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Effects of Pulsed Electric Fields on Cabernet Sauvignon grape berries and on the characteristics of wines

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Running title: Pulsed electric field treatment and wine
ABSTRACT

The treatment of Cabernet Sauvignon red grapes by Pulsed Electric Fields (PEF) is performed prior to vinification in order to enhance the extraction of polyphenols. PEF treatments of the longest duration and of the highest energy (E = 0.7 kV/cm, t_{PEF} = 200 ms, W = 31 Wh/kg) changed the structure of grape skins and produced a wine that was richer in tannins (34%), while treatment of the highest strength (E = 4 kV/cm, t_{PEF} = 1 ms, W = 4 Wh/kg) altered the visual appearance of phenolic compounds in the skins and led to greater extraction of the anthocyanins (19%). The PEF treatments caused the depolymerization of skin tannins, improving the diffusion of these decondensed tannins which are smaller. The PEF treatment of longest duration and of the highest energy had more impact on the parietal tannins and the cell walls of the skins while treatment of the highest strength modified more the vacuolar tannins. Changes in the operating parameters of the PEF treatment (E = 0.7 to 4 kV/cm, t_{PEF} = 1 to 200 ms, W = 4 to 31 Wh/kg) did not affect alcohol content, total acidity, nor volatile acidity in finished wines compared to the values of the control wine but seemed to cause a slight increase in pH (1-2%).

KEYWORDS: Pulsed Electric Fields, Extraction, Grape, Skin, Polyphenols
1) INTRODUCTION

During grape ripening, tissues differentiate; they organize themselves into a more optimal configuration that facilitates the extraction of intracellular compounds, including those of the skin, known for its content in molecules of oenological interest (Estruch, 2000; Scalbert et al., 2005). These molecules of interest are principally phenolic compounds, including anthocyanins, flavan-3-ols (monomers and polymers), flavonols, hydroxybenzoic and hydroxycinnamic acids, and also, in lower quantities, the aroma precursors called primary varietals (Fang et al., 2008). Anthocyanins and flavan-3-ols are largely located in the skin but also, for a part of the tannic components, in the seeds. With the exception of grape varieties known as dyers, the pulp is not rich in polyphenols, containing only a small quantity of phenolic acids and flavonols (Ribéreau-Gayon & Martinière, 1973). The degree of extraction of these compounds depends on many factors. The grape variety and the factors influencing their development, such as soil and climatic conditions, are critical in determining the extraction rate (Monagas et al., 2005). Moreover, it has been shown that high temperatures (Olivieri, 1991), sulfur dioxide content, the percentage of alcohol, winemaking techniques (Auw et al., 1996; Gao et al., 1997; Gómez-Plaza et al., 2000; Castillo-Sánchez et al., 2008), the addition of certain agents (polyvinylpyrrolidone, casein, gelatin) (Sims et al., 1995), skin maceration time (Gómez-Plaza, 2001), extreme pH (Sims & Morris, 1985), the degree of agitation and the non-compaction of pomaces all have an influence on the final concentrations and the stability of polyphenols in wines (Sun et al., 1999; Sacchi et al., 2005; Bautista-Ortínez et al., 2007).

Extraction is performed by using different practices and physico-chemical techniques associated at different stages of the winemaking process. Practices such as pumping over, cap punching, and rack and return are more traditional than carbonic maceration, sulphite...
maceration, thermal relaxation, flash-release or cryo-extraction (Castillo-Sánchez et al., 2006). The extraction may be carried out hot or cold, using enzymes, before and/or after fermentation (Sun et al., 2001). Not all these techniques are efficient, some modify the quality of the finished wine; others are costly in energy and time, which is an aberration in terms of present-day environmental issues (Castillo-Sánchez et al., 2006; Coffelt & Berg, 1965; Brown, 1975; Pardo et al, 1999; Revilla & González-SanJosé, 2002; Salinas et al., 2003; Bautista-Ortí et al., 2004; Revilla & González-SanJosé, 2003; Spranger et al., 2004; Bautista-Ortí et al., 2005). It is therefore important to investigate alternative techniques that are less time-consuming and more energy-efficient, while maintaining or optimizing the quality of extraction and the organoleptic characteristics of red wines.

According to recent studies, the development of an extraction process such as pulsed electric field (PEF) technology in the wine industry seems to be one of these alternatives (Praporscic et al., 2007; Puértolas et al., 2010c). Indeed, this method has proved highly efficient in terms of molecule extraction and yield (Liu et al., 2011; El Darra et al., 2012). Moreover, unlike processes of high-temperature thermal permeabilization, the application of PEF requires low power consumption, does not raise temperatures, and thus avoids the inconvenience of denaturing heat-sensitive cellular constituents. Furthermore, the time required for treatment with PEF (milliseconds) is much shorter than that required in other non-thermal processes such as the increase in hydrostatic pressure (several tens of minutes) (Corrales et al., 2008).

Finally, PEF technology can be applied in continuous flow production (Puértolas et al., 2010c) at a cost in the region of € 0.33/t for power consumption below 10 kJ/kg (Toepfl et al., 2006). The application of an electric field of several kilovolts and of very short duration to the newly destemmed and crushed harvest with a view to increasing the extraction of molecules of interest in wine was studied by López et al., (2009). In this study it was shown that the treatment of solids after pressing and separation of the juice by PEF increases juice yield,
significantly improves the quality of juice and wine and allows a reduction in the maceration time of the solids during the fermentation process. At the end of alcoholic fermentation, increases were observed in colour intensity (48%), in total polyphenol index (45%), in tannins (95%), in anthocyanins (43%) and also in the visual characteristics of wines treated with 5 kV/cm at a frequency of 1 Hz. The application of PEF to the solid parts of grape berries that have been lightly crushed and pressed does not change the other characteristics of the wine, such as the alcohol content, total acidity, pH, the concentration of reducing sugars, and volatile acidity (López et al, 2008a). Similar conclusions were arrived at for the different grape varieties tested. However, the extraction yield obtained by the application of PEF treatment to the different varieties of grapes was not identical. Finally, under the conditions tested, it appears that the PEF does not affect the lipid layer and lignocellulosic grape seeds (López et al, 2008b).

Understanding of the mechanisms by which PEF creates membrane pores is still imperfect but models describing this process have been put forward. The best known model states that when a cell is placed in a high-permittivity external medium, the presence of oppositely-charged particles on both sides of the cell membrane creates an electro-compressive force. When the applied electric field exceeds a critical value, the electro-compressive force becomes greater than the elastic force of the membrane which causes pores to appear in the membrane and/or causes the enlargement of existing pores. An increase in the amplitude of the electric field and/or the duration of its application causes an intensification of the permeabilization and irreversible destruction of the cell membrane (Barbosa-Cánovas et al., 1999). The explanation of PEF-induced effects on cells has also been approached through microscopic observations. Studies carried out on plants have confirmed that PEF permeabilizes membranes, thus allowing the expulsion of intracellular contents and leading to cell compaction without
significant impact on the cell walls (Bazhal et al., 2001; Fincan, 2003). However, there are no observations concerning grapes processed by PEF.

In the present research, Bordeaux cabernet sauvignon grapes were treated by pulsed electric fields and the effects on the organization of the cell envelope and cellular components were observed with the aim of determining whether these treatments facilitate the passage of phenolic compounds through the tonoplast, plasma membrane, and cell wall without modifying them. Monitoring of vinification and quantification of the compounds extracted using pulsed electric fields allowed the evaluation of the influence of the duration and the strength of the treatment.

2) MATERIALS AND METHODS

**Plant material.** Grapes *Vitis vinifera* L. var. Cabernet sauvignon, harvested in 2009 (Bourg sur Gironde, Gironde, France, label of origin Côtes de Bourg) were used for the research. All samples were manually harvested on the same day, in the morning when the temperature was low in order to preserve the organoleptic characteristics of the grapes. The day was decided upon when the analyses of the grapes were optimal (22 °Brix, titratable acidity: 5.25 g of tartaric acid/L).

**PEF equipment.** After destemming and crushing, a mass of m = 1-4 kg of Cabernet sauvignon grapes was placed between two parallel stainless-steel electrodes and PEF was subsequently applied. The PEF generator used (Hazemeyer, Saint-Quentin, France) provides maximum voltage and current of 5 kV and 1 kA respectively. This generator provides pulses of a near rectangular shape (Figure 1). N series of pulses were used for the treatment. Each series consisted of n pulses, pulse duration $t_i$, the time interval between pulses = $\Delta t$, and there
was a pause ($\Delta t_i$) after each train of pulses. The effective time of the PEF treatment was regulated by the variation of the number of series $N$ and is calculated as $t_{PEF} = n.N.t_i$. Electric field strength $E$ is evaluated as $E = U/d$, where $U$ is the applied voltage and $d$ is the distance between electrodes. The temperature at the centre of the sample was measured during PEF application by means of a teflon-coated thermocouple (Thermocoax, Suresnes, France).

The following parameters were used in the experiments: (i) $E = 0.7$ kV/cm, $t_{PEF} = 200$ ms and $W = 31$ Wh/kg ($n = 100$, $t_i = 100$ μs, $\Delta t = 10$ ms, $\Delta t_i = 10$ s, $N = 20$) and (ii) $E = 4$ kV/cm, $t_{PEF} = 1$ ms, and $W = 4$ Wh/kg ($n = 1$, $t_i = 10$ μs, $\Delta t = 10$ ms, $\Delta t_i = 10$ s, $N = 100$). These parameters were chosen in order to compare the effects of a long (200 ms) duration of PEF of low strength (0.7 kV/cm) with the effects of a treatment of a short duration (1 ms) and high strength (4 kV/cm).

Microscopic skin characterisation and cytological preparation for light microscopy (method described by Colin et al.) (2002). A first sampling of both treated and untreated berries was carried out immediately after two pulsed electric field (PEF) treatments (day 0) so as to observe the immediate effect of the treatment on the cellular structure. For each application of PEF ten berries were immediately prepared for microscopy as described by McManus (1948) for fixation, and by Thiéry (1967) for staining. Treated berries and control (untreated) berries were dissected into four sections each, always in the same way, and fixed (4°C for 90 mins in 2.5% glutaraldehyde in a phosphate buffer, pH 7.2 (v/v)) then washed in the same buffer and postfixied (4°C for 90 mins in osmium tetroxide). The berry sections were then progressively dehydrated in successive baths of ethyl alcohol from 40% (v/v) to absolute (100%), and were then embedded in epoxy resin (Epon). Semi-thin sections (2 μm thick) were stained using the Periodic Acid Schiff method (P.A.S.) for polysaccharide detection (Thiéry, 1967). Two hundred cross-sections were observed for each treatment and examined by means
of a light microscope (Olympus XI 50). Ten cross-sections per treatment were photographed using a digital camera (Olympus DP 70). The P.A.S.-stained sections were used to determine the general histocytological morphology and to evaluate the polysaccharide content of the walls based on the different shades of pink obtained (negative P.A.S. = light pink, positive P.A.S. = dark and intense pink). The semi-thin sections (200) were produced by the Bordeaux Imaging Center (BIC): the Aquitaine imagery platform (University of Bordeaux, France).

**Extraction of compounds from treated berries.** Fourteen frozen berries were peeled, and the skins were immediately immersed in liquid nitrogen and crushed manually in a mortar to obtain the fine powder. One part of this powder was directly used for the extraction and analysis of tannins of the total skin. Another part was used to get the cell-wall fraction according to the following step.

The extraction procedure of the cell-wall fraction of the powder skin is described by Gagné et al., 2006. The powder of total skin is suspended in a buffer of homogenization (Tris-HCl 0.2M, pH 7.5; 2.5% EDTA (w/v)) and centrifuged. Then, the pellet powder of total skin undergoes several cycles of centrifugation/homogenization successively in the buffer extraction, in the saccharose, in the extraction buffer, in the Triton X100, in the homogenization buffer. The final pellet, dried in an oven, corresponds to the cell-wall fraction of the total skin.

Phenolic compounds were extracted from powder of total skin or cell-wall fraction of total skin by a double maceration in methanol at 0.1% HCl (v/v), followed by a filtration PTFE filter, according to the method of Revilla et al., 1998. The tannins extraction of total skin corresponds to the vacuolar tannins while the tannins of cell-wall fraction corresponds to the tannins linked and embedded in the cell-walls of total skin.
**Winemaking process.** Microvinifications were carried out in 10L tanks with the same height/diameter ratio (38 cm/22 cm) as conventional industrial tanks. These dimensions were used in order to keep the same pomace/juice ratio as in wine industry installations. Measurements of pH and total acidity (TA) were carried out on the must in each tank immediately after PEF treatment (day 0) using the methods of the OIV (2012). Alcoholic and malolactic fermentations were initiated by inoculation with *Saccharomyces cerevisiae* yeast (Zymaflore FX10, Laffort, Bordeaux, France) at a dose of 15 g/hL and inoculation with *Oenococcus oeni* bacteria (VP41, MBR process LALVIN, Lallemand, Ontario, Canada) at a concentration of 5 g/hL. Throughout alcoholic fermentation a portable density meter DMA 35 (Anton Paar, Courtaboeuf, France) was used to monitor density and temperature by daily measurements up until the total degradation of sugars by the yeasts. The measurement of residual sugar concentrations (OIV, 2012) allowed confirmation of the end of this first fermentation. Alcohol content, TA and pH were measured in the wines at the end of alcoholic fermentation. The pomace was then pressed and the wines discharged into containers (5 L bottles) inerted by means of carbon dioxide, placed in a thermostatic chamber at 20 °C, and maintained under anaerobic conditions in order to optimize the malolactic fermentation. The degradation of malic acid into lactic acid was verified by means of thin layer chromatography. The wines were then sulphited at 5 g/hL and maintained in a thermostatic chamber at 20 °C. TA, volatile acidity (VA) and pH were monitored throughout malolactic fermentation, maturation on lees (133 days), and ageing, as well as in the finished wine three months after bottling (at 251 days). Free sulfur dioxide levels were measured at regular intervals during maturation by means of an eno 20 enotitrator (Oeno-bio, St. Martin le Viel, France) and corrected to 25 mg/hL. Finally the wines were racked, assembled (two bottles per treatment modality), adjusted for free sulfur dioxide, bottled, and stored at 10 °C.
**Analytical procedures.** Absorbance measurements were carried out using a double-beam spectrophotometer (Vasco V550) with a 1 mm or 10 mm light path in glass bowls in visible (420, 520, 550, 620 nm) and ultraviolet (280 nm) wavelengths. Colour intensities (CI) of the wines were calculated as the sum of the absorbances at 420, 520 and 620 nm with a 1 mm light path. Polyphenol content was evaluated by measurement of the Total Polyphenol Index (TPI) as determined by UV spectrophotometry at 280 nm with a 10 mm light path (Perkin-Elmer, Courtaboeuf, France). The method described by Ribéreau-Gayon & Stonestreet, (1966) was used for the analysis of proanthocyanidins in the wines. Anthocyanins analysis is based on a method using differences in pH and decoloration with sulfur dioxide (Ribéreau-Gayon & Stonestreet 1965). The identification and quantification of anthocyanins and proanthocyanidins in the bottled wines were determined by HPLC-DAD-Fluo. Anthocyanins and proanthocyanidins were analyzed by means of a liquid chromatography apparatus consisting of an autosampler connected to a UV diode array absorbance detector scanning from 250 to 700 nm (Thermo Finnigan, USA), followed by a spectrofluorometer and by a Finnigan Duo mass spectrometer with an electrospray interface (ESI) operating in positive mode. Separation was carried out using a 250 × 4.6 mm i.d. 4 µm Lichrospher RP-18 column (Merck, France), maintained at 30ºC. The mobile phases are A (water-formic acid, 95:5, v/v) and B (acetonitrile-formic acid, 95:5, v/v) (Table 1).

**Table 1.** Conditions for the elution gradient. Mobile phase A (water-formic acid, 95:5, v/v) and B (acetonitrile-formic acid, 95:5, v/v).

<table>
<thead>
<tr>
<th>t, min</th>
<th>0</th>
<th>3</th>
<th>15</th>
<th>50</th>
<th>51</th>
<th>54</th>
<th>55</th>
<th>65</th>
</tr>
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<tbody>
<tr>
<td>%B</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>3</td>
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The flow rate was maintained at 1 mL/min. After passing through the flow cell of the fluorospectrometer, the column eluate was split with only 20% being directed to the mass
spectrometer. Samples were filtered using 0.45 µm filters (Acrodisc) prior to the analysis. Injection volume was 20 µL. Anthocyanins were monitored at 520 nm (UV detection). As for proanthocyanidins, the fluorescence detector was set at λem 320 nm and λex 280 nm and UV detection was carried out at 280 nm. Full scan MS spectra were obtained from 150 to 2000 U. All the compounds were identified either by comparing their retention times with those of pure standards, on the basis of their elution order and retention time, or by the analysis of their absorbance and mass spectra. The Glories’ method was used (520nm) to determine the anthocyanins in the skin. Results are given in mg.g⁻¹ of skin (Ribéreau-Gayon & Stonestreet, 1965). The absorbance measurements were made with a spectrophotometer (Thermo Electron Corporation, Nicolet evolution 100). The fine composition of condensed tannins and mean degree of polymerization (mDP) of the total and cell-wall fraction of the skin were obtained by reverse phase HPLC after phloroglucinolyse, according to the method of Kennedy et al., 2001, described by Chira et al., 2010. The analysis conditions were as follows: column, 250 x 4.6μm, 5μm, ODS (Beckman, Roissy CDG, France); pre-column, 10 x 4.6mm, 5μm, BDS C18 (Thermo Hypersil); flow rate, 1ml/min; solvent A, water/acetic acid (95/5 v/v); solvent B, MeOH/acetic acid (95/5 v/v); gradient, 5% B from 0 to 30min, 20% B from 30 to 55min, 40% B from 55 to 60min, 90% B from 60 to 75min, 5% B from 75 to 80min; injection volume, 10µl; detection wavelength, 280nm; volume = 10µl.

Statistics. Variance analysis was used for data analysis. The statistical significance of the differences in the data was obtained using the Tukey test (α= 5%) or the Kruskal & Wallis Test (α= 0.1%). Data groups were distinguished in the tables with different letters. Data processing was carried out using XLSTAT (Addinsoft SARL, France) software.

3) RESULTS
**Evolution of must and wine characteristics**

After the application of the PEF treatment (day 0) (Table 2), the pH of the musts increased compared to the control. The respective values for the control, 0.7 kV/cm, and 4 kV/cm were 3.38, 3.55 and 3.61. During alcoholic fermentation, the pH of the must increased due to the crystallization of tartaric acid and the biological conversion of malic acid into lactate by the yeasts. Similarly, during malolactic fermentation, the bacteria metabolize the malate into a monoacid and provoke an increase in the pH of the wines. During this step arginine, the most concentrated amino acid in must and wine, is degraded, releasing ammoniac and increasing the pH. After 251 days the difference between the control and the treated wines were smaller but remained significant (1.6 and 1.9%). Contradictory results have been found by other authors. Marsellés-Fontanet et al (2011) reported of grape juice were not affected by PEF processing. Treatment of cabernet sauvignon grapes with a PEF of 5kV/cm (1Hz) did not modify the pH of the wines produced, as measured immediately after the end of fermentation (López et al., 2008a; López et al., 2009). Another study (Puértolas et al., 2010a) described a significant decrease in the pH of wines, as measured four months after bottling. Cabernet sauvignon grapes were treated with PEF of 5 kV/cm for 0.41 s. During the vinification of red grapes total acidity depends on pH. The total acidity of the control wine and the wines treated with 700 V/cm and 4 kV/cm increased over time from 0 to 133 days: 6%, 3% and 16% respectively. Between 133 days and 251 days, total acidity of the control wine remained constant. The values were not significatively different from those measured in the control wine, which corresponds to findings in other studies (López et al., 2008a; López et al., 2009). However, Puértolas et al (2010a) observed an increase in the total acidity of wine treated with PEF, measured at four months after bottling. Volatile acidity was analyzed only after the fermentations and bottling. During the fermentations, volatile acidity is the result of the
degradation of fatty acids by yeasts and bacteria. Thus, volatile acidity tends to increase during the fermentations. The results show that the volatile acidity of wines is not modified by the application of PEF. Similar results were obtained in different studies (López et al., 2008a; López et al., 2009; Puértolas et al., 2010a), even for measurements made four months after bottling. Alcohol content will depend on the initial sugar concentration in the must, fermentation temperature, and the types of yeast. Wine stability, its evolution during ageing, and certain of its sensory properties are influenced by alcohol content. In the present experiments, the alcohol content of the wines after bottling did not differ significantly. The wines produced after PEF presented only a slight increase in alcohol content (3% and 1% for 0.7 and 4 kV/cm respectively). Similar results have been described in available literature (López et al., 2008a; López et al., 2009). However, a significative decrease in alcohol content was observed by Puértolas after PEF treatment of grapes with 5 kV/cm (Puértolas et al., 2010a). The results obtained, combined with those presented in the literature, illustrate how difficult it is to arrive at conclusions concerning the effects of Pulsed Electric Fields on pH, total acidity, volatile acidity and alcohol content. Variations observed in the different studies may be the result of variations in a number of parameters independent of the PEF treatment relating to the quality and variety of the grapes, vinification and ageing conditions.

Table 2. Characteristics of the wines obtained from untreated (0 kV/cm), PEF-treated grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms). Analysis immediately after treatment (day 0), after malolactic fermentation (day 133) and at three months after bottling (day 251).

| Time (days) | Control E = 0 kV/cm | PEF-treated E = 0.7 kV/cm  
|            |                  |  |  
|            |                  | t_{PEF} = 200 ms |  
|            |                  | t_{PEF} = 1 ms |  
| pH         | 3.38 | 3.41 | 3.67 | 3.55 | 3.45 | 3.73 | 3.61 | 3.49 | 3.74 |  
| TA^a (g H_2SO_4/L) | 3.43 | 3.63 | 3.63 | 3.43 | 3.53 | 3.33 | 3.14 | 3.63 | 3.68 |  
| VA^b (g CH_3COOH /L) | - | 0.25 | 0.52 | - | 0.26 | 0.44 | - | 0.24 | 0.44 |  
| Alcohol content (v/v) | - | 12.13 | - | 12.50 | - | 12.30 |  

^aTotal acidity
Volatile acidity

**Evolution of colour intensity and total polyphenol index**

The colour of the red wines, given by the measurement of its colour intensity (CI), was measured from immediately after PEF treatment (day 0) to one month after bottling (day 232). Phenolic content was also determined using the total polyphenol index (TPI) (Figure 2). Colour intensity was reduced by the addition of sulfites and also in the wines with low pH. In this study the wines were of similar pH and the concentrations in sulfites in the wines were the same. Therefore, the differences observed between the wines treated with PEF and the control wines are the result of the application of the pulsed electric fields. During vinification the CI is higher in the wines treated with PEF than that of the control wine, especially for the wine treated at 0.7 kV/cm. A classic hyperchromic effect was observed in the colour of the three wines. A rapid increase over the first twenty days was followed by a slight decrease and then stabilization. The colour intensity of the musts freshly treated by PEF at 4 Wh/kg and 31 Wh/kg was respectively 86% and 168% higher than that of the control must. The increase observed in the wines remained constant at approximately 10% (4 kV/cm, 4 Wh/kg) and 24% (0.7 kV/cm, 31 Wh/kg). The hyperchromic effect may be explained by the enhanced extraction of anthocyanins at the beginning of the fermentation process. Thus, the decrease in colour intensity is due to the co-precipitation of the anthocyanins associated to the berry cells and yeasts and also to the modification of must pH during the fermentation process creating colourless anthocyanins. After bottling (day 205) the colour intensity of the wines obtained with the application of PEF at 0.7 kV/cm and 4 kV/cm was higher (26% and 13% respectively) than in the control wine. It thus appears that anthocyanins are extracted more intensely and more rapidly with PEF treatment than is the case with traditional maceration.

After decreasing slightly at the end of the fermentation process, the increased CI remained constant after bottling. However, for López et al. (2008a, 2009), colour intensity may decrease
over time until it attains a value similar to that of the control wine. A significant increase in the total polyphenol index (TPI) was observed for all the wines throughout the alcoholic fermentation process, reaching maximum values at 20 days. Following this increase, a slight decrease was observed, followed by stabilization of the values. The initial increase in the TPI, as compared with the control, was 83% and 55% for the musts treated at 0.7 kV/cm and 4 kV/cm respectively (day 0). After the alcoholic and malolactic fermentations (day 40) the TPI remained higher in the wines treated with PEF: 16% and 11% for treatments at 0.7 kV/cm and 4 kV/cm respectively. After bottling the differences between the wines were less significant but remained higher in the treated wines than in the control wine, even though the difference between the two treated wines was reduced and tended towards a similar value (12% for 0.7 kV/cm and 14% for 4 kV/cm). Although the different tests give values for the increase in the TPI that vary from the results of tests described in the literature, all authors have observed an increase in this parameter with the application of Pulsed Electric Fields on grapes as compared to traditional vinification methods (Corrales et al., 2008, López et al, 2009; López et al, 2008a; López et al., 2008b Puértolas et al, 2010a; Puértolas et al., 2010b, Puértolas et al, 2010c). It can, therefore, be concluded that polyphenolic compounds are extracted more effectively and more rapidly with PEF treatment. The total polyphenol index principally represents the anthocyanin and polyphenol content extracted at different kinetics in traditional vinification.

**Extraction of polyphenolic compounds of wine**

A durable increase in colour intensity and the total polyphenol index following PEF treatment was observed in the test wines. Anthocyanidins, localized in the external hypodermis of the grape skin, were extracted within the first few days of maceration, before the tannins. Figure 3 shows the concentrations in total anthocyanidins in the different wines at three months after
bottling as well as the associated photos of the musts immediately after the application of the PEF treatment. The control musts are also presented. Anthocyanidins are less concentrated in the control wine than those in the wines obtained from PEF-treated grapes. The musts were red after the PEF treatment whereas the control must was yellow. Must colour is a function of anthocyanidin content. The concentration of anthocyanidins in the wines made from PEF-treated grapes was 9% and 19% higher than that observed in the control wine for 0.7 kV/cm and 4 kV/cm respectively. The total extraction of anthocyanidins in the wines, the colour of the musts, and the colour intensity of the wines are all a function of the parameters of the PEF treatment. It was observed that anthocyanidins extraction was greater in the wine produced from grapes treated with 4 kV/cm even though the colour intensity of this wine was lower than that of the wine produced from grapes treated with 0.7 kV/cm. The increase in the colour intensity of the wine following treatment at 0.7 kV/cm (26% as compared to the control wine) is greater than the increase in anthocyanin content (9% as compared to the control wine). Thus, colour intensity is not only related to free anthocyanins but is also related to those linked to other polyphenol compounds (Hermosín-Gutiérrez et al., 2005). The anthocyanidins were analysed by HPLC (Table 3). Similar HPLC profiles were obtained for the three different bottled wines. Extraction is not selective as other authors have previously observed for wines after alcoholic fermentation (López et al., 2009) and at four months after bottling (Puértolas et al., 2010a). The malvidin-3-o-glucoside, malvidin-3-o-acetyl and malvidin-3-o-copumaroyl concentrations are all higher indifferently of the treatment carried out on the grapes. Anthocyanidin content, with the exception of malvidin-3-glucoside and malvidin-3-acetyl (0.7 kV/cm), was significantly higher in the wine treated at 4 kV/cm. These concentrations were lower at 0.7 kV/cm but remain higher than that observed in the control wine. However, other authors have found a non-significant increase in the anthocyanidins for delphinidin-3-o-glucoside, peonidin-3-o-glucoside, and peonidin-3-o-coumaroylglucoside.
(Puértolas et al., 2010a). These observations can be explained principally by the different parameters used for the treatment of the grapes or pomaces and the vinification process. The values of the anthocyanidins concentrations obtained for the different wines in this study are in accordance with the extraction of the anthocyanins measured by spectrophotometry but are in contradiction with the classification of the wines as a function of colour intensity. This latter parameter depends not only on anthocyanin content but also on tannin content and their interactivity. High-strength treatments \((E = 4 \text{ kV/cm}, t_{PEF} = 1\text{ms})\) produce a greater extraction of anthocyanins but the colour intensity of these wines could be similar to that of wines produced from grapes following PEF at low strength and long treatment times \((E = 0.7 \text{ kV/cm}, t_{PEF} = 200 \text{ ms})\). It is possible that high-strength PEF treatment may modify the tridimensional structure of the anthocyanins or their electron configuration, creating a lower colour intensity than in the wine produced from grapes treated at 0.7 kV/cm even though the concentration in anthocyanins is higher. Proanthocyanidins are a class of compounds that have been variously described as anthocyanogens, leucoanthocyanidins, flavan-3,4-diols, condensed tannins, and tannins. They show a great diversity of structures, from simple dimers to extremely complex molecules. Figure 4 represents the results of the spectrophotometric analysis of the proanthocyanidins in the three wines at three months after bottling. The tannin concentrations in the wines obtained after PEF treatment at 4 kV/cm and 0.7 kV/cm are significantly higher than those in the control wine, i.e. 18% and 34% respectively. These results are in accordance with previous experiments on cabernet sauvignon grapes (López et al., 2009; Puértolas et al., 2010a; Puértolas et al., 2010b). Tannins and anthocyanins represent the essential proportion of the polyphenolic compounds extracted, which provides an understanding of the high values of TPI obtained in the wines produced from grapes treated at 0.7 kV/cm. Furthermore, tannin concentrations in the wine produced from grapes treated at 0.7 kV/cm may explain the higher values obtained for colour intensity due to the optimized
interaction between tannins and anthocyanins resulting in a more stable colour. Proanthocyanidins in the wines were analysed by chromatography in order to identify and quantify the two monomeric molecules catechin and epicatechin and the dimers B1, B2 and B4. The results of the analysis of the three HPLC chromatograms of the control wine and the wines produced after PEF treatment at 0.7 kV/cm and 4 kV/cm on the cabernet sauvignon grapes are given in the Table 3. Higher concentrations were observed in all the wines for (+)-catechin, but (-)-epicatechin, a more efficient wine copigment, was more effectively extracted by the application of PEF treatment. Concentrations of (-)-epicatechin were higher with PEF treatment at 0.7 kV/cm than those obtained at 4 kV/cm. Tannins, therefore, are the major compounds of the polyphenols. The TPI is more directly related to concentrations of proanthocyanidins than to concentrations of anthocyanidins. Thus, the wine obtained from grapes treated by PEF at 0.7 kV/cm has not only a higher concentration in tannins but is also the wine with the higher TPI. The colour stability of red wine is improved by the polymeric pigments resulting from interactions between anthocyanins and tannins. These interactions are enhanced by PEF treatment at 0.7 kV/cm, which gives high concentrations of proanthocyanidins and greater colour intensity. Total specific energy at 0.7 kV/cm for 200 ms was 31 Wh/kg and only 4 Wh/kg for 4 kV/cm for 1 ms. This observation may explain the results observed on the grape skins and on the analytical results. Further complementary experimentation is necessary in order to define the impact of treatment strength, impulse duration, the number of pulsations, and the number of trains of pulses on the extraction of the different compounds and their stability in the wines.

**Table 3.** Mean concentration (mg/L) of anthocyanin compounds and tannins in the wines (at six months after bottling) obtained from untreated (0 kV/cm) and PEF-treated (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms) red grapes.
### Anthocyanins

<table>
<thead>
<tr>
<th>Anthocyanins</th>
<th>Concentration (mg/L)</th>
<th>Untreated</th>
<th>$E=0.7 \text{ kV/cm}$ $t_{PEF} = 200 \text{ ms}$</th>
<th>$E=4 \text{ kV/cm}$ $t_{PEF} = 1 \text{ ms}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delphinidin-3-o-glucoside</td>
<td>12.8 ± 0.0</td>
<td>14.5 ± 0.2</td>
<td>17.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Petunidin-3-o-glucoside</td>
<td>13.2 ± 0.0</td>
<td>14.9 ± 0.9</td>
<td>18.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Peonidin-3-o-glucoside</td>
<td>5.4 ± 0.2</td>
<td>5.6 ± 0.1</td>
<td>7.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Peonidin-3-o-glucoside</td>
<td>159.3 ± 0.9</td>
<td>155.4 ± 4.6</td>
<td>180.4 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Peonidin-3-o-acetyl</td>
<td>2.5 ± 0.3</td>
<td>3.5 ± 0.0</td>
<td>3.9 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Malvidin-3-o-acetyl</td>
<td>77.5 ± 6.4</td>
<td>73.3 ± 0.1</td>
<td>86.2 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Peonidin-3-o-coumaroyl</td>
<td>0.8 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Malvidin-3-o-coumaroyl</td>
<td>13.6 ± 0.2</td>
<td>14.3 ± 1.3</td>
<td>15.9 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

### Tannins

<table>
<thead>
<tr>
<th>Tannins</th>
<th>Concentration (mg/L)</th>
<th>Untreated</th>
<th>$E=0.7 \text{ kV/cm}$ $t_{PEF} = 200 \text{ ms}$</th>
<th>$E=4 \text{ kV/cm}$ $t_{PEF} = 1 \text{ ms}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>20.1 ± 0.5</td>
<td>26.9 ± 1.6</td>
<td>23.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Procatechin</td>
<td>10.1 ± 0.3</td>
<td>15.7 ± 0.4</td>
<td>13.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>21.1 ± 0.7</td>
<td>23.7 ± 0.2</td>
<td>22.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Procyanidin B2</td>
<td>9.0 ± 0.2</td>
<td>13.7 ± 0.2</td>
<td>10.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Procyanidin B4</td>
<td>3.6 ± 0.0</td>
<td>4.8 ± 0.0</td>
<td>4.3 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

### Histo-cytological grape berry structure

The effects of PEF on the histo-cytological structure of the grape berries were observed by means of light microscopy for two PEF treatments. The observations were performed immediately after the application of PEF (day 0) in order to observe the immediate effects of PEF treatments on the cell structure of the grape berries. Figure 5 shows a comparison of the cross-section of untreated (0 kV/cm) versus PEF-treated (PEF1: 0.7 kV/cm for 200 ms) grape berries in light microscopy with P.A.S. staining. The skin-cell layers of the treated berry are much compacted in comparison with those of the untreated berry. The greatest degree of compaction is observed in the deepest layers of the skin, which corresponds to the cell layers of the inner hypodermis. On the one hand, this phenomenon is related to the external electrical field which induced higher transmembrane potentials in larger cells, and on the other hand, the rise in potential is proportional to the increase in cell size (Janositz & Knorr, 2010). The skin of the harvested berries is made up of an external epidermis with small cells and a thin external hypodermis. Contrary to the skin, the pulp is very well developed and contains large...
cells (Pratt, 1071; Hardie et al., 1996; Ollat et al., 2002). The cells of the inner hypodermis, which are located at the boundary between the skin and the pulp, become pulp cells after thinning of their cell walls (Fougère-Rifot et al., 1996), becoming the largest cells of the pericarp (Hardie et al., 1996; Ollat et al., 2002; Fougère-Rifot et al., 1997). Thus, within the pericarp, the cells of the inner hypodermis are the largest cells and they have a very thin wall. This is the pericarp level that will preferentially propagate the potential of the external electrical field, especially as the fineness of the cell walls means that the protective effect of the walls is limited (Janositz & Knorr, 2010).

Figure 6 shows a comparison between the cross-section of untreated (0 kV/cm) and PEF-treated (PEF2: 4 kV/cm for 1 ms) grape berries in light microscopy with P.A.S. staining. In contrast to PEF1 ($E = 0.7$ kV/cm, $t_{PEF} = 200$ ms, $W = 31$ Wh/kg) observations, the skin-cell layers of the treated berry are slightly more distended than those of the untreated berry. The skin-cells are less affected and the cell walls seem to have been less affected by the PEF2 treatment ($E = 4$ kV/cm, $t_{PEF} = 1$ ms, $W = 4$ Wh/kg) as compared to the previous case ($E = 0.7$ kV/cm, $t_{PEF} = 200$ ms, $W = 31$ Wh/kg). PEF2 treatment ($E = 4$ kV/cm, $t_{PEF} = 1$ ms, $W = 4$ Wh/kg) was of greater intensity, much shorter, and with a lower total energy consumption compared to the PEF1 treatment ($E = 0.7$ kV/cm, $t_{PEF} = 200$ ms, $W = 31$ Wh/kg). Thus, PEF2 treatment ($E = 4$ kV/cm, $t_{PEF} = 1$ ms, $W = 4$ Wh/kg) may have less impact on the cell walls and/or membranes (Ersus & Barrett, 2010; Fincan & Dejmek, 2002). Furthermore, it was observed that the appearance of vacuolar phenolic compounds had changed as compared to the control berry, perhaps denoting that PEF treatment affects their chemical composition. In this case the composition of musts and wines may also be affected. Treatment with $E = 0.7$ kV/cm and $t_{PEF} = 200$ ms ($W = 31$ Wh/kg) induced a significant change in the structure of the berry skin. Cell walls were greatly modified and membranes were certainly subjected to electroporation, which probably resulted in enhanced release of the compounds of interest in winemaking.
Treatment with $E = 4$ kV/cm and $t_{PEF} = 1$ ms, ($W = 4$ Wh/kg) as compared to treatment at 0.7 kV/cm, induced, on the one hand, the formation of the skin-cell layers that were slightly more distended than those of untreated berries; on the other hand it induced a change in the appearance of vacuolar phenolic compounds, which influenced the characteristics of the musts and wines produced from the treated berries.

**Composition of grapes skins after treatment PEF**

Concentrations of polyphenols and the mean degree of polymerization (mDP) of tannins were measured in berry skins immediately after PEF treatment and the values as measured are given in Table 4. These tannins correspond to the vacuolar tannins that are more easily extracted than parietal tannins. The skins of berries treated at 4 kV/cm during 1 ms ($W = 4$ Wh/kg) contained higher total tannins than those of the control sample. Furthermore, these tannins were less condensed than those in the control sample, their mDP being smaller (see Table 4). It can be seen that treatment at 4 kV/cm ($t_{PEF} = 1$ ms, $W = 4$ Wh/kg) induced wide-scale breakdown of molecules and high tannin decondensation (very low mDP), which explains the significant increase in the total amount of tannins in the treated skins. The skins of the berries processed by the treatment at 0.7 kV/cm ($t_{PEF} = 200$ ms, $W = 32$ Wh/kg) contained slightly higher total tannins than the skins of the control berries, and their mean degree of polymerization was also lower than in the control batch.

Table 4 also gives the concentration and the mDP of tannins localized in the cell-walls of the grape skins. These tannins correspond to the parietal tannins that are less easily extracted than vacuolar tannins. There was no significant difference between the control group of berries and the group treated at 4 kV/cm during 1 ms ($W = 4$ Wh/kg). However, in the case of treatment at 0.7 kV/cm ($t_{PEF} = 200$ ms, $W = 32$ Wh/kg), the concentrations of total tannins in the cell-walls
of the skin were much higher than in the control whereas the mDP was lower: there was a decondensation of tannins affected by the treatment of longer duration.

Table 4. Tannins composition and anthocyanins of total skin and cell-wall of skin following PEF treatment

<table>
<thead>
<tr>
<th>Fraction of total skin</th>
<th>Fraction of cell-wall of skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vacuolar Tannins (mg/g of skin)</td>
</tr>
<tr>
<td>Control</td>
<td>15.30 ± 2.45 a*</td>
</tr>
<tr>
<td>4 kV/cm</td>
<td>47.51 ± 12.88 b*</td>
</tr>
<tr>
<td>0.7 kV/cm</td>
<td>22.05 ± 3.41 c*</td>
</tr>
</tbody>
</table>

<sup>a</sup>significant difference in Kruskal & Wallis Test (α= 0.1%)

<sup>a</sup>mDP: mean degree of polymerization

An analysis of anthocyanins in the grape skins was also carried out, the results of which are given in Table 4. The PEF treatment that had the greatest effect on the concentrations of anthocyanins was the treatment at 0.7 kV/cm during 200 ms (W = 31Wh/kg). Berries subjected to this treatment had significantly lower levels of anthocyanins than berries in the control batch, which means that this treatment induced a more complete release of the anthocyanins contained in skin cells compared to control-batch berries. Treatment of 1 ms at 4 kV/cm (W = 4 Wh/kg) had a similar but less pronounced effect, which remained, however, significant compared to the control.

Further to PEF treatment of the crushed and destemmed grape berries and their juice, the concentrations of anthocyanins and tannins in the musts were quantified (Table 5). The concentrations of anthocyanins and tannins were slightly lower in the must treated at 4 kV/cm during 1 ms (W = 4 Wh/kg) and higher in the juice treated during 200 ms at 0.7 kV/cm (W = 31 Wh/kg) as compared to values measured in the juice of the control batch.
Table 5. Anthocyanins, proanthocyanidins concentrations; colour intensity (CI') and Index of Total Polyphenols (ITP) in juice (one day after PEF treatment).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>$E = 4 \text{kV/cm}$</th>
<th>$E = 0.7 \text{kV/cm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins (mg/L)</td>
<td>57.79 ± 0.67 ab*</td>
<td>40.35 ± 1.57 b*</td>
<td>87.59 ± 1.05 a*</td>
</tr>
<tr>
<td>Proanthocyanidins (mg/L)</td>
<td>20.18 ± 0.30 ab*</td>
<td>17.41 ± 0.17 b*</td>
<td>22.33 ± 0.11 a*</td>
</tr>
<tr>
<td>Colour Intensity (CI')</td>
<td>0.58 ± 0.03 a*</td>
<td>1.08 ± 0.11 ab*</td>
<td>1.57 ± 0.06 b*</td>
</tr>
<tr>
<td>Index of Total Polyphenols (ITP)</td>
<td>18 ± 2 a*</td>
<td>27 ± 2 ab*</td>
<td>32 ± 1 b*</td>
</tr>
</tbody>
</table>

*significant difference in Kruskal & Wallis Test ($\alpha = 0.1\%$)

Colour characteristics of the juices were also studied. The colour intensity and the index of total polyphenols in the musts are given in Table 5 which shows that, following PEF treatment, the colour intensity and the index of total polyphenols were increased as compared to the values found in the control juice.

5) DISCUSSIONS

In view of the results, it can be confirmed that the tannins affected by high-intensity, short duration PEF treatment are not the parietal tannins, but the vacuolar tannins. High-intensity, short-duration treatment resulted in a decondensation of vacuolar tannins and, thus, a greater concentration of total tannins in the skins. This finding was confirmed by microscope observations in which vacuolar tannins were seen to be more highly diffused following treatment than those of the control batch of berries at the same stage.

This indicates a lower rate of extraction of tannins from the skins during maceration. In effect the cells are still protected by their walls which are not degraded by this type of PEF treatment, which means that the kinetics of tannin extraction remained limited. This was confirmed by the analysis carried out on the must after treatment, in which the concentrations of polyphenols were found to be similar to those of the control juice. Moreover, the vacuolar
tannins in the berries were still observable by microscope after 14 days of maceration in the juice.

However, the values found for colour intensity and the index of total polyphenols in the post-treatment musts were greater than those measured in the control must. In addition, the wine made from grapes treated with this modality of PEF was richer in polyphenols and in colour than wine made from control berries. PEF treatment of high intensity and short duration had little impact on skin cell-walls and thus had a lesser influence on the release of compounds.

The results reveal that PEF treatment of lower strength for a longer duration induced a rupture of polymer chains and a moderate decondensation of the tannins together with a greater release of anthocyanins than in the control must. The tannins were only slightly affected and slightly more fragmented than those of the control-batch berries. The concentrations of vacuolar tannins and parietal tannins in berry skins treated by PEF were higher than those measured in the control berries. However, this low-intensity, long-duration PEF treatment mainly affected parietal tannins. Light-microscopy observation of the grape berries immediately after PEF treatment showed that treated berries were characterized by a greatly degraded skin in which the cell-walls appeared to have been burnt in places. The parietal system was more severely impacted by the PEF treatment of lower intensity and longer duration than by the previous treatment. This suggests a more rapid release of cellular tannins, the parietal system being disrupted and thus becoming more permeable to intra-cellular compounds. Analysis of the must demonstrated that the concentrations of polyphenols together with the values of the colour intensity and the index of total polyphenols were significantly higher than in the control must. This result is consistent with microscope observations made after 14 days of maceration in which skin cells were seen to be completely drained, containing no traces of tannins. Furthermore, the wine made from grapes treated by this modality of PEF also had a higher polyphenolic content and colour intensity than the
control wine. This PEF treatment strongly impacted the cell walls, leading to a rapid diffusion of intracellular compounds into the must.

The extraction of tannins during maceration is facilitated by PEF treatment of the grapes. The tannins are decondensed and their distribution in the juice is improved. However, the characteristics of tannin astringency are related to the mean degree of polymerization of those tannins (McRae & Kennedy, 2011). Thus, their PEF-induced decrease in size in the must and in the wine is not without consequence. In effect, triangular tests and hedonic tests were carried out after one year of ageing in bottle in order to compare the control wine with wine made from grapes treated during 1 ms at 4 kV/cm ($W = 4 \text{ Wh/kg}$) and wine made from grapes treated at 0.7 kV/cm ($t_{PEF} = 200 \text{ ms}, W = 32 \text{ Wh/kg}$). Test results show that these wines were perceived as significantly different at the 5% and 1% thresholds according the statistical analyses. Nevertheless, the expression of hedonic preferences showed that tasters were divided in their choice of the “best” wines (Delsart et al., 2012). PEF treatment of grapes also enhanced the kinetics of anthocyanin extraction in the must during maceration (López et al., 2008a). Several studies have demonstrated that the concentrations of anthocyanins extracted from grapes by PEF treatment in the musts are retained in the resulting wines (López et al., 2008b, Puértolas et al., 2010b). With knowledge of the different characteristics of the tannins extracted by means of PEF treatment, the tannin-anthocyanin complexation (Jurd, 1969) could be modified. Nevertheless, contrary to what might be expected, the wines made from PEF-treated grapes possessed a colour which remained stable over time as well as constant levels of polyphenols that were above those measured in the control wine.

Several questions remain to be answered concerning, for example, the complexation of anthocyanins and tannins in the must and wine further to PEF treatment, and also the modification of the oxidizability of these musts and wines following PEF treatment given that...
oxidizability is directly related to the antioxidant properties of polyphenols (Oliveira et al., 2011).

6) CONCLUSIONS

PEF treatment induces the depolymerization of grape-skin tannins resulting in a lower mDP and a total tannin content that is higher than in the untreated berry. The tannins in the musts and the wines from PEF-treated grapes were found in greater quantities than in the control due to both the diffusion of decondensed tannins being facilitated by the decrease in the size of these molecules and to the increased permeability of berry cell envelopes. PEF treatment also enhances the release of skin anthocyanins resulting in lower concentrations in the skin of treated berries and in higher concentrations in the must and the wine as well as a higher colour intensity in treated must and wine. However, the choice of treatment modality is of prime importance: PEF treatment of a few kilovolts and of short duration has an impact mainly on vacuolar tannins, whereas treatment of lower intensity but of much longer duration has a greater impact on the parietal tannins and the cell-walls of the skin, resulting in enhanced kinetics of polyphenol extraction.

ABBREVIATIONS

PEF pulsed electric fields
P.A.S. periodic acid schiff
ESI electrospray interface
TPI total polyphenol index
CI colour intensity
TA total acidity
VA volatile acidity

7) ACKNOWLEDGEMENTS

This study received financial support from the Conseil Interprofessionnel des Vins de Bordeaux (CIVB) and the French Agency for Environment and Energy Management (ADEME). The authors wish to express their appreciation to Professor P.L. Teissedre who provided the opportunity to perform the HPLC measurements in his laboratory.

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Sauvignon freshly fermented model wines obtained after different maceration times. LWT - Food Science and Technology, 42(7), 1225-1231.


FIGURE CAPTIONS

Figures:

Figure 1. Experimental set-up. Diagrams of PEF equipment (a) and PEF pulsing protocol (b).

Figure 2. Evolution of colour intensity (a) and total polyphenol index (b) versus time for untreated (0 kV/cm) and PEF-treated grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms). Measurements taken after treatment (day 0), during vinification, and in the bottled wine.

Figure 3. Anthocyanin content of wine (at three months after bottling) obtained from untreated (0 kV/cm) and PEF-treated grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms).

Figure 4. Concentration of tannins in wine (at three months after bottling) for untreated (0 kV/cm) and PEF-treated (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms) red grapes.

Figure 5. Cross-section of A: untreated (0 kV/cm) and B: PEF-treated (PEF1: 0.7 kV/cm for 200 ms) grape berries in light microscopy with P.A.S. staining. Observations made immediately after treatment (day 0). (E: epidermis; eH: external hypodermis; iH: inner hypodermis; S: skin; P: pulp).

Figure 6. Cross-section of A: untreated (0 kV/cm) and C: PEF-treated (PEF2: 4 kV/cm for 1 ms) grape berries in light microscopy with P.A.S. staining. Observations made immediately after treatment (day 0). (E: epidermis; eH: external hypodermis; iH: inner hypodermis; S: skin; P: pulp).
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Figure 3. Colour of the musts immediately after treatment and anthocyanins content of the wines (at three months after bottling) obtained from untreated (0 kV/cm) and PEF-treated grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms)
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FIGURE CAPTIONS

Tables:

Table 1. Conditions for the elution gradient. Mobile phase A (water-formic acid, 95:5, v/v) and B (acetonitrile-formic acid, 95:5, v/v).

Table 2. Characteristics of the wines obtained from untreated (0 kV/cm) and PEF-treated cabernet sauvignon grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms). Analysis immediately after the treatment (day 0), after malolactic fermentation (day 133) and at three months after bottling (day 251).

Table 3. Mean concentration (mg/L) of anthocyanins compounds and tannins in the wines (after six months of bottling) obtained from untreated (0 kV/cm) and PEF-treated (0.7 kV/cm during 200 ms and 4 kV/cm during 1 ms) red grapes.

Table 4. Tannins composition and anthocyanins of total skin and cell-wall of skin following PEF treatment

Table 5. Anthocyanins, proanthocyanidins concentrations; colour intensity (CI') and Index of Total Polyphenols (ITP) in juice (one day after PEF treatment).
**Table 1.** Conditions for the elution gradient. Mobile phase A (water-formic acid, 95:5, v/v) and B (acetonitrile-formic acid, 95:5, v/v).

<table>
<thead>
<tr>
<th>t, min</th>
<th>0</th>
<th>3</th>
<th>15</th>
<th>50</th>
<th>51</th>
<th>54</th>
<th>55</th>
<th>65</th>
</tr>
</thead>
<tbody>
<tr>
<td>%B</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of the wines obtained from untreated (0 kV/cm), PEF-treated grapes (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms). Analysis immediately after treatment (day 0), after malolactic fermentation (day 133) and at three months after bottling (day 251).

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>PEF-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E = 0 \text{kV/cm}$</td>
<td>$E = 0.7 \text{kV/cm}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$I_{PEF} = 200 \text{ ms}$</td>
</tr>
<tr>
<td>pH</td>
<td>3.38</td>
<td>3.41</td>
</tr>
<tr>
<td>TA</td>
<td>3.43</td>
<td>3.63</td>
</tr>
<tr>
<td>VA</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Alcohol content (v/v)</td>
<td>-</td>
<td>12.13</td>
</tr>
</tbody>
</table>

$^a$Total acidity

$^b$Volatile acidity
Table 3. Mean concentration (mg/L) of anthocyanin compounds and tannins in the wines (at six months after bottling) obtained from untreated (0 kV/cm) and PEF-treated (0.7 kV/cm for 200 ms and 4 kV/cm for 1 ms) red grapes.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Untreated</th>
<th>$E=0.7$ kV/cm $t_{PEF} = 200$ ms</th>
<th>$E=4$ kV/cm $t_{PEF}= 1$ ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delphinidin-3-o-glucoside</td>
<td>12.8 ± 0.0</td>
<td>14.5 ± 0.2</td>
<td>17.2 ± 0.2</td>
</tr>
<tr>
<td>Petunidin-3-o-glucoside</td>
<td>13.2 ± 0.0</td>
<td>14.9 ± 0.9</td>
<td>18.6 ± 0.6</td>
</tr>
<tr>
<td>Peonidin-3-o-glucoside</td>
<td>5.4 ± 0.2</td>
<td>5.6 ± 0.1</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>Peonidin-3-o-glucoside</td>
<td>159.3 ± 0.9</td>
<td>155.4 ± 4.6</td>
<td>180.4 ± 6.2</td>
</tr>
<tr>
<td>Peonidin-3-o-acetyl</td>
<td>2.5 ± 0.3</td>
<td>3.5 ± 0.0</td>
<td>3.9 ± 0.0</td>
</tr>
<tr>
<td>Malvidin-3-o-acetyl</td>
<td>77.5 ± 6.4</td>
<td>73.3 ± 0.1</td>
<td>86.2 ± 1.6</td>
</tr>
<tr>
<td>Peonidin-3-o-coumaroyl</td>
<td>0.8 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>1.3 ± 0.0</td>
</tr>
<tr>
<td>Malvidin-3-o-coumaroyl</td>
<td>13.6 ± 0.2</td>
<td>14.3 ± 1.3</td>
<td>15.9 ± 0.0</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>20.1 ± 0.5</td>
<td>26.9 ± 1.6</td>
<td>23.9 ± 1.3</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>10.1 ± 0.3</td>
<td>15.7 ± 0.4</td>
<td>13.1 ± 0.1</td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>21.1 ± 0.7</td>
<td>23.7 ± 0.2</td>
<td>22.3 ± 0.5</td>
</tr>
<tr>
<td>Procyanidin B2</td>
<td>9.0 ± 0.2</td>
<td>13.7 ± 0.2</td>
<td>10.1 ± 0.3</td>
</tr>
<tr>
<td>Procyanidin B4</td>
<td>3.6 ± 0.0</td>
<td>4.8 ± 0.0</td>
<td>4.3 ± 0.1</td>
</tr>
</tbody>
</table>
Table 4. Tannins composition and anthocyanins of total skin and cell-wall of skin following PEF treatment

<table>
<thead>
<tr>
<th></th>
<th>Fraction of total skin</th>
<th>Fraction of cell-wall of skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vacuolar Tannins (mg/g of skin)</td>
<td>mDP(^a)</td>
</tr>
<tr>
<td>Control</td>
<td>15.30 ± 2.45 a(^*)</td>
<td>13.69</td>
</tr>
<tr>
<td>4 kV/cm</td>
<td>47.51 ± 12.88 b(^*)</td>
<td>1.26</td>
</tr>
<tr>
<td>0.7 kV/cm</td>
<td>22.05 ± 3.41 c(^*)</td>
<td>7.92</td>
</tr>
</tbody>
</table>

* significant difference in Kruskal & Wallis Test (\(\alpha=0.1\%\))

\(^a\)mDP: mean degree of polymerization
Table 5. Anthocyanins, proanthocyanidins concentrations; colour intensity (CI’) and Index of Total Polyphenols (ITP) in juice (one day after PEF treatment).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E = 4 kV/cm ( t_{PEF} = 1 \text{ ms} )</th>
<th>E = 0.7 kV/cm ( t_{PEF} = 200 \text{ ms} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins (mg/L)</td>
<td>57.79 ± 0.67 ab*</td>
<td>40.35 ± 1.57 b*</td>
<td>87.59 ± 1.05 a*</td>
</tr>
<tr>
<td>Proanthocyanidins (mg/L)</td>
<td>20.18 ± 0.30 ab*</td>
<td>17.41 ± 0.17 b*</td>
<td>22.33 ± 0.11 a*</td>
</tr>
<tr>
<td>Colour Intensity (CI’)</td>
<td>0.58 ± 0.03 a*</td>
<td>1.08 ± 0.11 ab*</td>
<td>1.57 ± 0.06 b*</td>
</tr>
<tr>
<td>Index of Total Polyphenols (ITP)</td>
<td>18 ± 2 a*</td>
<td>27 ± 2 ab*</td>
<td>32 ± 1 b*</td>
</tr>
</tbody>
</table>

*significant difference in Kruskal & Wallis Test (\( \alpha = 0.1\% \))
The treatment of Cabernet Sauvignon red grapes by Pulsed Electric Fields (PEF) is performed prior to vinification in order to enhance the extraction of polyphenols. PEF treatments of the longest duration and of the highest energy (E = 0.7 kV/cm, t_{PEF} = 200 ms, W = 31 Wh/kg) changed the structure of grape skins and produced a wine that was richer in tannins (34%), while treatment of the highest strength (E = 4 kV/cm, t_{PEF} = 1 ms, W = 4 Wh/kg) altered the visual appearance of phenolic compounds in the skins and led to greater extraction of the anthocyanins (19%). The PEF treatments caused the depolymerization of skin tannins, improving the diffusion of these decondensed tannins which are smaller. The PEF treatment of longest duration and of the highest energy had more impact on the parietal tannins and the cell walls of the skins while treatment of the highest strength modified more the vacuolar tannins. Changes in the operating parameters of the PEF treatment (E = 0.7 to 4 kV/cm, t_{PEF} = 1 to 200 ms, W = 4 to 31 Wh/kg) did not affect alcohol content, total acidity, nor volatile acidity in finished wines compared to the values of the control wine but seemed to cause a slight increase in pH (1-2%).