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Assessing the efficacy of PEF treatments for improving polyphenol extraction during red wine vinifications

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ABSTRACT

The influence of the electric field intensity and pulse width on the improvement of total polyphenol index (TPI) and colour intensity (CI) during extraction in an ethanolic solution (30%) and during fermentation-maceration has been investigated in different grape varieties: Grenache from two harvesting times, Syrah and Tempranillo. The aim of this study was to develop a procedure to establish the PEF treatment conditions that cause enough permeabilization in the skin cells of different grape varieties to obtain a significant improvement in the vinification process in terms of increment on the polyphenol content or reduction of maceration time.

Results obtained in this investigation indicate that extraction of polyphenols in a solution of ethanol (30%) for 2 h could be a suitable procedure to know if the PEF technology is effective for improving extraction of polyphenols from the grapes during vinification and to determine the most suitable PEF treatment conditions to obtain this objective. Improvement in the extraction during vinification only was observed with those grapes and under treatment conditions in which the improvement of the polyphenol extraction was higher than 40%. Other interesting observation from this research is the highest efficacy of PEF when treatments of the same duration are applied using longer pulses. Therefore, in a continuous process, where the flow processed is determined by the frequency applied by the PEF generator, it is possible to increase the processing capacity of the PEF installation.

Industrial relevance: Benefits from PEF treatment of the grapes before the maceration step in the vinification process have been demonstrated. Nevertheless, the characteristics of the grapes may change in different vintages and grape varieties. Therefore, it is of high importance to be able to determine the optimum PEF conditions in order to obtain the desired benefit during the vinification. The rapid method developed permits to determine PEF process parameters before the application of the PEF treatment with the objective of facilitating the phenolic extraction and therefore, reducing the maceration time. In these cases, it would be possible to remove the skins from the rest of the wine earlier, and therefore, increase the processing capacity of the winery.

1. Introduction

A driving force to maintain and enhance the competitiveness of food industry is technological innovation. The development of innovations capable of improving wine quality and processing sustainability are increasingly attracting for wineries. Pulsed electric field technology (PEF) is an innovative processing technology with potential applications in wineries to accelerate and/or increase the extraction of phenolic compounds during the maceration-fermentation step of red winemaking (Luengo, Franco, Ballesteros, Álvarez, & Raso, 2014). Red wine quality is strongly affected by the phenolic compounds which are responsible for the sensory characteristics such as colour and taste, aging properties, and antioxidant properties which may play a positive role in human health (Moreno-Arribas & Polo, 2005).

Application of PEF technology for improving extraction of polyphenols results particularly interesting in those vintages in which the concentration of these compounds in the grape skins is poor or the polyphenol extraction is difficult. Another advantage of PEF treatments is that it permits reducing the time of contact of the grape pomace with the fermenting must to obtain a given polyphenol concentration in the wine, thus increasing the productivity of the winery (Puértolas, Saldaña, Condón, Álvarez & Raso, 2010).

During PEF processing, a mix of must, skin and seeds obtained after de-stemming and lightly mechanically crushing the whole bunches is subjected to short pulses (μs) of high voltage (kV). When exposed to a sufficiently strong electric field, the cell membrane of grape skins undergoes electroporation, which renders it permeable to molecules such as polyphenols that are otherwise unable to cross it (Cholet et al., 2014).

PEF application to improve extraction of phenolic compounds during red winemaking has been deeply investigated in different grape varieties, using moderate electric fields (0.5–1 kV/cm) and treatment

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times in the range of 40–100 ms (Delsart et al., 2012, Delsart et al., 2013; El Darra, Grimi, Maroun, Louka, & Vorobiev, 2013) or higher electric fields (1–10 kV/cm) and treatment times in the range of 100 μs (Lopez, Puértolas, Álvarez, Condón & Raso, 2008; López, Puértolas, Condón, Álvarez & Raso, 2008; Donsi, Ferrari, Fruilo, & Pataro, 2010). These studies have demonstrated that electroporation of the cells of the grape skins by PEF depends on the grape varieties but also that the physicochemical composition of the grape, that may differ for different vintages or even during the harvesting period, could influence the PEF effect.

The release of polyphenols during the maceration-fermentation step and the conversion of sugars of the must into ethanol is a slow process that takes several days. Therefore, the effectivity of the PEF treatment in the improvement of the polyphenol content of the wine only can be observed some days after the treatment. On the other hand, if the electroporation of the grape skins is not intense enough, effects observed during the first days of maceration may disappear at the end of the maceration-fermentation step. Therefore, evaluating in a short period of time the electroporation degree of the grape skins in terms of improvement in the polyphenol release could result of interest to define the required PEF treatment conditions to maximize polyphenol extraction minimizing energetic costs. Different methods such as microscopic observations of cell integrity, measurement of the liquid release, evaluation of the conductivity of the exuded liquid, analysis of textural parameters of treated tissues or impedance measurement have been proposed to assess electroporation of plant cells (Donsi et al., 2010; Puértolas, Luengo, Álvarez, & Raso, 2012). However it has not been demonstrated that these methods were effective estimating the effect of PEF when subsequent processing steps are required.

The aim of this study was to develop a procedure to establish the PEF parameters that cause enough permeabilization in the skin cells of different grape varieties to obtain a significant improvement in the vinification process in terms of increment on the polyphenol content or reduction of maceration time. The influence of the electric field intensity and pulse width on the improvement of polyphenol extraction has been investigated in three grape varieties, Syrah, Tempranillo and Grenache.

2. Material and methods

2.1. Grape samples

Grapes from *Vitis vinifera* L. var. Syrah, Tempranillo and Grenache, from the certified origin Campo de Borja (Aragon, north-east Spain), were harvested from the 2015 vintage. The grapes were manually harvested in good sanitary conditions during their optimal ripening stage. In the case of Grenache grapes, they were harvested during their optimal ripening stage (Grenache 1) and two weeks later (Grenache 2).

Total acidity, pH, °Brix, and total phenols were analyzed in the must (OIV, 1990). Phenols at pH 3.2 and 1.0 and extractability of phenols were obtained by macerating a grape homogenate (Ika labortechnik A10, Staufen, Germany) for 4 h at two different pH values (3.2 and 1.0), according to the method described by Saint-Cricq de Gaulejac, Vivas, and Glories (1998).

The grapes were transported in 20-kg boxes from the field to the laboratory for the subsequent experiments. Then, the grapes were destemmed and crushed, and the grape juice was separated for treating the skins by PEF. The proportion of grape juice and pomace was measured in order to maintain this proportion during the vinifications.

2.2. PEF equipment

The PEF unit used in this investigation (EPULSUS®-PM1-10, Energy Pulse System, Lisbon, Portugal) is a Marx generator that can apply monopolar square waveform pulses with a frequency up to 200 Hz. The maximum output voltage and current were 10 kV and 180 A, respectively. The pulse width can be modified, ranging from 5 to 100 μs. It is a compact PEF generator with 800 × 600 × 400 mm as dimension and only 80 kg of weight that can be used both at lab and pilot plant scale, and can be controlled directly from its touchscreen. The actual voltage, current and pulse duration were measured using a high voltage probe (Tektronix, P6015A, Wilsonville, OR, USA) and a current probe (Stangenes Industries Inc. Palo Alto, CA, USA), respectively, connected to an oscilloscope (Tektronix, TDS 220,Wilsonville, OR, USA).

The PEF treatments were applied to a parallel plate electrodes treatment chamber of 19.6 cm^2 of electrode area and 2 cm of gap. Batches of 50 g of grapes were treated at 1, 3 and 5 kV/cm for 100 μs of total treatment time, and the corresponding number of pulses of 5, 20, 50 and 100 μs of pulse width were applied. The specific energy applied was 0.14, 1.26 and 3.5 kJ/kg at electric fields of 1, 3 and 5 kV/cm respectively.

2.3. Extraction in ethanol solution

50 g of grapes of untreated and PEF treated grapes were placed in 250 mL Erlenmeyer flasks containing 100 mL of a 30% v/v ethanol solution, at room temperature without agitation. Previous results indicated that a 30% v/v of ethanol solution showed the highest difference of absorbance at 280, 420, 520 and 620 nm after 2 h of extraction at room temperature between the untreated grapes and PEF treated grapes (data not shown). Samples of 1 mL were taken during 120 min and centrifuged at 8640*g* for 90 s (Minispin®plus, Eppendorf, Hamburg, Germany). The absorbance was measured at 280 nm for the total polyphenol index (Eq. (1)) and 420, 520 and 620 for the colour index (Eq. (2)) in a spectrophotometer (Unicam UV500, Unicam Limited, Cambridge, UK) according to Glories' methods (Glories, 1984; Glories & Agustin, 1990):

$$TPI = Abs_{280} \times \mathrm{DF} \tag{1}$$

$$CI = (Abs_{420} + \mathrm{Abs}_{520} + \mathrm{Abs}_{620}) \times \mathrm{DF} \tag{2}$$

where *TPI* is the total polyphenol index, *CI* is the colour index, Abs_λ is the absorbance at the corresponding length wave (λ) of 280, 420, 520 and 620 nm and *DF* is the dilution factor.

Each experiment was carried out by triplicate.

2.4. Vinifications

Laboratory fermentations were performed in 500 mL Erlenmeyer flasks that were opened to the atmosphere by a small orifice of 1 mm of diameter. 100 g of grapes were mixed with the corresponding proportion of grape juice. The fermentation was carried out at by a commercial preparation of yeast of *Saccharomyces cerevisiae* (Lalvin, Ontario, Canada) at an initial concentration of 10^6 CFU/mL. The fermentation temperature was controlled at 25 °C and the weight of the samples was monitored once a day, until the measured weight was constant at least two consecutive days. The weight loss is related to the loss of CO_2 during the fermentation. The duration of the fermentation-maceration step was 10 days for all the grapes. Every day a sample of 1 mL was taken and the TPI and CI were measured as described previously.

Each vinification was carried out by triplicate.

2.5. Statistical data treatment

The results represent the mean ± standard error of the mean, of the analysis performed on the three flasks containing samples receiving the same treatment. A *t*-test was conducted to assess significant differences between vinifications conducted with untreated and PEF-treated gapes along maceration–fermentation time. The differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Grape characterization

Experiments to define PEF processing parameters that cause the required electroporation in the grape skin cell to obtain a significant improvement polyphenol release during vinification were conducted with three different grape varieties and, in the case of Grenache, with grapes harvested in two different moments. It is well known that the release of polyphenols during fermentation-maceration is influenced not only by the total polyphenol content in the grapes, that may depend on factors affecting the berry development such as soil, geographical location and weather conditions, but also on the cell wall structure and morphology of the skin cells that are intrinsic characteristics determined by the grape variety (Jackson & Lombard, 1993; Jones & Davis, 2000).

Table 1 shows the results of the analysis carried out on the grapes used in this investigation. Acidity was higher in Syrah than Tempranillo and Grenache and the total sugar content expressed as °Brix and the pH was similar in Syrah, Tempranillo and Grenache 1. However the °Brix of the Grenache 2 grapes was much higher because they were harvested two weeks later. In order to evaluate the total content of polyphenols the grapes were macerated for 4 h at pH 1.0 and 3.2. At pH 1.0 a complete degradation of the membranes of cell skin and vacuoles occurs facilitating the complete release of phenolic compounds while at pH 3.2 the extraction was conducted in similar conditions as those occurring during maceration in vinification process. The TPI determined at pH 1.0 was higher for Tempranillo than for Syrah and Grenache 1 grapes while this index was higher for the Grenache 2 grapes, harvested later. Similar results were obtained for the TPI determined at pH 3.2. Tempranillo grapes had the highest TPI value and the TPI for the Grenache 2 grapes was higher than Syrah and Grenache 1 grapes, harvested before. No significant differences were observed between the conductivity of the most of the different varieties or of the same variety harvested in different moments (1.5 ± 0.1 mS/cm). Conductivity of the must affects the performance of PEF modulators by influencing the electrical resistance of the treatment chamber. Energy requirements to generate a given electric field strength are lower as lower is the resistance of the treatment chamber. The conductivity of the grape must as compared with other food is quite low indicating that specific energetic requirements for this application may be lower than for others (Heinz, Álvarez, Angersbach, & Knorr, 2001)

3.2. Influence of PEF treatments of different electric field strength and pulse width on the extraction of polyphenols from different grapes varieties in ethanol solution

Fig. 1A and B shows the influence of the electric field strength and pulse width on the polyphenol index and the colour intensity of the solution of ethanol (30%) containing PEF pre-treated grapes of the three varieties after 2 h of extraction. The main procedures to measure total polyphenols in wineries are the Folin-Ciocalteu index (FCI) and the Total Polyphenol Index (TPI). Both are spectrophotometric methods, however, whereas the FCI requires the use of reagents and the incubation of the sample for 30 min, the determination of the TPI simply consist in a direct measure of the absorbance of the sample at 280 nm - wavelength that corresponds to the maximum of absorbance of the benzene ring. As the ultimate goal of this paper is to set up a procedure to evaluate grape electroporation the faster procedure to measure polyphenols was chosen. On the other hand, the determination of the colour intensity of wine and must is other direct spectrophotometric measured conducted in the wineries consisting in the sum of 420, 520, 620 nm absorbance.

For the four grape varieties, it was observed that the application of a PEF treatment of 1 kV/cm of intensity did not increase de polyphenol extraction at any pulse width investigated. No differences were observed in the TPI for the treatments applied at different pulse width as compared with the control. This lack of effect of the PEF could be a consequence of the low total specific energy of the treatments applied at 1 kV/cm (0.14 kJ/kg). Other authors have observed improvement in polyphenol extraction during red winemaking at lower electric fields but applying pulses in the range of milliseconds with much higher specific energies (50 kJ/kg) (El Darra et al., 2013). The electroporation of the grape skin cell by PEF treatments of 3 kV/cm increased the TPI as compared with the untreated grapes for the treatments applied at higher pulse widths. The TPI of the treated samples as compared with the control increased 30.1%, 13.8%, 19.1% and 33.3% for the treatments applied at 100 μs in the Syrah Tempranillo, Grenache 1 and Grenache 2 varieties respectively. For the three grape varieties, the pre-treatment of the grapes increased the TPI when the PEF treatment was applied using pulses higher than 5 μs at 5 kV/cm. For Syrah these treatments increased the polyphenol extraction between a 46% (5 pulses of 20 μs) to 70% (1 pulse of 100 μs), for Grenache 1 between a 38% (5 pulses of 20 μs) to 63% (1 pulse of 100 μs), Grenache 2 between a 37% (5 pulses of 20 μs) to 46% (1 pulse of 100 μs), and for Tempranillo variety between a 8% (5 pulses of 20 μs) to 24% (1 pulse of 100 μs). It was observed that although the total duration (100 μs) and as consequence the total specific energy (3.5 kJ/kg) of the different treatments was the same the electroporation was more effective by decreasing the number of pulses and increasing the pulse width.

The influence of the pulse width on electroporation caused by PEF of microbial and eukaryote cells at a fixed total treatment time has not been widely investigated. Some authors observed that at a constant quantity of the applied specific energy pulse width did not have a significant effect on microbial inactivation by PEF (Raso, Alvarez, Condón, & Sala, 2000; Sampedro, Rivas, Rodrigo, Martínez, & Rodrigo, 2007) but others reporter higher microbial inactivation when pulses applied were wider (Martín-Belloso et al., 1997; Abram, Smelt, Bos, & Wouters, 2003). However, in these studies higher inactivation could be consequence of the increment of the temperature of the treatment medium when wider pulses were applied (Saldaña et al., 2010). Microbial inactivation requires the application of higher specific energy (high electric field strengths and longer treatments) than electroporation of eukaryotes so higher increment of the temperature when wider pulses were applied could cause the higher efficacy of PEF. In the case of plant cells results obtained in this investigation are in contradiction with those reported by Ersus, Oztop, McCarthy, and Barret (2010) that observed that the ion leakage from onion tissue decreased with decreasing pulse number and increasing pulse widths. However the higher polyphenol release from grape tissues when longer pulses were applied confirm observation of De Vito, Ferrari, Lebovka, Shynkaryk, and Vorobiev (2008) that reported that samples of sugar beet and apple tissues

Table 1
Physicochemical characteristics of the grapes at harvesting time.

	pH	Total acidity (g/L)	°Brix	Phenols at pH 1 (OD 280 nm)	Phenols at pH 3.2 (OD 280 nm)	Extractability (%)
Syrah	3.2	6.5	23.4	23.4	16.6	29.3
Tempranillo	3.2	5.1	22.7	32.9	28.0	15.0
Garnacha 1	3.2	5.1	23.8	20.6	17.7	13.8
Garnacha 2	3.5	4.8	31.3	28.0	22.3	20.5

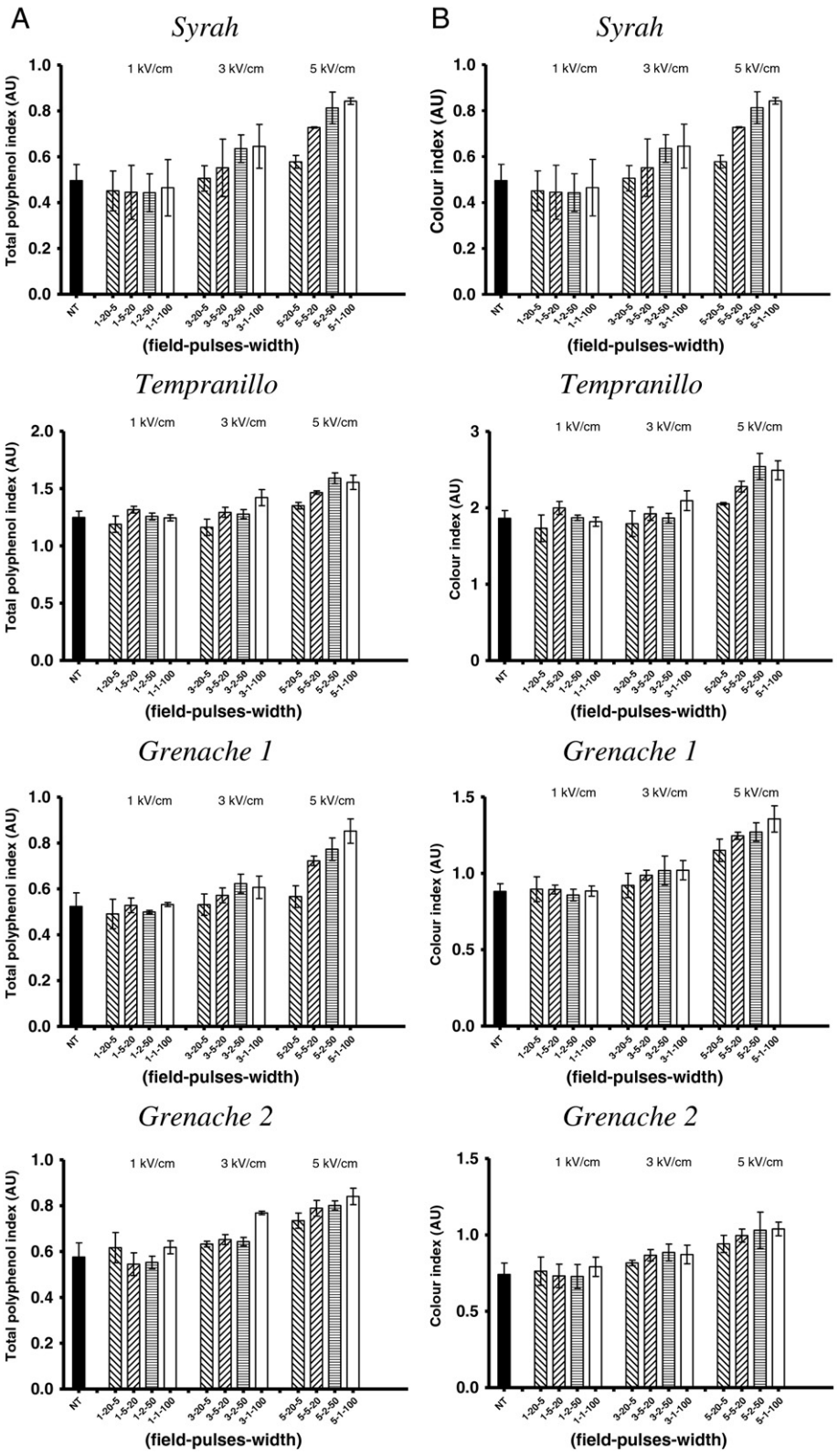

Fig. 1. Total polyphenol index (A) and color index (B) after 120 minutes of extraction in ethanol solution (30%) for the untreated (NT) and PEF treated grapes of Syrah, tempranillo, Grenache 1 and 2. For each bar, PEF treatments are indicated as electric field-number of pulses-pulse width in kV/cm, number of pulses and μs, respectively.

exposed to the same PEF treatment time showed higher electroporation in terms of cell disintegration index when the pulse duration was wider. The higher efficacy of longer pulses has been related to the fact that an efficient electroporation of the cell membranes requires pulses of longer duration, as compared to the membrane charging time, in order to reach the maximum transmembrane voltage (Bazhal, Lebovka, & Vorobiev, 2003).

Fig. 1B shows that influence of the PEF treatment of different electric field strength and pulse width on the colour intensity (CI) of the extraction medium containing grapes of the three varieties follows a pattern

similar to the TPI. No significant differences were observed in the CI for the treatments applied at 1 kV/cm as compared with the control. At 3 kV/cm the treatment increased the CI for the Syrah variety. Significant differences were observed between the control and the samples treated by pulses longer than 5 μs. However for the other two varieties, 3 kV/cm only increased significantly the CI using a pulse width of 100 μs for the Tempranillo variety. Similarly to the effect observed for the TPI, for the three varieties the treatment at 5 kV/cm significantly increased the CI especially when the treatments were applied using the wider pulses. For Syrah these treatments increased the CI between a 93%(5 pulses of 20 μs) to 121% (1 pulse of 100 μs), for Grenache 1 variety between a 40% (5 pulses of 20 μs) to 59% (1 pulse of 100 μs), for Grenache 2 variety between a 34% (5 pulses of 20 μs) to 40% (1 pulse of 100 μs) and for Tempranillo variety between a 24% (5 pulses of 20 μs) to 36% (1 pulse of 100 μs).

The correspondence observed between the TPI and CI could be related with the fact that the main compounds of the grape skins responsible of the CI are anthocyanins that are the pigments responsible of the colour of red grapes and represents one of the main phenolic compounds present into the cells of the grape skins.

3.3. Influence of PEF treatments of different electric field strength and pulse width on the extraction of polyphenols from different grapes varieties during vinification

The evolution of the TPI of the fermenting must containing untreated grapes and PEF treated grapes under the same treatment conditions that in the extraction experiment in the ethanol solution (30%) for Syrah, Grenache1, Grenache 2 and Tempranillo varieties are shown in Figs. 2–5 respectively. In all cases, the maceration-fermentation process was extended for 10 days. After this period of time, the weight of the flask containing the fermenting must was constant indicating the end of the fermentation.

The evolution of the TPI along the maceration-fermentation process depended on the grape variety and on the intensity of the PEF treatment applied. For the Syrah variety, the maximum value for the TPI was

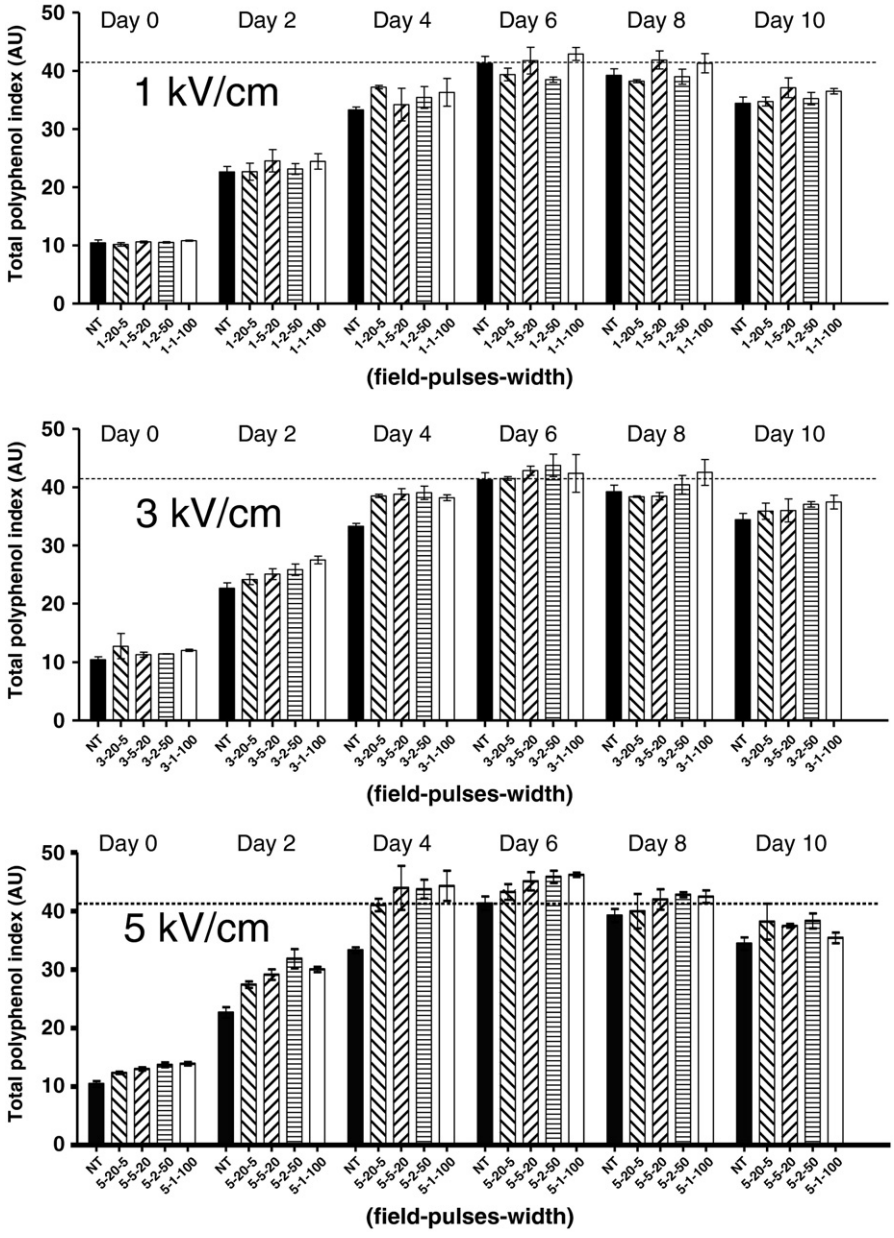

Fig. 2. Total polyphenol index (TPI) observed during the fermentation-maceration of the untreated (NT) and PEF treated Syrah grapes. For each bar, PEF treatments are indicated as electric field-number of pulses-pulse width in kV/cm, number of pulses and μs, respectively. The dotted line represents the maximum TPI obtained for the untreated samples.

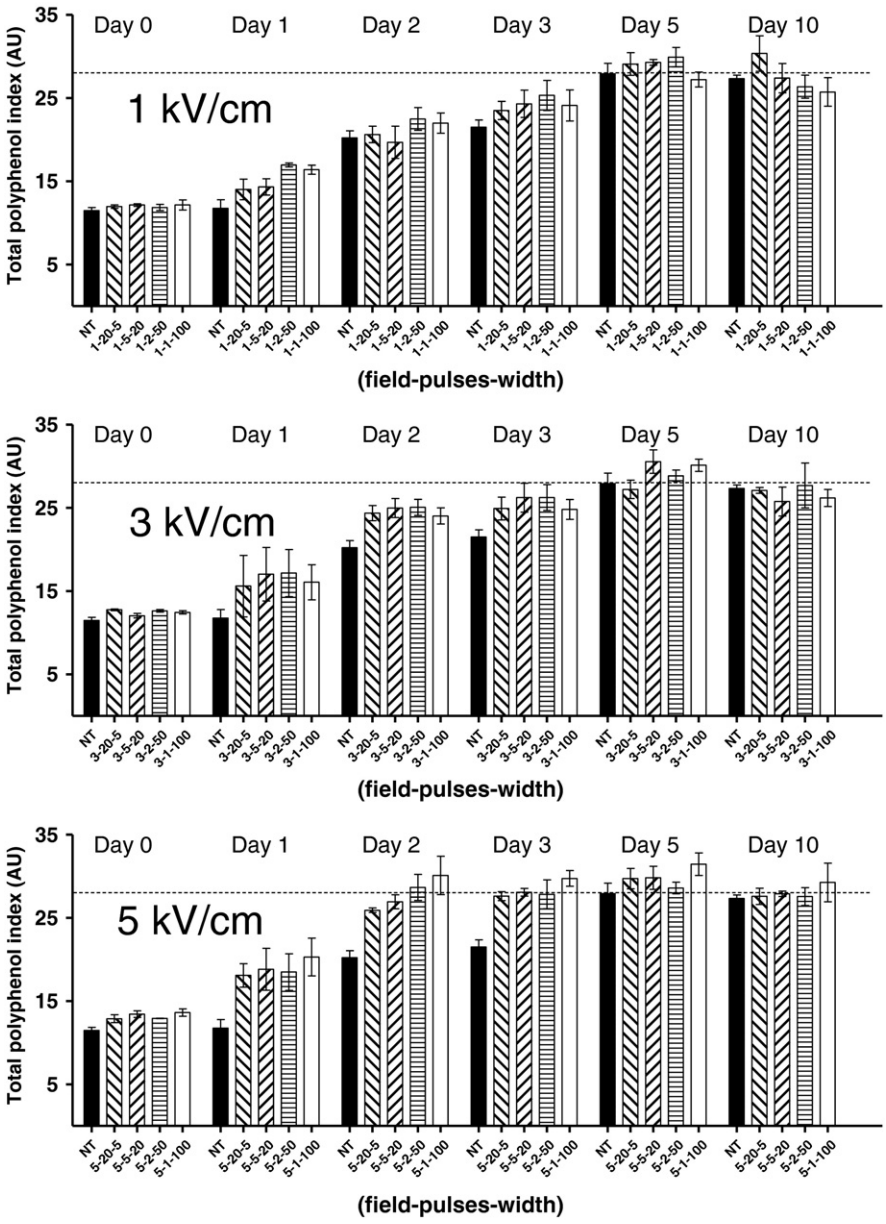

Fig. 3. Total polyphenol index (TPI) observed during the fermentation-maceration of the untreated (NT) and PEF treated Grenache 1 grapes. For each bar, PEF treatments are indicated as electric field-number of pulses-pulse width in kV/cm, number of pulses and μs, respectively. The dotted line represents the maximum TPI obtained for the untreated samples.

achieved after 6 days of maceration fermentation for the untreated and PEF treated samples at 1 and 3 kV/cm. After this maximum, TPI slightly decreased in all cases. This fall of polyphenols at the end of the maceration-fermentation process is attributed to their attachment to other compounds and to their precipitation causing the decrease of free polyphenols in dissolution. While no significant differences were observed between the control and PEF treated samples at 1 kV/cm along the fermentation-maceration time, the TPI after 4 days of maceration was higher for the PEF treated samples at 3 kV/cm than for the control. However, differences in TPI between untreated and treated samples disappeared after 6 days of maceration-fermentation. In the case of the samples treated at 5 kV/cm, the TPI of the PEF treated samples was higher than for the control even after 6 days of maceration-fermentation. On the other hand, the TPI of the PEF samples after 4 days of maceration fermentation was similar or slightly higher than for the control after 6 days of maceration-fermentation. These results indicate that the PEF treatment could reduce the maceration time to obtain the highest phenolic concentration in the wine for 2 days. Fig. 3 shows that the maximum value for TPI was achieved after 5 days of maceration-fermentation for the untreated and PEF treated samples at 1 and 3 kV/cm of Grenache 1 samples and then the value of TPI in the fermenting must was maintained practically constant until the end of the fermentation. Similarly to Syrah grapes, the treatment at 1 kV/cm was ineffective to significantly increase the TPI along maceration-fermentation. On the other hand the differences observed between untreated and treated samples at 3 kV/cm during the 3 first days of maceration fermentation disappeared after 5 days. In the case of the samples treated at 5 kV/cm, the PEF treatment was very effective for improving TPI of fermenting must during the first 3 days of maceration-fermentation but the differences between the control and PEF treated samples disappear after 5 days of maceration-fermentation. However, the maximum value for the TPI of the control that was obtained after 5 days of maceration fermentation was achieved after 2 days for the samples treated with pulses of 50 and 100 μs and after 3 days for the

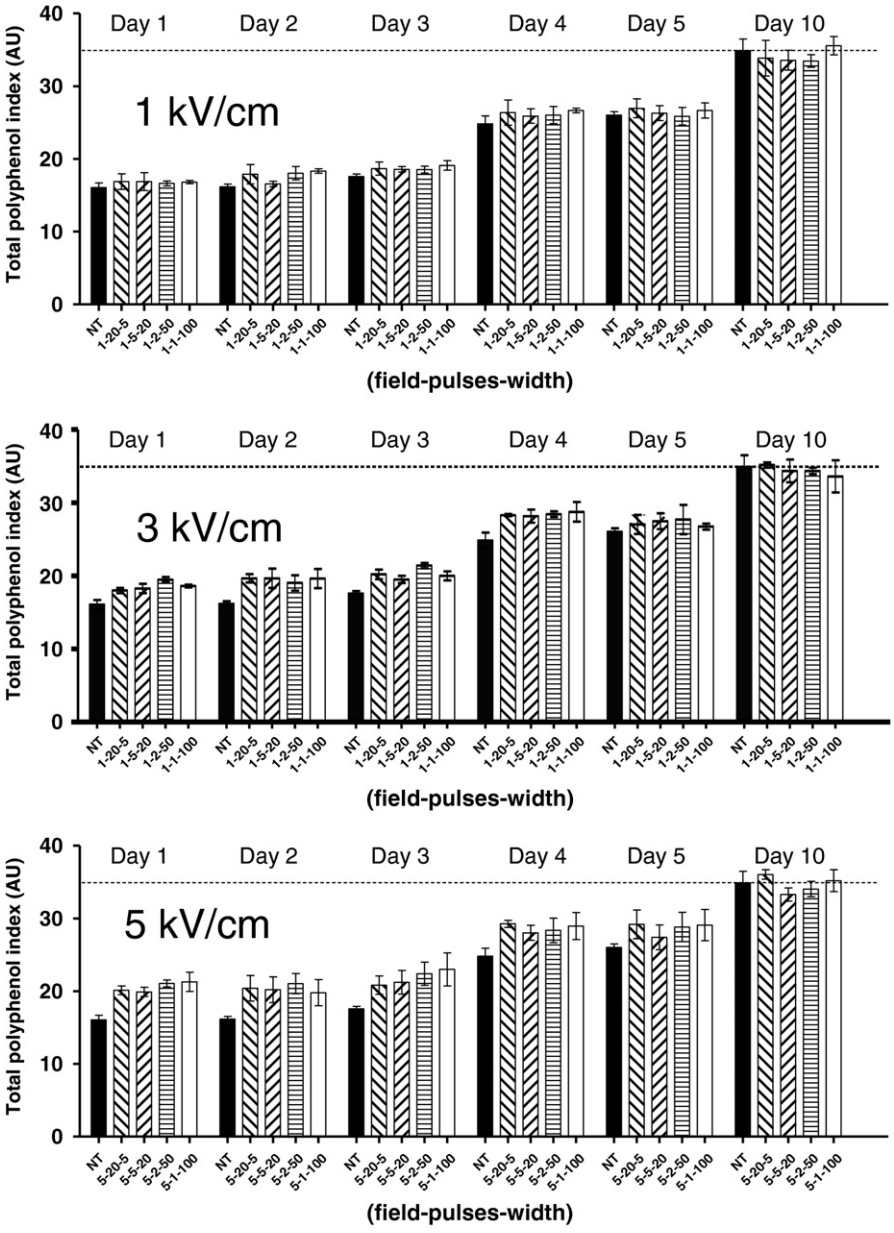

Fig. 4. Total polyphenol index (TPI) observed during the fermentation-maceration of the untreated (NT) and PEF treated Grenache 2 grapes. For each bar, PEF treatments are indicated as electric field-number of pulses-pulse width in kV/cm, number of pulses and μs, respectively. The dotted line represents the maximum TPI obtained for the untreated samples.

rest of the PEF treatments. Results obtained indicate that the pretreatment of the grapes of Grenache 1 by PEF could reduce maceration time between 2 and 3 days depending of the width of the pulses applied. Concerning the influence of PEF intensity and pulse width on polyphenol extraction from the Grenache grapes harvested later (Fig. 4), it was observed a slightly higher polyphenol release for the samples treated by PEF at 3 and 5 kV/cm until the first 4 days of maceration-fermentation. However, the differences in TPI observed between treated and untreated samples would not allow reducing maceration time for the samples PEF treated without affecting the TPI in the final wine. Finally, Fig. 5 shows that for the Tempranillo variety, the PEF treatments applied to the grapes at different electric field strengths and pulse widths did not significantly increase TPI at any moment of maceration-fermentation process.

As it happened during the extraction experiments conducted in a 30% ethanol solution, the evolution of the colour index (CI) followed a similar trend than the evolution of the TPI in the must during the vinifications for all the grape varieties studied (data not shown).

3.4. Relationship between the PEF effect on the polyphenol release from different varieties of grapes in the ethanol solution and during fermentation-maceration

Results obtained regarding the extraction of polyphenols in the alcoholic solution for 2 h showed that the effect of PEF treatments in the improvement of the polyphenol release was much higher in Syrah and Grenache 1 grapes than in Grenache 2 and Tempranillo grapes. In all cases, the most significant effect of PEF was observed at the highest electric field strength and when longer pulses were applied. In Syrah and Grenache 1 grapes a treatment of 5 pulses of 20 μs at 5 kV/cm increased the TPI around 40–45% while when 1 pulse of 100 μs was applied the observed increment was around 60–70%. In the case of Grenache 2 and Tempranillo the highest increment in the TPI of the extraction medium was 46 and 25% respectively after applying 1 pulse of 100 μs at 5 kV/cm. When these results were compared with the total phenols measured in the grapes it was observed that the PEF treatment was more effective in

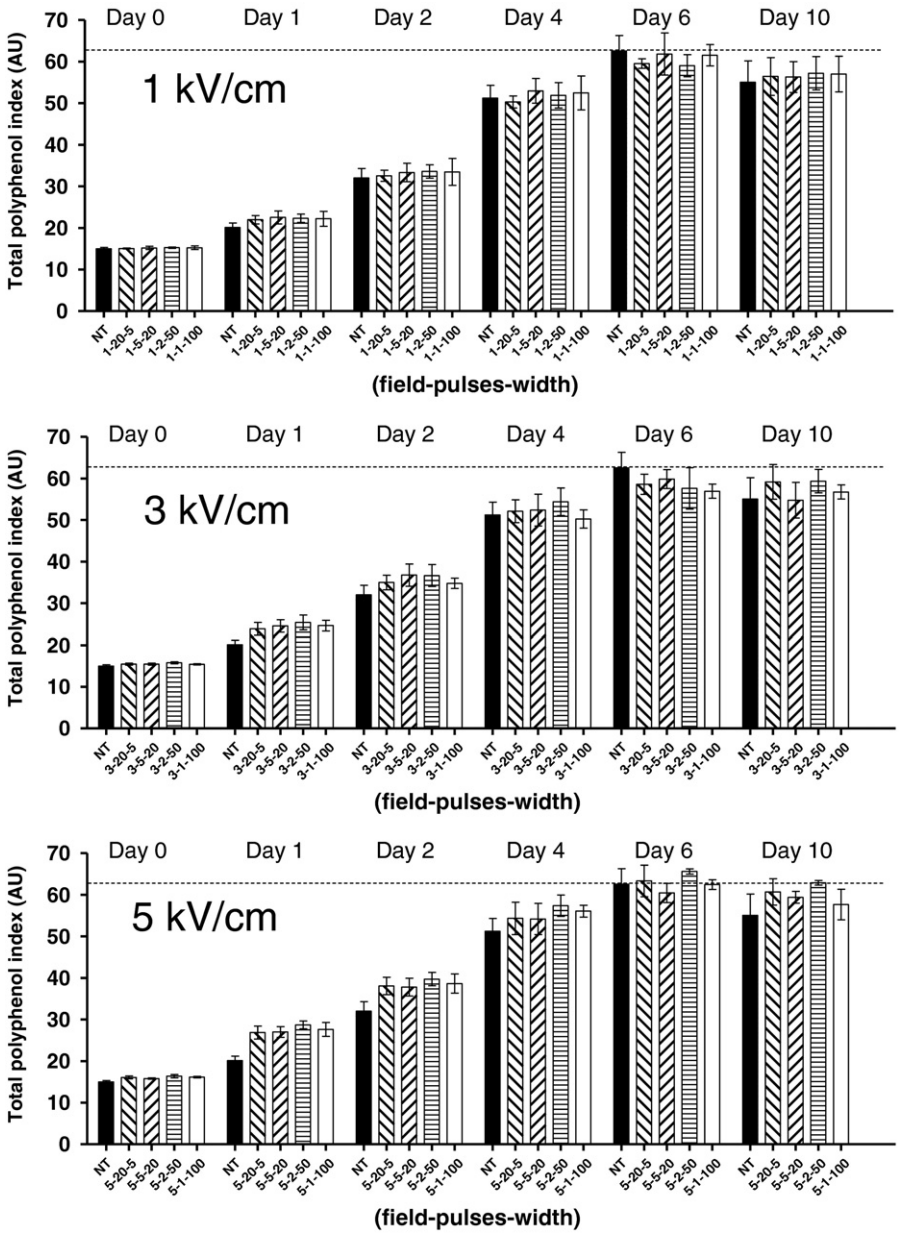

Fig. 5. Total polyphenol index (TPI) observed during the fermentation-maceration of the untreated (NT) and PEF treated Tempranillo grapes. For each bar, PEF treatments are indicated as electric field-number of pulses-pulse width in kV/cm, number of pulses and μs, respectively. The dotted line represents the maximum TPI obtained for the untreated samples.

that grapes in which the total polyphenol content obtained by macerating the grapes at pH 3.2 and 1.0 was lower. These results confirm previous observations that indicated that the PEF treatment might be more useful when the extraction of the phenolic compounds from the grape skins is hampered or its total polyphenol content is lower (Puértolas, López, Condón, Álvarez & Raso, 2010).

When results obtained in ethanol solution were compared with the polyphenol release during maceration-fermentation it was observed that the treatment was ineffective in those grapes (Grenache 2 and Tempranillo) in which the extraction in the alcohol solution was lower but effective in those grapes (Syrah and Grenache 1) and under those PEF treatment conditions in which the highest effect on polyphenol extraction was observed in the ethanol solution for 2 h. From a practical point of view the key advantage of the application of the PEF treatment in these grapes was the reduction of the duration of the maceration during vinification between 2 and 3 days. The efficiency of the transfer of polyphenols from grape skins to the fermenting must depends on the extent of cell degradation. In the first days of maceration-fermentation the extraction was more effective in the PEF treated samples that in the control samples because the electroporation of the cells facilitates the polyphenol release. However differences in the TPI in the fermenting must containing untreated and PEF treated grapes becomes smaller along maceration-fermentation because the alcohol produced during fermentation contributes to the disorganization of the cell envelopes of the grape skins facilitating the polyphenol release from the untreated grapes. Currently wineries are interested in reducing the duration of the time of contact of the grape skins with the fermenting must during maceration-fermentation. Grape skins represent a huge volume in the fermentation tanks, therefore, the removal of the grape skins increases the amount of fermenting must that can be stored in the tanks increasing the production capacity of the wineries without increasing the number of fermentation tanks.

As conclusion this investigation demonstrates that an extraction of polyphenols in a solution of ethanol (30%) for 2 h could be a procedure to know if the PEF technology is effective for improving extraction of polyphenols from the grapes that are arriving to the winery during

vinification and to determine the most suitable PEF treatment conditions to obtain this objective. Other interesting observation from this research is the highest efficacy of PEF when treatments of the same duration are applied using longer pulses. Longer pulses permit reducing the number of pulses to be applied for a given treatment time. Therefore in a continuous process, where the flow processed is determined by the frequency applied by the PEF generator, it is possible to increase the processing capacity of the PEF installation.

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