Anthocyanins and tannins are directly linked to quality attributes such as colour. Thus, any increase or decrease in the quantities of these compounds will have an impact on wine colour.

The colour intensity of wines treated with PEF was similar to control wines, whereas the HVED treatments resulted in a decrease of the CI. These results are coherent with the concentrations of anthocyanins and tannins found in the wines. Results obtained in other juices such as watermelon, tomato, and strawberry juices show a reduction in browning and an enhancement of lightness, brightness, and redness further to processing by PEF (Aguiló-Aguayo *et al.* 2009; Aguiló-Aguayo *et al.* 2010). The ability of PEF technology to inactivate oxidative enzymes is a possible explanation of these more colourful wines. As described above, the oxidative phenomena produced during HVED treatments are significant and can degrade polyphenol compounds (Boussetta *et al.* 2011). Puértolas *et al.* (2009) conducted the only study carried out so far into the microbial stabilization of red wine by means of PEF treatment. They indicate that no significant changes in colour nor odour were observed in musts and wine upon tasting further to PEF treatment at the highest intensities (from 16 to 31 kV cm^{-1}, number of pulses from 0 to 100, specific energy per pulse from 1·02 to 3·77 kJ l^{-1}, and a frequency of 1 Hz). Similar results have been found in products other than wine. There was no significant difference in colour between nontreated samples and a cranberry juice treated with 40 kV cm^{-1} for 150 ms (Jin and Zhang 1999) or a yoghurt-based drink containing a strawberry fruit syrup (Evrendilek *et al.* 2004), indicating that the changes induced by PEF treatments are relatively minor.

Finally, the total polyphenol index refers to the total quantity of phenolic compounds. Control wines and PEF-treated wines contained the same quantity of polyphenols while the phenolics content of wines treated by HVED decreased, which is coherent with the preceding results.

Acknowledgements

The authors thank the 'Conseil Interprofessionnel des Vins de Bordeaux (CIVB) – 33000 Bordeaux' and the Agency for Environment and Energy Management

(ADEME) for their financial support. The authors would like to thank Alan Metcalfe for English corrections made to this text.

Conflict of Interest

No conflict of interest declared.

References

Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. (2009) Flavour retention and related enzyme during storage of strawberry juices processed by high-intensity pulsed electric fields or heat. *Food Chem* **116**, 59–65.

Aguiló-Aguayo, I., Soliva-Fortuny, R. and Martín-Belloso, O. (2010) Color and viscosity of watermelon juice treated by high-intensity pulsed electric fields or heat. *Innov Food Sci Emerg Technol* **11**, 299–305.

Aronsson, K., Lindgren, M., Johansson, B.R. and Rönner, U. (2001) Inactivation of microorganisms using pulsed electric fields: the influence of process parameters on *Escherichia coli*, *Listeria innocua*, *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. *Innov Food Sci Emerg Technol* **2**, 41–54.

Balasubramaniam, V.M., Farkas, D. and Turey, E.J. (2008) High pressure processing. *Food Technol* **62**, 33–38.

Boussetta, N., Deloison, V., Pochez, F., Falcimaigne-Cordin, A. and Lanoisellé, L. (2011) Valorisation of grape pomace by the extraction of phenolic antioxidants: application of high voltage electrical discharges. *Food Chem* **128**, 364–370.

Buzrul, S. (2012) High hydrostatic pressure treatment of beer and wine: a review. *Innov Food Sci Emerg Technol* **13**, 1–12.

Chatonnet, P., Dubourdieu, D., Boidron, J.N. and Pons, M. (1992) The origin of ethylphenols in wines. *J Sci Food Agric* **60**, 165–178.

Corrales, M., Toepfl, S., Butz, P., Knorr, D. and Tauscher, B. (2008) Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. *Innov Food Sci Emerg Technol* **9**, 85–91.

Costa, A., Barata, A., Malfeito-Ferreira, M. and Loureiro, V. (2008) Evaluation of the inhibitory effect of dimethyl dicarbonate (DMDC) against wine microorganisms. *Food Microbiol* **25**, 422–427.

Couto, J.A., Neves, F., Campos, F. and Hogg, T. (2005) Thermal inactivation of the wine spoilage yeasts *Dekkera/Brettanomyces*. *Int J Food Microbiol* **104**, 337–344.

Crouigneau, A.A., Feuillat, M. and Guilloux-Benatier, M. (2000) Influence of some factors on autolysis of *Oenococcus oeni*. *Vitis* **39**, 167–171.

Crowell, E.A. and Guymon, J.F. (1975) Wine constituents arising from sorbic acid addition, and identification of 2-ethoxyhexa-3,5-diene as source of geranium-like off-odor. *Am J Enol Vitic* **2**, 97–102.

Delsart, C., Ghidossi, R., Poupot, C., Cholet, C., Grimi, N., Vorobiev, E., Milisic, V. and Mietton-Peuchot, M. (2012) Enhanced extraction of phenolic compounds from Merlot grapes by pulsed electric field treatment. *Am J Enol Vitic* **63**, 205–211.

Delsart, C., Cholet, C., Ghidossi, R., Grimi, N., Gontier, E., Gény, L., Vorobiev, E. and Mietton-Peuchot, M. (2013) Effects of pulsed electric fields on Cabernet Sauvignon grape berries and on the characteristics of wines. *Food Bioproc Tech* **7**, 424–436.

Delsart, C., Grimi, N., Boussetta, N., Miot Sertier, C., Ghidossi, R., Mietton Peuchot, M. and Vorobiev, E. (2015) Comparison of the effect of pulsed electric field or high voltage electrical discharge for the control of sweet white must fermentation process with the conventional addition of sulfur dioxide. *Food Res Int*, In Press. doi:10.1016/j.foodres.2015.04.017.

Divol, B. and Lonvaud-Funel, A. (2005) Evidence for viable but nonculturable yeasts in botrytis-affected wine. *J Appl Microbiol* **99**, 85–93.

Dols-Lafargue, M., Lee, H.Y., Le Marrec, C., Heyraud, A., Chambat, G. and Lonvaud-Funel, A. (2008) Characterization of *gtf*, a glucosyltransferase gene in the genomes of *Pediococcus parvulus* and *Oenococcus oeni*, two bacterial species commonly found in wine. *Appl Environ Microbiol* **74**, 4079–4090.

Donsì, F., Ferrari, G., Fruilo, M. and Gianpiero, P. (2010) Pulsed electric field-assisted vinification of Aglianico and Piedirosso grapes. *J Agric Food Chem* **58**, 11606–11615.

Dubois, P. (1994) Les arômes des vins et leurs défauts. *Revue Française d'œnologie* **145**, 27–40.

Dunn, J.E. and Pearlman, J.S. (1987) Method and apparatus for extending the shelf life of fluid food products. *United States Patent* No. 4695472.

Eglinton, J.M. and Henschke, P.A. (1999) The occurrence of volatile acidity in Australian wines. *Aust Grapegrow Winemak* **426a**, 7–12.

El Rayess, Y., Albasi, C., Bacchinc, P., Taillandier, P., Raynal, J., Mietton-Peuchot, M. and Devatine, A. (2011) Cross-flow microfiltration applied to oenology: a review. *J Memb Sci* **382**, 1–19.

Evrendilek, G.A., Yeom, H.W., Jin, Z.T. and Zhang, Q.H. (2004) Safety and quality evaluation of yogurt-based drink processed by a pilot plant PEF system. *J Food Process Eng* **27**, 197–212.

Eynard, N., Rols, M.P., Ganeva, V., Galutzov, B., Sabri, N. and Teissié, J. (1997) Electrotransformation pathways of prokaryotic and eukaryotic cells: recent developments. *Bioelectrochem Bioenerg* **44**, 103–110.

Garde-Cerdán, T., González-Arenzana, L., López, N., López, R., Santamaría, P. and López-Alfaro, I. (2013) Effect of different pulsed electric field treatments on the volatile

composition of Graciano, Tempranillo and Grenache grape varieties. *Innov Food Sci Emerg Technol* **20**, 91–99.

Geneix, C., Lafon-Lafourcade, S. and Ribéreau-Gayon, P. (1983) Effet des acides gras sur la viabilité des populations de *Saccharomyces cerevisiæ*. *C R Hebd Séances Acad Sci* **296**, 943–947.

Giner-Segui, J., Elez-Martínez, P. and Martín-Belloso, O. (2009) Modelling within the Bayesian framework, the inactivation of pectinesterase in gazpacho by pulsed electric fields. *J Food Eng* **95**, 445–452.

Grahl, T. and Märkl, H. (1996) Killing of microorganisms by pulsed electric fields. *Appl Microbiol Biotechnol* **45**, 148–157.

Grimi, N., Praporscic, I., Lebovka, N. and Vorobiev, E. (2007) Selective extraction from carrot slices by pressing and washing enhanced by pulsed electric fields. *Sep Purif Technol* **58**, 267–273.

Guerrero, R.F. and Cantos-Villar, E. (2015) Demonstrating the efficiency of sulphur dioxide replacements in wine: a parameter review. *Trends Food Sci Technol* **42**, 27–43.

Heinz, V., Alvarez, I., Angersbach, A. and Knorr, D. (2002) Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for process design. *Trends Food Sci Technol* **12**, 103–111.

Heinz, V., Toepfl, S. and Knorr, D. (2003) Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatment. *Innov Food Sci Emerg Technol* **4**, 167–175.

Huang, K., Tian, H., Gai, L. and Wang, J. (2012) A review of kinetic models for inactivating microorganisms and enzymes by pulsed electric field processing. *J Food Eng* **111**, 191–207.

Jackson, R.S. (2008) *Wine science, Principles and Applications*, 3rd edn. San Diego, CA: Academic Press.

Jin, Z.T. and Zhang, Q.H. (1999) Pulsed electric field inactivation of microorganisms and preservation of quality of cranberry juice. *J Food Process Preserv* **23**, 481–497.

Liu, X., Yousef, A.E. and Chism, G.W. (1997) Inactivation of *Escherichia coli* 0157:H7 by the combination of organic acids and pulsed electric fields. *J Food Saf* **16**, 287–299.

Lonvaud-Funel, A. (2001) Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiol Lett* **199**, 9–13.

Loske, A.M., Alvarez, U.M., Hernández-Galicia, C., Castaño-Tostado, E. and Prieto, F.E. (2002) Bactericidal effect of underwater shock waves on *Escherichia coli* ATCC 10536 suspensions. *Innov Food Sci Emerg Technol* **3**, 321–327.

Marsellés-Fontanet, R., Puig, A., Olmos, P., Mínguez-Sanz, S. and Martín-Belloso, O. (2009) Optimising the inactivation of grape juice spoilage organisms by pulse electric fields. *Int J Food Microbiol* **130**, 159–165.

Millet, V. and Lonvaud-Funel, A. (2000) The viable but non-culturable state of wine micro-organisms during storage. *Lett Appl Microbiol* **30**, 136–141.

Mine, Y. and Zhang, J.W. (2002) Comparative studies on antogenity and allergenity of native and denatured egg white proteins. *J Agric Food Chem* **50**, 2679–2683.

Molinari, P., Pilosof, A.M.R. and Jagus, R.J. (2004) Effect of growth phase and inoculum size on the inactivation of *Saccharomyces cerevisiae* in fruit juices, by pulsed electric fields. *Food Res Int* **37**, 793–798.

Morgan, T.R., Laudone, U.P., Heston, W.P.W. and Fair, W.R. (1988) Free radical production by high energy shock waves-comparison with ionizing radiation. *J Urol* **139**, 186–189.

Morren, J., Roodenburg, B. and De Haan, S.W.H. (2003) Electrochemical reactions and electrode corrosion in pulsed electric field (PEF) treatment chambers. *Innov Food Sci Emerg Technol* **4**, 285–295.

Navarro, L., Zarazaga, M., Sáenz, J., Ruiz-Larrea, F. and Torres, C. (2000) Bacteriocin production by lactic acid bacteria isolated from Rioja red wines. *J Appl Microbiol* **88**, 44–51.

O. I. V. (2013) *Recueil des méthodes internationales d'analyses des vins et des moûts*. Office International de la vigne et du vin. (ed. 2013), Paris, France.

Plumridge, A., Hesse, S.J.A., Watson, A.J., Lowe, K.C., Stratford, M. and Archer, D.B. (2004) The weak acid preservative sorbic acid inhibits conidial germination and mycelia growth of *Aspergillus niger* through intracellular acidification. *Appl Environ Microbiol* **70**, 3506–3511.

Puértolas, E., López, N., Condón, S., Raso, J. and Álvarez, I. (2009) Pulsed electric fields inactivation of wine spoilage yeast and bacteria. *Int J Food Microbiol* **130**, 49–55.

Rein, M.J. and Heinonen, M. (2004) Stability and enhancement of berry juice color. *J Agric Food Chem* **52**, 3106–3114.

Renouf, V. (2006) *Description et caractérisation de la diversité microbienne durant l'élaboration du vin: interactions et équilibres – relation avec la qualité du vin*. PhD thesis, Institut national polytechnique de Toulouse, Toulouse, France.

Renouf, V. and Lonvaud-Funel, A. (2007) Development of an enrichment medium to detect *Dekkera/Brettanomyces* bruxellensis, a spoilage wine yeast, on the surface of grape berries. *Microbiol Res* **161**, 154–167.

Ribéreau-Gayon, P. and Stonestreet, E. (1964) La constitution des tanins du raisin et du vin. *C R Acad Agric* **7**, 662–671.

Ribéreau-Gayon, P. and Stonestreet, E. (1965) Le dosage des anthocyanes dans le vin rouge. *Bull Soc Chim Fr* **419**, 2649–2652.

Rojo-Bezares, B., Sáenz, Y., Zarazaga, M., Torres, C. and Ruiz-Larrea, F. (2007) Antimicrobial activity of nisin against *Oenococcus oeni* and other wine bacteria. *Int J Food Microbiol* **116**, 32–36.

Smith, M.T. and Grinsven, A.M. (1984) *Dekkera anomala* sp. nov., the teleomorph of *Brettanomyces anomalus*, recovered from spoiled soft drinks. *Antonie Van Leeuwenhoek* **50**, 143–148.

Stanley, D.W. (1991) Biological membrane deterioration and associated quality losses in food tissue. *Crit Rev Food Sci Nutr* **30**, 487–553.

Surgiarto, A.T. and Sato, M. (2001) Pulsed plasma processing of organic compounds in aqueous solution. *Thin Solid Films* **386**, 295–299.

Toepfl, S., Heinz, V. and Knorr, D. (2006) Application of pulsed electric fields technology for the food industry. In *Food Engineering Series, 2006, Pulsed Electric Fields Technology for the Food Industry Fundamentals and Applications* ed. Raso, J. and Heinz, V. pp. 197–221. NY, USA: Springer Science+Business Media, LLC.

Van der Walt, J.P. (1970) The genus *Dekkera* van der Walt. In *The Yeasts, a Taxonomic Study*, 2nd edn ed. Lodder, J., pp. 154–165. Amsterdam: North Holland Publ. Co.

Wouters, P., Álvarez, I. and Raso, J. (2001) Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends Food Sci Technol* **12**, 112–121.

Zimmermann, U. (1986) Electrical breakdown, electropermeabilization and electrofusion. *Rev Physiol Biochem Pharmacol* **105**, 175–256.

Zimmermann, U., Pilwat, G. and Riemann, F. (1974) Dielectric breakdown of cell membranes. *Biophys J* **14**, 881–899.