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Red wine pilot-scale vinification assisted by pulsed electric fields: effect on sensory and physico-chemical quality of Syrah and Tempranillo varieties (*Vitis vinifera* L.)

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ABSTRACT

Pulsed electric field (PEF) assisted vinification was applied in a pilot-scale winery for the production of red wines from Syrah and Tempranillo grape vine varieties. The grape mash obtained after destemming and crushing the grapes was subjected to pulsed electric fields with 2 kV/cm of electrical field intensity and specific energies of 2 kJ/kg and 2.8 kJ/kg for Syrah and Tempranillo, respectively. The long-term effects of the PEF assisted extraction on the physico-chemical parameters and sensory quality of the finished wines were investigated after 1.5 years of wine ageing. For sensory, discriminative triangle tests were carried out for each wine variety to assess any effect of PEF on colour and flavour.

PEF did not affect most of the physico-chemical parameters analysed, such as density, alcoholic strength by volume, total acidity, total SO_2, total dry extract, and tanning capacity. However, wines made with grapes treated with PEF have a higher pH value, turbidity, and electrical conductivity compared to control wines. The physico-chemical results showed that the permeabilisation of the grape skin caused by the PEF treatment led to an increase in total phenols, including flavonoid phenols and anthocyanins, in relation to wines made with grapes without PEF treatment. In agreement, the results of chromatic parameters showed that PEF increased the value of the a* parameter, corresponding to a more intense red colour, decreased the value of the L* parameter, corresponding to a darker wine, and increased the C* parameter, known as chroma, referring to the saturation of a colour.

The sensory results showed that PEF promotes changes in colour and flavour (aroma and taste) of wines. The colour sensory results are in agreement with the analytical colour assessed by the chromatic characteristics and anthocyanin contents. Sensory characterisation of control and PEF wines, and panellists' comments from triangle tests, favoured the wines vinified with PEF, which revealed a more intense red colour, fuller-bodied and rounder, and with greater intensity and persistence in the aftertaste aroma.

KEYWORDS: electroporation, PEF, extraction, Tinta Roriz, Aragonez, colour, flavour, red wine

INTRODUCTION

In recent decades, with an increased demand from consumers for minimally processed, high-quality products, the food industry has undergone technological innovations. The challenges in the wine industry are related to economic aspects, such as yield in must and the behaviour of alcoholic fermentation, as well as quality parameters, such as aromatic profile and sensory attributes (Fauster *et al.*, 2020). Winemaking techniques play a crucial role in the quality and ageing stability of wine. Among them, in red wine vinification, the extraction of phenolic compounds has a significant effect on organoleptic quality and colour stability. The colour of red wines is one of the most important attributes for the evaluation of wine organoleptic characteristics, serving as a key indicator of its quality. It provides the consumer with visual information about the body, age, and evolution of the wine during storage, with any alteration in colour potentially indicating possible defects (Araújo *et al.*, 2005). From a chemical perspective, the colour of red wines is strongly related to their phenolic composition, particularly with the pigments extracted from the grape and other pigments formed through reactions with anthocyanins during the fermentation and ageing processes (Jackson, 2008; Singleton, 1987).

According to Cerpa-Calderón and Kennedy (2008), during traditional winemaking, only a portion of the solid components of the grape are transferred to the must. As polyphenols affect the sensory quality of red wine, past studies have focused on techniques influencing the release of these compounds from the grape to the must, such as varying maceration temperatures and times, thermovinification, carbonic maceration, and the use of pectolytic enzymes (Pinelo *et al.*, 2006; Sacchi *et al.*, 2005). However, these techniques, which require effective temperature control, may demand high energy consumption, long operation times, and can negatively influence the sensory quality of the wine (Delsart *et al.*, 2012). Therefore, enologists are increasingly focused on the application of non-thermal physical treatments for wine production, ageing, and preservation, thus allowing a reduction in SO_2 additions during wine production and the preservation of the original pigments and taste volatile compounds of the wine (Morata *et al.*, 2017; Van Wyk & Silva, 2019). Among all non-thermal technologies, the application of pulsed electric fields (PEF) is one of the most attractive (Silva & Van Wyk, 2021; Silva *et al.*, 2024). Puértolas *et al.* (2009) demonstrated that PEF treatment was more effective in enhancing the extraction of phenolic compounds during the vinification of *Vitis vinifera* L. cv. Cabernet-Sauvignon than enzymatic maceration using commercial pectolytic enzyme preparations, namely Lallzyme EX and Lallzyme OE (Lallemand Inc., Ontario, Canada).

The principle of PEF processing involves the application of multiple high-intensity electric field pulses of microseconds to a liquid or semi-solid food placed between two electrodes, which induces electroporation of the membranes of eukaryotic and prokaryotic cells (Buckow *et al.*, 2013). PEF treatment induces a transmembrane potential difference. When the potential difference reaches a critical value, electrical breakdown and mechanical changes in the membrane occur (Zimmermann *et al.*, 1974). As a result, pores are formed in the cell membrane, increasing its permeability and allowing the transport of intracellular components to the outside of the cell (Yang *et al.*, 2016). Beyond a certain threshold of electric field intensity and processing time, irreversible cell damage occurs, culminating in cell death, making it also a useful tool for inactivating the microorganisms associated with wine spoilage. This electroporation effect on cell membranes, which promotes the temporary or permanent formation of pores, depends on the applied PEF parameters (electric field intensity, pulse duration, number of pulses, total treatment time) and the characteristics of the cells, such as the size and shape (Alzahrani *et al.*, 2022; Tylewicz, 2020).

The phenomenon of electroporation of grape skins can enhance the extraction of precursor molecules for aromatic compounds contained in the skins, thereby contributing to the improvement of the aromatic profile of wines produced from grapes treated with PEF (Arcena *et al.*, 2021; Maza *et al.*, 2019). Several studies have shown that the application of a PEF treatment to grapes increases and accelerates the extraction of phenolic compounds and, consequently, the colour intensity during the winemaking of different grape varieties, including Graciano, Tempranillo, Grenache, Mazuelo, and Cabernet-Sauvignon (Ilona *et al.*, 2018; López *et al.*, 2008a; López *et al.*, 2008b; López-Alfaro *et al.*, 2013; Morata *et al.*, 2017; Puértolas *et al.*, 2010; Ricci *et al.*, 2018). Thus, with the resolution OIV-OENO 634-2020, the International Organization of Vine and Wine (OIV) approved the use of this technology at the grape level, to increase or speeding up the extraction of valuable substances, such as polyphenols, available nitrogen for yeasts, aroma compounds (including precursors and other compounds of interest located inside the grape cells), and, in the case of red varieties, also reducing maceration time (OIV, 2020).

The main goal of this study was to apply pilot-scale PEF treatment to the grape mash (juice, seeds and skins) to enhance the extraction from red grape varieties Syrah and Tempranillo, in particular, the comparison of sensory properties of wines produced with PEF assisted extraction and the conventional extraction method. The long-term impact of PEF extraction on wine quality was investigated after ageing the wine for a period of approximately 1.5 years. The specific objectives were to investigate the effect of PEF assisted vinification (extraction) on the finished wines' quality as follows:

i. Physico-chemical parameters of Syrah and Tempranillo monovarietal wines, including standard analyses, global phenolic compounds, and chromatic characteristics.

ii. Sensory characteristics of Syrah and Tempranillo wines, and overall impact of grape mash PEF treatment on colour and flavour, assessed through triangle tests.

Regarding colour, a discussion comparing the results of sensorial, chromatic characteristics and global phenolic contents was also performed.

MATERIALS AND METHODS

1. Grapes

The grapes (*Vitis vinifera* L.) of the Syrah and Tempranillo (also known as Tinta Roriz and Aragonez, in Portugal) varieties were chosen for this study. Syrah was cultivated in the wine region of Beira Interior (Portugal) and Tempranillo in the Dão wine region (Portugal). In 2021, the grapes were manually harvested into 15 kg boxes and transported to the pilot-scale winery located in Gouveia, Portugal. For the production of the single-variety Syrah and Tempranillo wines, 1 tonne of grapes of each variety was acquired. Upon reception, harvested grapes were divided into two batches of 500 kg. Grapes of each variety were harvested from a single plot, thus reducing terroir-related variability that might affect the results, with one fraction being treated with pulsed electric fields and the other remaining untreated, serving as the control.

2. Pilot-scale PEF equipment to potentiate the extraction of important components from grapes

A processing line equipped with pulsed electric field (PEF) technology, like the one installed in Gouveia, Portugal, consists of a high-voltage pulse generator, a control and monitoring unit, and a continuous flow treatment chamber integrated into the line. With this setup, the product, in this case must consisting of crushed and destemmed grapes, is pumped through and subjected to PEF treatment. A solid-state Marx generator was used to generate electric pulses (Redondo & Silva, 2009). The high-voltage pulse generator (model EPULSUS-PM1A-10) was designed and set by EnergyPulse Systems (Lisbon, Portugal) (Figure 1). Depending on the objective, the pulse generator allows the application of electric fields with an intensity up to 10 kV, pulse widths ranging from 1 to 200 µs, with currents up to 200 A, with a maximum average power of 3 kW. A DN50 collinear treatment chamber composed of three stainless steel electrodes (*i.e.*, with the central electrode connected to the high-voltage output of the generator, while the outer electrodes were grounded) was used (Figure 1, right). With this setup, it is possible to achieve a maximum field strength of 2 kV/cm inside the transducer. The chamber was set vertically to avoid air entering the PEF treatment chamber and electrical discharges.

3. Vinification and PEF treatments' parameters

Two vinifications were carried out for each grape variety: the control vinification obtained without PEF and the vinification with PEF assisted extraction. The first step of the vinification process involved the mechanical destemming and crushing of grape berries, commencing with the transfer of grape transport containers into the CMA Lugana 1 destemmer-crusher unit (Figure 2). The resulting stream was composed of the grape juice, skins, and seeds. At this moment, the Syrah grape must (juice) presented a pH of 3.59, total acidity of 5.85 g/L, 13 % of probable alcohol, relative density or specific gravity of 1.093, 198.15 g/L of glucose and fructose, and 3.15 g/L of malic acid. The chemical characteristics of Tempranillo grapes were as follows: pH 3.51, total acidity 7.5 g/L, probable alcohol 13.2 %, relative density 1.099, glucose + fructose 204.15 g/L, and malic acid 3.56 g/L.

The resulting grape mash (comprising both solid and liquid fractions of the grapes) was pumped (Peristaltic Pump, CME PPC 200) through the PEF treatment chamber. An electric field intensity of 2 kV/cm was applied, using monopolar pulses, with the specifications indicated in Table 1, for the batches that required PEF treatment. The PEF treatment parameters were chosen based on the flow rate, preliminary studies, and previous publications. In addition, the frequency and pulse width chosen for PEF treatment of each grape variety were different because of differences in the electrical conductivities. Control batches underwent the same vinification protocol, except for the PEF treatment, in which the generator was disconnected during the operation.



FIGURE 1. Pulsed electric fields (PEF) generator, control and monitoring unit EPULSUS-PM1A-10 (left). PEF treatment chamber with collinear configuration, used to potentiate extraction before pressing (right).

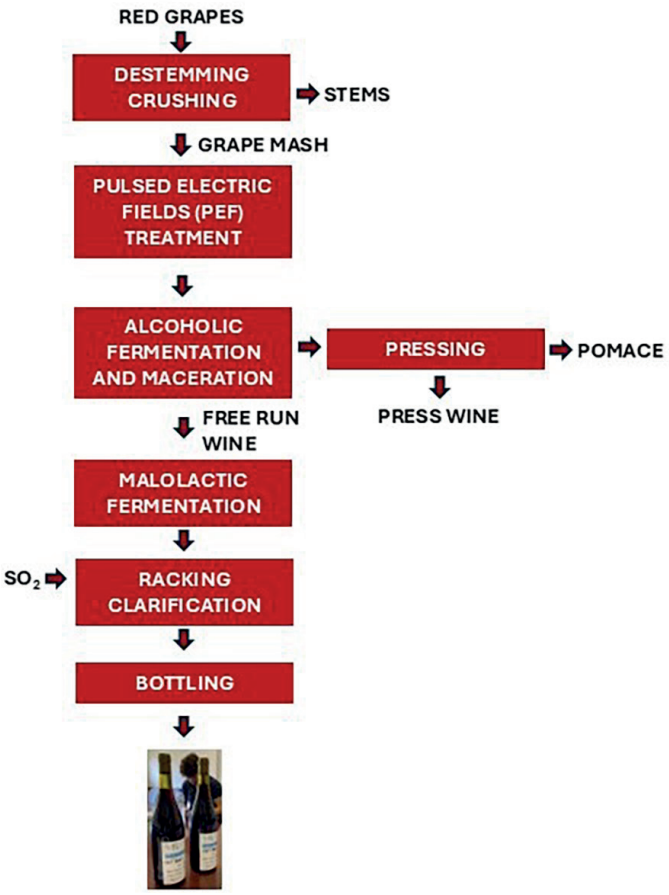


FIGURE 2. Diagram of the red winemaking process with the application of pulsed electric fields (PEF) to the grape mash composed of juice, grape seeds, and grape skins.

TABLE 1. Parameters used in the PEF treatments applied to destemmed and crushed grapes of varieties Syrah and Tempranillo (electric field intensity of 2 kV/cm).

	Voltage (kV)	Current (A)	Pulse width (μs)	Frequency (Hz)	Flow rate (tonne/h)	Specific energy (kJ/kg)
Syrah	10	90	25	150	4.5	2.0
Tempranillo	10	80	50	100	4.5	2.8

The experiment was conducted using a continuous flow system, with a 50 mm diameter (DN50) and a distance of 50 mm between the stainless-steel electrodes. The grape mash, with and without PEF treatment, was pumped into different stainless-steel vats, where 25 g/hL of a commercial ADY *Saccharomyces cerevisiae,* with a killer factor of 1 (Fermol® Complete Killer Fru, AEB Group, Portugal), and 30 g/hL of yeast nutrients, rich in ammonium and thiamin (Enovit, AEB Group, Portugal), were added. The maceration step took place simultaneously with alcoholic fermentation during the first four days, where grape juice was in contact with the solid parts of the grape (skins and seeds). During maceration, punch down was carried out twice a day for better extraction. On the fourth day, the extraction of the free run wine and the pressing of the solid parts of the grape using a discontinuous pneumatic horizontal press Bucher Vaslin® XPro 5 (Chalonnes-sur-Loire, France) were carried out (Figure 2). The liquid fractions obtained from the free run and the pressing have different tank destinations, as press wine was not used for this study.

During alcoholic fermentation, each vat was monitored twice a day through measurements of density and temperature. The alcoholic fermentations/macerations were carried out at 20 ± 2 °C for Syrah and 18 ± 2 °C for Tempranillo. Alcoholic fermentation was considered complete when the relative density reached values below 1.000, with glucose + fructose levels < 2 g/L. Subsequently, the wine underwent spontaneous malolactic fermentation. When it was deemed complete, free SO_2 was adjusted for 35 mg/L with 6 % sulfurous solution and fining treatments with bentonite (Bentogram, 30 g/hL) were applied for stabilisation, followed by the respective racking. During processing, the exposure to oxygen was reduced by inertization with CO_2 gas. Finally, bottling was carried out. For this investigation, only the free run *(saignée)* wine was used.

The monovarietal wines, Syrah and Tempranillo (control and PEF treated samples), were derived from the free run juice. Once ready, the wines were bottled and aged up to 1.5 years (until May 2023). Then, standard physico-chemical, phenolic composition, chromatic characterisation and sensory analyses were carried out at the Enology Laboratory of the Ferreira Lapa building, at the School of Agriculture (ISA), University of Lisbon.

4. Determination of standard physico-chemical wine parameters

The standard physico-chemical characterisation of the wine includes the following parameters: pH, electrical conductivity, density, total acidity, volatile acidity, sulfur dioxide (total, free, and bound), total dry extract, alcoholic strength by volume, reducing substances, and turbidity. Their determination allows an understanding of whether the treatment with pulsed electric fields affects these parameters commonly used for red wine characterisation.

The pH was measured by potentiometry, following the official OIV method type I (OIV-MA-E-AS313-15), using a potentiometer Orion Star A211 (Thermo Fisher Scientific, Waltham, MA, USA). Electrical conductivity was measured using the Orion Star A212 (Thermo Fisher Scientific, Waltham, MA, USA) benchtop conductivity meter, following the official OIV method type IV (OIV-MA-F1-01). For the determination of density, the official OIV method type IV (OIV-MA-AS2-01B) by areometry (hydrometry) was applied. For the determination of total acidity, method type I (OIV-MA-AS313-01) was used, which is based on a titration with sodium hydroxide in the presence of an indicator (bromothymol blue). Volatile acidity was determined using the official method type I (OIV-MA-AS313-02), described by the OIV (2025), which involves the separation of volatile acids by distillation, followed by titration of the distillate with sodium hydroxide. The result was corrected to exclude the interference of sulfur dioxide.

To determine the free, bound, and total sulfur dioxide an internal method adapted from the Ripper method was used, following the official OIV (2025) method (OIV-MA-AS323-04B, type IV), where free SO_2 was determined by potentiometric titration with iodide, and total SO_2 by potentiometric titration with iodide following alkaline hydrolysis, induced by the addition of a base (NaOH). The bound SO_2 value was calculated from the difference between the free fraction and the total sulfur dioxide.

The total dry extract was determined using the official OIV method type IV (OIV-MA-E-AS2-03B) (OIV, 2025). The alcoholic strength by volume was determined by distillation and areometry, following the official OIV method type IV (OIV-MA-AS312-01B) (OIV, 2025). The determination of reducing substances was performed using the official OIV method type IV (OIV-MA-AS311-01A) (OIV, 2025). The determination of turbidity was performed using the official OIV method type IV (OIV-MA-AS2-08) by nephelometric analysis, using a Hach 2100N IS Turbidimeter (Loveland, CO, USA).

5. Determination of total phenols (flavonoids and non-flavonoids), total anthocyanins, and tanning capacity

Total phenols were determined according to the Ribéreau-Gayon (1970) method, which involves spectrophotometric absorbance (λ = 280 nm) measurement of the centrifuged and diluted wine sample, at a 1/100 dilution. Total phenolic content was expressed in mg of gallic acid equivalent per litre of wine (mg GAE/L), obtained from a previously determined calibration curve of concentration of gallic acid (mg/L) *vs* absorbance. A Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used for the absorbance readings using distilled water for blank. The flavonoid and non-flavonoid phenols were quantified according to the methodology developed by Kramling and Singleton (1969) with a few modifications as described by Silva *et al.* (2024). The concentration of flavonoid phenols was obtained by subtracting non-flavonoid phenols from total phenols.

The total anthocyanin content was determined using the method proposed by Ribéreau-Gayon and Stonestreet (1965), based on the difference between absorbance readings at a wavelength of 520 nm of solutions with and without the addition of sodium metabisulfite. The results were expressed in mg of malvidin 3-glucoside/L calculated through a calibration curve which relates different concentrations of this compound with different absorbance readings.

Tanning capacity was determined according to the method described by De Freitas and Mateus (2001).

6. Analysis of wine chromatic parameters

The determination of the chromatic parameters of the wine was performed using the official OIV method type I (OIV-MA-AS2-11) (OIV, 2025). The spectrophotometric method, CIELab, allows the determination of chromaticity coordinates from transmittance readings at 5 nm wavelength intervals along the 380–780 nm spectrum, according to the Commission internationale de l'éclairage. The chromatic characteristics of a wine are then defined by the colorimetric or chromaticity coordinates: lightness (L*), red/green colour component (a*), blue/yellow colour component (b*), and their derived colour parameters: chroma (C*) and hue (H*). A Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used. In addition, the total colour difference (ΔE) between control and PEF treated samples was calculated and discussed (Sulaiman *et al.*, 2017).

7. Sensory analysis

Wine samples were presented in standard clear glasses recommended by ISO for wine tasting. The monovarietal red wines, Syrah and Tempranillo, control and PEF treated, were subjected to a summary sensory analysis, using a tasting sheet (Laboratório Ferreira Lapa, 2023). The analysis was conducted with eight experienced panellists and included visual, olfactory, and gustatory assessments.

In addition, two triangle tests were conducted for each wine variety, with 30 untrained panellists, to determine if there was a perceptible sensory difference in colour (one triangle test) and flavour (smell and taste, one triangle test). The triangle tests and statistical analysis of results followed the ISO Standard Nº 4129:2021 methodology (International Organization for Standardization, 2021). This test is a forced-choice procedure, meaning the taster must randomly select one sample (among the three samples presented), even if no difference was detected between them. The three wine samples were presented in random order for each panellist to avoid biased answers. At the end, the tasters were invited to answer some questions about the wine samples, including the degree of difference between the samples (none, slight, moderate, high) and which one they preferred (the two identical samples, the different sample, no preference). A section for comments was also added, where tasters could provide relevant information about the wine samples.

8. Statistical analysis

To investigate whether PEF treatment had any effect on the quality characteristics of the finished wine, the results obtained from the physico-chemical analyses (control and PEF treated wine samples) were subjected to a one-way analysis of variance (ANOVA) with a single factor (wine treatment) and two levels (control wine; wine produced with PEF assisted extraction), with two replicates (two different bottles). For this purpose, Excel® software (version 2013, Microsoft Corporation, Redmond, USA) was used.

The data analysis and statistical treatment were performed separately for each grape variety and each physico-chemical parameter, as the aim of this study is to evaluate the effect of PEF treatment on various physico-chemical parameters related to wine quality, and not to compare two different red wines from two different grape varieties.

The statistical analysis of the sensory triangle test was based on binomial distribution tables, which indicate the minimum number of correct responses required to achieve a specific statistical significance, depending on the number of participants, according to the International Organization for Standardization (2021).

RESULTS AND DISCUSSION

1. Effect of PEF extraction on physico-chemical parameters of wine

1.1. Standard wine parameters

The results of the conventional physico-chemical parameters of red wine samples obtained with PEF and the control red wine samples (without PEF treatment) for the Syrah and Tempranillo varieties are presented in Table 2.

Statistical analysis revealed that density, alcoholic strength by volume, total acidity, volatile acidity, total SO_2, and total dry extract were not affected by the PEF extraction treatment, as no statistically significant differences were observed between the values of these parameters ($p > 0.05$). Regarding the values of electrical conductivity, pH, turbidity, free SO_2 in Syrah wine, and the concentration of reducing

TABLE 2. Effect of PEF assisted extraction (2 kV/cm) on standard wine parameters obtained from Syrah and Tempranillo grapes.[a]

Parameter	Syrah		Tempranillo	
	Control	PEF	Control	PEF
Electrical conductivity (mS/cm)	2.91 ± 0.01	3.21 ± 0.01 *	3.23 ± 0.01	3.39 ± 0.01 *
pH	3.67 ± 0.00	3.77 ± 0.00 *	3.78 ± 0.00	3.83 ± 0.00 *
Density (g/mL)	0.993 ± 0.000	0.994 ± 0.000	0.993 ± 0.000	0.993 ± 0.000
Total acidity (g tartaric acid/L)	5.04 ± 0.08	4.82 ± 0.03	4.93 ± 0.08	4.70 ± 0.01
Volatile acidity (g acetic acid/L)	0.50 ± 0.01	0.51 ± 0.01	0.57 ± 0.02	0.54 ± 0.02
Total SO_2 (mg/L)	38 ± 0	41 ± 3	34 ± 2	35 ± 2
Free SO_2 (mg/L)	19 ± 0	22 ± 1 *	17 ± 0	17 ± 0
Total dry extract (g/L)	32.5 ± 0.2	32.6 ± 0.4	33.6 ± 0.4	33.8 ± 0.2
Alcoholic strength by volume (% vol)	12.2 ± 0.1	11.9 ± 0.1	12.8 ± 0.1	12.9 ± 0.0
Reducing substances (g/L)	1.5 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.8 ± 0.0 *
Turbidity (NTU)	24 ± 3	64 ± 1 *	12 ± 3	21 ± 0 *

[a] Mean ± standard deviation values from two different wine bottles. For each wine grape variety, an asterisk * signal in the same row means PEF treatment affects that physico-chemical parameter for a significance level of 0.05.

substances in Tempranillo wine, a significant increase in these parameters was observed in wine obtained from PEF treated grapes. López *et al.* (2008a), Maza *et al.* (2019), and Puértolas *et al.* (2009), through treatments with higher intensities, between 4 and 10 kV/cm, concluded that the treatments did not have a significant effect on pH value, alcoholic strength by volume, total acidity, volatile acidity, and reducing substances. The majority of studies conducted with different grape varieties have shown that these analytical parameters are not affected by PEF. On the other hand, Garde-Cerdán *et al.* (2013) observed that pH and total acidity values were affected by PEF, but the observed differences were so small that they had no practical implications.

The increase in pH observed with PEF (Table 2) may be related to the phenomenon of electroporation caused by PEF, where there could be a greater extraction of potassium, but also calcium and magnesium from the grape skins into the must, consequently leading to an increase in pH (Jackson, 2008). Therefore, in wines made from grapes treated with PEF, as well as in certain wine regions, where vineyard practices and winemaking processes tend to increase pH, special attention must be given to this parameter. High pH values can affect colour and microbial stability.

The electrical conductivity (EC) of a wine is directly proportional to the concentration of ions present in it. The main factors influencing the EC of a solution include its ionic concentration, migration rate, and the charges of cations and anions. In addition to phenolic compounds, wine contains large amounts of metal cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and organic and inorganic anions, which can not only affect the wine sensory characteristics and stability but also contribute to the increase in its EC (Yan *et al.*, 2017). EC values of Syrah and Tempranillo wines (control and PEF treated samples) are presented in Table 2. The values are significantly higher in PEF wine samples, which can be explained by the release of ions into a soluble/free form during the PEF extraction treatment of the grape mash, consequently affecting the electrical properties of the wine, *i.e.*, increasing its EC.

Turbidity is a crucial wine quality parameter, and it is due to the presence of several macromolecules like proteins, polysaccharides, other suspended solids, and even microorganisms or some polymerised phenolic compounds involving tannins. According to Ribéreau-Gayon *et al.* (2006), the results obtained (Table 2) regarding turbidity show that all samples are turbid, as they have a turbidity value higher than 8 NTU. These high values may be related to wine subjected to high temperatures during storage. The increase in turbidity observed between the untreated samples and the PEF treated samples for both grape varieties (Table 2) may be related to the electroporation phenomenon caused by PEF, and a greater extraction of macromolecules from the grape skins into the must. For these reasons, ensuring wine stability before bottling is a crucial step in the winemaking process and represents a significant challenge for winemakers.

1.2. Total phenols (flavonoids and non-flavonoids), total anthocyanins, and tanning capacity

Polyphenols are very important constituents, particularly in red wines, due to their sensory properties, especially astringency, colour, and bitterness (Sun & Spranger, 2015). The effect of the electroporation phenomenon induced by PEF to accelerate and/or enhance the extraction of phenolic compounds during red wine production has been investigated. Studies conducted with different grape varieties (Alfrocheiro, Garnacha, Tempranillo, Merlot, Cabernet-Sauvignon) have concluded that PEF is an advantageous technique for facilitating the release of polyphenols located in the cytoplasm of the grape skin cells into the must or wine (Delsart *et al.*, 2012; López *et al.*, 2008b; López *et al.*, 2009; López-Giral *et al.*, 2023; Puértolas *et al.*, 2009; Puértolas *et al.*, 2010; Redondo *et al.*, 2012). The results obtained in this study confirm that the application of a PEF treatment to the grapes, after they have been destemmed and crushed, improved the extraction of total phenols, flavonoid phenols, and total anthocyanins.

The concentrations of total phenols (TP) in the Syrah and Tempranillo wines, both control and PEF wine samples (2 kV/cm), are shown in Figure 3A, with overall values slightly higher for Syrah. The PEF treatment resulted in an increase from 715 to 862 mg of gallic acid/L for Syrah wine and from 589 to 722 mg of gallic acid/L for Tempranillo wine. The low total phenolic content observed overall, when compared with values reported in other studies with red wines, may be explained by the winemaking approach adopted. Firstly, the wine was produced exclusively from the free-run liquid without pressing *(saignée)*, which inherently limits phenols' extraction, as these pigments are predominantly located in the grape skins. Secondly, the maceration period lasted only four days, which is considerably shorter than the typical duration required for sufficient anthocyanin diffusion. In a study using the same grape variety, Tempranillo, a higher concentration of TP was also observed in wines made from grapes treated with PEF, with increases of 7 % in monopolar and 13 % in bipolar PEF treatments compared to the control (Aguiar-Macedo *et al.*, 2024). Donsì *et al.* (2011) observed that PEF treatment, with an electric field intensity of 3 kV/cm and a specific energy of 20 kJ/kg applied to Aglianico grapes, led to an increase in TP concentration from 1,100 to 2,400 mg of gallic acid/L in the resulting wine.

Total phenols are subdivided into two groups: flavonoid phenols, which include flavonols, flavanonols, condensed tannins, and anthocyanins, and non-flavonoid phenols, which encompass phenolic acids and other phenolic derivatives, such as stilbenes. In grapes, flavonoids are primarily synthesised in the skin and seed. Flavonols and anthocyanins are mainly located in the skin, while condensed tannins are predominantly synthesised in the seeds and stems (Jackson, 2008).

The values of flavonoid phenols concentration in the Syrah and Tempranillo wines are significantly different between the control and PEF wine samples, with PEF treatment

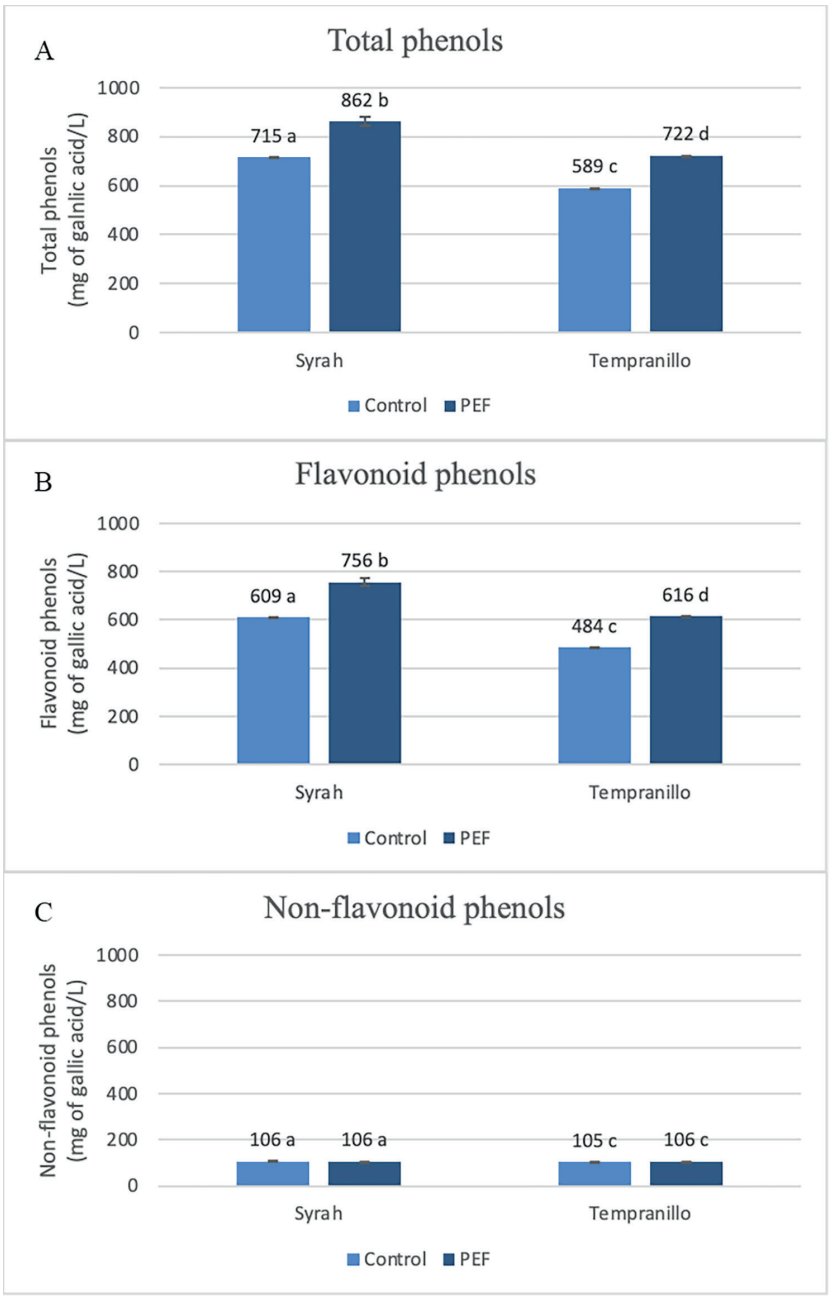


FIGURE 3. Total phenols (A), flavonoid phenols (B), and non-flavonoid phenols (C) concentrations in Syrah and Tempranillo wines: control *vs* PEF assisted extraction. Syrah and Tempranillo are presented in two distinct groups, and different letters within the same group indicate significant differences at a 0.05 significance level.

resulting in an increase from 609 to 756 mg of gallic acid/L and from 484 to 616 mg of gallic acid/L in the Syrah and Tempranillo wines, respectively (Figure 3B). This significant increase in flavonoid phenols appears to be related to the PEF permeabilisation of the grape skin cell membranes and the subsequent release of intracellular components, as this group of phenolic compounds is predominantly found in the grape skin. On the other hand, the results for non-flavonoid phenols in the Syrah and Tempranillo wines did not show significant differences between the control and PEF treated samples (Figure 3C), which can be explained by the low transfer of these compounds from the grape to the wine, as is the case with stilbenes (Sun *et al.*, 2006).

Colour is a fundamental property of red wine quality, primarily dependent on the extraction of anthocyanins. In Syrah and Tempranillo wines, PEF extraction treatment (2 kV/cm, 2 and 2.8 kJ/kg) resulted in an increase in anthocyanin concentrations from 127 to 194 mg malvidin 3-glucoside/L and from 66 to 115 mg malvidin 3-glucoside/L, respectively (Figure 4). Donsì *et al.* (2011) found that PEF treatment with an electric field intensity of 3 kV/cm and a specific energy of 20 kJ/kg applied to Aglianico grapes resulted in an increase in anthocyanin concentration from 300 to 395 mg/L in the wine. In agreement with these results and at lower electric field intensities (0.5 kV/cm), wines produced from Merlot grapes treated with PEF showed an increase from 880 to 1,100 mg/L (Delsart *et al.*, 2012).

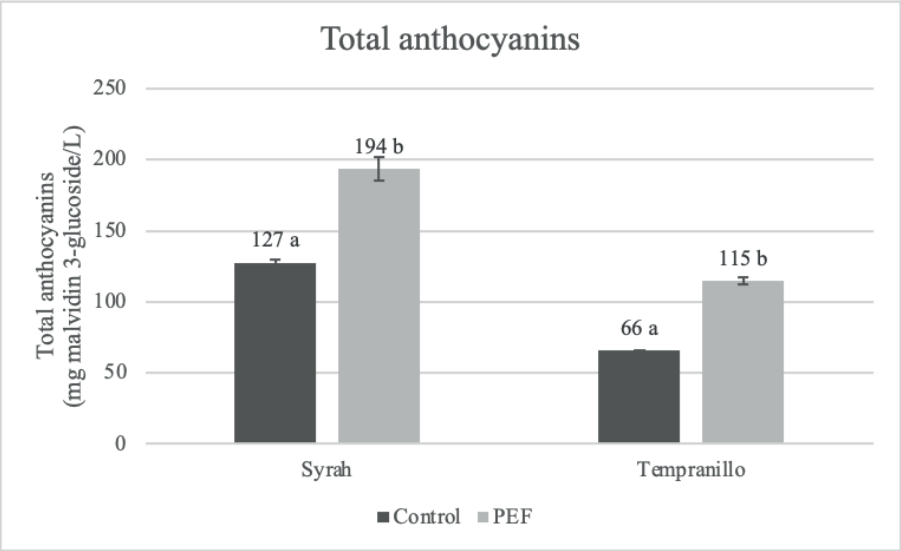


FIGURE 4. Total anthocyanins concentration in Syrah and Tempranillo wines: control *vs* PEF assisted extraction. Syrah and Tempranillo are presented in two distinct groups, and different letters within the same group indicate significant differences at a 0.05 significance level.

The relatively low anthocyanin concentrations observed in this study can be attributed to winemaking factors, as discussed previously for total phenols. In addition, the wine was analysed in May 2023, approximately 1.5 years after the harvest, which occurred in 2021. During this extended ageing period, anthocyanins undergo numerous chemical transformations, including oxidative degradation, polymerisation, and condensation with tannins and flavanols. These reactions lead to the formation of more stable pigments but also cause a marked decline in the concentration of free anthocyanins measurable by standard analytical methods (Pérez-Magariño & González-San José, 2004).

Tanning capacity is a parameter that allows the estimation of a wine's potential astringency. It is characterised by the ability of certain phenolic compounds, namely tannins, to interact with proteins and form insoluble aggregates, thus influencing the astringent character of the wine. The results for Syrah control and PEF wines were 43.3 ± 2.2 NTU and 41.7 ± 1.8 NTU, respectively, whereas for Tempranillo, 40.5 ± 0.9 NTU and 37.7 ± 0.6 NTU were obtained. This parameter was not significantly affected ($p = 0.05$) by pulsed electric fields for both grape varieties, contrary to what was reported previously by Fartouce (2014).

1.3. Chromatic parameters

Table 3 shows the results of the colorimetric parameters from the CIELab method performed on the Syrah and Tempranillo wines. Overall colour difference (ΔE) between control and PEF wine samples was 5.3 for Syrah and 3.8 for Tempranillo, indicating distinct but not great differences. Those colour differences were also detected by the sensory panel (see colour sensory results in section 2.2). The slightly higher changes in colour registered for Syrah with PEF extraction were in agreement with anthocyanin results, where the magnitude of anthocyanin difference between control and PEF wines was also larger for Syrah. The L* parameter is a measure of luminosity, where its values range from 0 (black) to 100 (transparent). It can be observed that the lowest L* values were obtained in the samples treated with PEF, indicating that wines vinificated with PEF are darker (deeper colour) than the control wines (Table 3). This result may be consistent with the increased colour intensity observed in the sensory analyses. Empirically, an increase in colour intensity leads to a lower luminosity value.

The values of the a* parameter in PEF wines are higher than those in the control Syrah and Tempranillo wines (Table 3). The increase in this parameter in the PEF treated samples represents an increase in red colour intensity. These results are consistent with total anthocyanins in the following section, as total anthocyanins are the main type of phenolics responsible for the red colour of red wines. For the b* parameter, which represents yellow colour at values greater than zero, there were no significant changes with PEF treatment in both grape varieties (Table 3). The C* parameter, referred to as chroma or saturation, indicates the intensity of a colour, where higher values reflect greater colour vivacity. Regarding this parameter, it can be observed that the PEF wine samples from both grape varieties have higher values compared to the control wines (Table 3), which is consistent with the sensory analysis results discussed later. In line with the results obtained with Syrah and Tempranillo grapes, wines produced from Garnacha grapes treated with PEF at an electric field intensity of 4 kV/cm showed a trend of decreasing L* component and a trend of increasing a* and C* components (Maza *et al.*, 2019).

Regarding the H* component of CIELab, it is noteworthy that the wines made with PEF treatment from both grape varieties show a slightly lower value compared to the control wines, which means they have a lower hue (Table 3). In Syrah wine, unlike what happens in Tempranillo, the difference in the H* parameter value between the control and the PEF treated samples was not statistically significant.

TABLE 3. Results for L*, a*, b*, C*, and H* colour parameters of Syrah and Tempranillo wines (control wine produced without PEF and, PEF wine produced with 2 kV/cm PEF assisted extraction).#

CIELab colour parameter	Syrah		Tempranillo	
	Control	PEF	Control	PEF
L*	83.0 ± 1.1	78.5 ± 0.1 *	90.8 ± 0.2	87.9 ± 0.2 *
a*	16.58 ± 0.15	19.41 ± 0.12 *	7.58 ± 0.09	10.14 ± 0.06 *
b*	6.11 ± 0.32	6.71 ± 0.05	6.68 ± 0.10	6.90 ± 0.07
C*	17.67 ± 0.26	20.54 ± 0.10 *	10.10 ± 0.13	12.27 ± 0.09 *
H*	0.35 ± 0.01	0.33 ± 0.00	0.72 ± 0.00	0.60 ± 0.00 *

Syrah and Tempranillo results are presented in two distinct groups. For each wine grape variety, * signal in the same row means PEF treatment affects that colour coordinate for a significance level of 0.05.

2. Effect of PEF extraction on sensory parameters of finished wines

2.1. Sensorial characterisation of Syrah and Tempranillo wines

The monovarietal Syrah control red wine presented a ruby red colour and appeared cloudy. On the olfactory examination, it was intense with sufficient persistence, highlighting fruity aromas of red fruits and a vegetal note. In the gustatory phase, the wine was light in alcohol, with good acidity, a light body, and lacking astringency, in accordance with the values obtained for the tanning capacity. In the aftertaste aroma, a mild intensity with low persistence is evident. The Syrah PEF wine sample exhibited similar characteristics to the previous one, differing in its colour, which was a more intense red, and in the aftertaste aroma, where it showed higher intensity and persistence. It was also observed that the Syrah wine assisted by PEF was rounder in terms of its smoothness.

The monovarietal Tempranillo control red wine appeared clear and was characterised by a brick red ruby colour. In the olfactory examination, it displayed a good aromatic intensity and persistence, with notes of red fruits and a vegetal aroma. In terms of the gustatory examination, it was dry in terms of sugars, with high acidity and a light body. The tasting also revealed a wine devoid of tannicity, with a slight bitter taste and low astringency. In the aftertaste aroma, it exhibited low intensity and persistence. The Tempranillo wine assisted by PEF showed similar characteristics to the previous one, differing in colour, with a more intense red hue (ruby red). In the olfactory examination, a milky/buttery attribute was detected. The tasting also demonstrated that the PEF assisted wine sample was fuller-bodied and had a greater intensity in the aftertaste aroma. No defects were detected in any of the wines analysed.

2.2. Triangle test to compare the colour of finished wines vinificated with PEF *vs* conventional extraction

Regarding the effect of pulsed electric fields (2 kV/cm) on the colour of the single variety Syrah and Tempranillo wines, Table 4 presents the results of the triangle test conducted.

According to the International Organization for Standardization (2021), for a total of 30 tests, the minimum number of correct responses required to conclude that there is a significant difference between the samples is 15 for $\alpha = 0.05$; 17 for $\alpha = 0.01$; and 19 for $\alpha = 0.001$. Since we obtained a value higher than 17 correct responses for both wines, we can conclude that there are significant colour differences, at a significance level of 0.01, between the wines produced with and without the application of pulsed electric fields. This result is in agreement with analytical colour CIELab results presented in Table 3 and calculated ΔE (corresponding to a "distinct colour"), where PEF extraction decreased L*, and increased a* and C*, in both Syrah and Tempranillo wines.

In both monovarietal wines, in the comments section, many panellists mentioned that the sample corresponding to the PEF extraction appeared darker, with a greater preference for that sample. This fact can be explained by the phenomenon of electroporation provided by the technology, which causes the formation of pores in the cell membrane, increasing its permeability and enabling the transport of intracellular components to the exterior of the cell (Yang *et al.*, 2016), such as phenolic compounds, particularly anthocyanins, which are the main pigments responsible for the red colour of young red wines (Ilona *et al.*, 2018; López *et al.*, 2008a; López-Alfaro *et al.*, 2013; Morata *et al.*, 2017; Ricci *et al.*, 2018).

2.3. Triangle test to compare the flavour of finished wines vinificated with PEF *vs* conventional extraction

Table 5 presents the results obtained from the triangle test on the aroma and taste of monovarietal Syrah and Tempranillo wines produced with and without the aid of PEF.

Similar to the triangle test on colour, we obtained a value higher than 17 correct responses for both wines. Thus, we can conclude that there is a significant difference in flavour between the wines produced with and without the application of pulsed electric fields, at a significance level of 0.01.

In both monovarietal wines, in the comments section, many tasters mentioned that the wine sample obtained with PEF extraction appeared more velvety, with a more complex and intense aroma and taste, and there was a greater preference for that sample.

TABLE 4. Results of the triangle test on the overall colour change of monovarietal Syrah and Tempranillo wines produced with and without the application of pulsed electric fields.

	Number of correct answers	Number of incorrect answers	Total number of tests conducted
Syrah	23	7	30
Tempranillo	25	5	30

TABLE 5. Results of the triangle test on the overall flavour (aroma and taste) of monovarietal Syrah and Tempranillo wines produced with and without the application of PEF.

	Number of correct answers	Number of incorrect answers	Total number of tests conducted
Syrah	21	9	30
Tempranillo	19	11	30

CONCLUSION

This study demonstrates the positive impact of PEF treatment on the quality characteristics of red wine obtained from Syrah and Tempranillo grape varieties. The application of the PEF treatment at an intensity of 2 kV/cm to destemmed and crushed grapes influenced the permeabilisation of the grape cell plasma membrane, mainly facilitating the release of phenolic compounds located in the cytoplasm into the must through the formed pores. This results in higher values of total phenols, flavonoid phenols, and total anthocyanins, and consequently, a positive effect on the colour of the wine. Therefore, PEF treatment could contribute to reduce the duration of maceration time during winemaking and, consequently, presents a competitive advantage by increasing production capacity and reducing the necessity of other extraction processes, such as pectolytic enzymes, thermal treatments, or mechanical processing (*i.e.*, *remontage*, *délestage*…), a critical step in red wine production that involves higher energy, workload, and time consumption.

The sensory characterisation of control and PEF treated wine samples showed that all wines were acceptable without defects. The discriminative triangle sensory tests performed to assess overall organoleptic changes showed significant differences between the wines produced with and without PEF technology. The triangle tests and the summary sensory analysis favoured the PEF treated wine samples, which were characterised by a more intense red colour, a fuller and rounder body, and greater intensity and persistence in the aftertaste aroma.

Future research should explore the impact of PEF assisted vinification on other grape varieties/wines and optimise PEF protocols to enhance wine quality. Moreover, since most varietal aroma compounds are concentrated in the grape skins, it would be important to compare volatile and aroma precursor levels in wines made with and without PEF, especially for aromatic varieties. Sensory preference tests could further clarify consumer acceptance of wines produced using PEF technology. Additionally, given the critical role of nitrogen in fermentation and its decline due to climate change, assessing Yeast Assimilable Nitrogen (YAN) extraction from PEF treated grapes is essential to understand its effects on fermentation dynamics and wine composition.

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