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Original Article

The Effect of Different Pulsed Electric Field Treatments on Producing High Quality Red Wines

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Abstract

The aim of this study was to apply different Pulsed Electric Field (PEF) treatments in the pre-maceration stage of the mash which derives from 'Pinot Noir' and 'Merlot' grapes that were harvested in the Crişana-Santimreu vineyard, Romania, in 2016, in order to increase the content of total phenols, flavonoids, monomeric anthocyanin pigment and colour intensity of 'Pinot Noir' and 'Merlot' wines. The electrical and mechanical parameters that represent the variables in this experiment were: the distance between the drums, different voltages (7-8 kV), and different frequencies (178-344 Hz). The wines obtained were also analyzed in terms of the antioxidant capacity using two different methods. All PEF treatments applied in the pre-maceration stage resulted in an increase in bioactive compounds content and colour intensification. Of the five PEF treatments tested, the PEF treatment using the distance between the drums of 2.5 mm, U = 8 kV, the frequency f = 344 Hz, pulse durations of 300 s resulted in a wine with a content of total phenols 2 times and 1.5 times higher than the control sample in the case of 'Pinot Noir' and 'Merlot', respectively. Also, this type of PEF treatment also resulted in an extraction of the total flavonoids as efficiently as 1.8 times and 1.4 times, respectively, in the case of 'Pinot Noir' and 'Merlot', respectively. PEF treatment is a technology suitable for extracting phenols from grapes and so this technology can be used in the food industry to obtain wines rich in bioactive compounds with antioxidant capacity.

Keywords: antioxidant, 'Merlot', 'Pinot Noir', PEF, total phenols

Introduction

In recent years, the food industry, especially the wine industry, has been looking for innovative solutions to deliver high quality products and, as far as possible, with low production costs. Regarding the quality of a red wine, it depends on several factors, including phenols content, antioxidant capacity, anthocyanin monomer content, color, flavor, and sensory properties (Clodoveo *et al*., 2016). One innovative solution is the non-thermal technology, pulsed electric field (PEF), which was initially used as an alternative method for pasteurizing liquid foods (milk and derivatives, egg products, juices etc.) (Puértolas *et al*., 2013; Kumar *et al*., 2016). In recent years, this technology has been used to extract as efficiently as possible compounds of interest from different fruits and vegetables. Recent studies have demonstrated the effectiveness of PEF treatment for the extraction of phenols (López *et al*., 2008; López-Alfaro and Garde-Cerdán, 2015; López-Giral *et al*., 2015) and volatile (Garde-Cerdán *et al*., 2013) compounds from grape pomace. By using the pulsed electric field (PEF), an increase in cell membrane permeability (by an electroporation process) is achieved, with a mass transfer through which the compounds of interest located in the cells migrate into the liquid phase (Toepfl *et al*., 2006; Vorobiev and Lebovka, 2010).

In grapes, polyphenols are the most important bioactive compounds, especially because of the multitude of biological effects they present (Xia *et al*., 2010). The phenolic compounds present in grapes are phenolic acids, flavanols, flavonols, anthocyanins, and stilbenes. Pigments responsible for the red color of grapes, anthocyanins, are mainly found in the skin of grapes, in the outer layers of the hypodermic

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tissue, mainly in the vacuoles, and they improve the color of the wine (Barcelo *et al*., 1994).

In the case of traditional winemaking, only 40% of anthocyanins and 20% of tannins from grape skins are transferred to wine (Boulton, 2003; Cerpa-Calderon and Kennedy, 2008). This limited extraction is due in particular to insufficient permeabilization of cell walls and cytoplasmic membranes. There are currently many studies that focus on implementing technologies capable of penetrating cellular barriers and increasing the content of polyphenols in red wines (Sacchi *et al*., 2005; Puértolas *et al*., 2010).

By thermal winemaking a cell structure disruption is obtained, the anthocyanins and tannins are released instantly, this method is applied for a short time (30-40 min), the maximum temperature is 70 °C, followed by cooling before fermentation (Parenti *et al*., 2004; Kelebek *et al*., 2007). Increasing the temperature may in turn affect the quality of the wine.

Another method used in winemaking is to add exogenous preparations of pectinases, hemicellulases and celluloses during the fermentation process (Muñoz *et al*., 2004). Pectinases attack pectic substances in grapes, thus releasing compounds responsible for wine flavor and color (Cabaroglu *et al*., 2003).

The aim of this study was to apply different pulsed electric field (PEF) treatments (consisting in different electrical and mechanical parameters) in the pre-maceration stage of the must which derives from 'Pinot Noir' and 'Merlot' grapes that were harvested in the Crişana-Santimreu vineyard, Romania, in 2016. The red wines obtained were then analyzed in terms of their content in bioactive compounds (total phenols, total flavonoids, total anthocyanins), antioxidant capacity (by two methods, DPPH, FRAP) and chromatic properties. Based on the data obtained, a multivariate data analysis (Principal Component Analysis, PCA) was applied in order to identify the most effective PEF treatment, depending on the variety of grapes, to obtain a wine of superior quality in terms of bioactive compounds, antioxidant capacity, and colour.

Materials and Methods

Samples, PEF treatments and winemaking process

The study was conducted in 2016, on two varieties of grapes, 'Pinot Noir' (PN) and 'Merlot' (MT) (50 kg of each variety), harvested from the Crişana-Santimreu vineyard – Romania, at optimum maturity. The grapes were declustered and crushed, and the obtained mash was divided according to the PEF treatment applied. Five PEF treatments have been applied that differ in the distance between the drums (7 and 8 mm, respectively), pulse duration (seconds), and electrical parameters (voltage, frequency). Table 1 shows the coding of the samples according to the mechanical and electrical parameters applied to the samples. For each variety, an untreated sample that represents the control sample (PN_M and MT_M), was kept. After the treatment, the must was obtained by pressing with the mechanical laboratory press.

Determination of oenological parameters of wines

The measurements of the physico-chemical parameters of the wines obtained (alcohol, density, total acidity, tartaric acid / L), volatile acidity (g acetic acid / L), pH, malic acid (g / L), glucose + fructose (g / L) were made using the OenoFoss analytical instrument.

Analysis of bioactive compounds from red wines

- *Total phenolic analysis (TPh)*

Total phenolic compounds of wines were evaluated by the Folin –Ciocalteu method (Singleton *et al*., 1999). The wine diluted sample (100 µl) was mixed with 1.750 ml distilled water, 0.2 ml Folin-Ciocalteu reagent (dilution 1:10, v/v), and 1.0 ml of 7.5% Na_2CO_3 solution, and was incubated at room temperature, in the dark, for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer Shimadzu mini UV-VIS. The calibration curve was linear for the range of concentrations between 0.1-0.5 mg/ml gallic acid. The total phenolic content of the samples was expressed as mg gallic acid equivalents (GAE)/L.

- *Analysis of total flavonoids (TFlav)*

Total flavonoid (TFlav) content of the wine samples was determined using the aluminium chloride colorimetric method (Pękal and Pyrzynska, 2014). 1 mL of the sample (or standard) was mixed with 4 mL water and 3 mL of $NaNO_2$ 5% solution (w/v) and after 5 min, 0.3 mL 10% $AlCl_3$ (w/v) was added. A sample was mixed and 6 minutes later was neutralized with 2 mL of 1M NaOH solution. The solution was mixed and left for 10 minutes at room temperature and its absorbance was recorded at 510 nm. Catechin was used as a standard, and the results were expressed as mg catechin equivalents (CE)/L.

- *Total monomeric anthocyanin pigment content (MAP)*

Total monomeric anthocyanin pigment content (MAP) was determined according to the pH-differential method

Table 1. Sample coding and mechanical and electrical parameters applied during PEF treatment

Parameter	PN_M/ MT_M (control)	Treatment 1 PN_PEF_1 MT_PEF_1	Treatment 2 PN_PEF_2 MT_PEF_2	Treatment 3 PN_PEF_3 MT_PEF_3	Treatment 4 PN_PEF_4 MT_PEF_4	Treatment 5* PN_PEF_5 MT_PEF_5
Distance between the drums [mm]	-	7	7	2.5	2.5	2.5
Voltage [kV]	-	7	8	7	8	8
Pulse duration [s]	-	150	300	150	300	300
Frequency [Hz]	-	178	344	178	344	344

Note:* In the case of treatment 5, the samples have undergone centrifugation prior to PEF treatment

(Giusti and Wrolstad, 2001). Two dilutions of each sample were performed, one in phosphate buffer KCl 0.025M (pH=1) and another one in acetate buffer 0.4M (pH=4.5). The samples were maintained 20 minutes for balancing, after which their absorbances at λ_{max} and 700 nm were read. The absorbance of the diluted samples was calculated according to the formula: $A=(A\lambda_{max}\text{-}A700)_{pH\ 1.0}\text{-}(A\lambda_{max}\text{-}A700)_{pH\ 4.5}$, and the MAP concentration in must and wine samples was calculated using the formula: MAP (mg/L)=(A×MW×DF×1000)/(ε×1), where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity. The results were expressed as malvidin-3-glucoside equivalents (MW=493.2; ε=28,000 $M^{-1}cm^{-1}$).

Estimation of antioxidant capacity of samples

- *DPPH(2,2-diphenyl-2-picryl-hydrazyl-hydrate) assay*

The DPPH assay was determined using the method proposed by (Brand-Williams *et al*., 1995) with some modifications. Briefly, an aliquot of 100 µl diluted sample or standard (Trolox) was mixed with 1.4 ml of DPPH solution (80 µM) and 1 ml ethanol. The homogenate was shaken vigorously and the decrease in the absorbance of the resulting solution was monitored at 515 nm for 5 min on a spectrophotometer (Shimadzu 1240 mini UV-VIS). The results were expressed as mmol Trolox equivalents (TE)/L.

- *FRAP (Ferric Reducing Antioxidant Power) assay*

The FRAP assay was applied according to the method of Benzie and Strain (1996) with some modifications. The working FRAP solution was freshly prepared by mixing 300 mM acetate buffer, 20 mM $FeCl_3 \cdot 6\ H_2O$ solution, and 10 mM TPTZ solution (10/1/1, v/v/v). The diluted wine or standard (Trolox, 50-500 µM) (100 µl) was allow to react with 500 µl FRAP solution and 2 ml distilled water, for 1 h, in the dark. Absorbance was measured at 595 nm using the spectrophotometer Shimadzu 1240 mini UV-VIS. The results were expressed in mmol Trolox equivalent (TE)/L.

Chromatic analysis of wines

The wine chromatic parameters involved the VIS spectra of the samples from 380 to 720nm. The spectra were measured with non-diluted samples (i.e. at native wine pH) in 10 mm cuvette with Shimadzu UV 1700 PharmaSpec series. For the chromatic parameters L*, a*, b*, C* and h* were recorded the transmittance spectra and for wine colour index (CI), wine tint (Tint), percentage of red (%Red) and percentage of blue (%Blue) were measured the absorbance spectra. The transmittance spectra were converted in XYZ standard colour coordinates by using of theirs convolution with standard 10° colour matching functions specific to human eyes, followed by mathematically integration and normalisation. The R, G, B, L*, a*, b*, C*, h* chromatic coordinates were evaluated respecting the CIE (Commission Internationale d'Éclairage) transformation equations. Wine colour intensity shows how "dark" the wine it is and it was calculated based on equation: $CI = A_{420} + A_{520} + A_{620}$, where A_λ represents the absorbance at wavelength λ. Wine tint is a measure of the "how much colour" is present in the wine and it was calculated based on equation: $Tint = A_{420} / A_{520}$ (Ho *et al*., 2001; Puértolas *et al*., 2010).

The colour differences, ΔE, were done pair-wisely between all wine samples, according the equation:

$$\Delta E_{12} = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{1/2} \quad (1).$$

Statistical data

Experimental data was statistically analysed using Minitab 16 statistical software (Minitab Inc. Pennsylvania, USA). The bioactive compounds, antioxidant capacities, and separately the chromatic parameters were processed by one-way analysis of variance (ANOVA) (P = 0.05). Mean value pairwise comparisons were analysed with Tukey's test (P = 0.05). The high quality of wines is expressed as higher levels of bioactive compounds and antioxidant capacity. Furthermore, the chromatic parameters were involved in measuring the quality of wine.

The multivariate analysis (principal component analysis, PCA) was used. The multivariate analysis was performed with PAST version 3.12 (Paleolontological Statistical software, Hammer and Harper, 2005).

Results and Discussion

Starting from 50 kg of grapes vinified under laboratory conditions after declustering, crushing, and pressing, we obtained 34 L of grape juice in the case of 'Pinot Noir' grapes and 36 L in the case of 'Merlot' grapes. After the application of PEF, the quantity of must increased by 1.5% and 2% in the case of 'Pinot Noir' and 'Merlot', respectively. After applying PEF, Leong *et al*., 2016 have obtained for 'Pinot Noir' grapes an increase in grape juice yield, an effect attributable to cell membrane permeabilization after the application of PEF. Cellular destruction results in the acceleration of mass transport from the grape pulp to the juice. Praporscic *et al*., 2007 proved that the application of PEF to white grapes ('Sauvignon', 'Muscadelle', and 'Semillon') resulted in an increase in the yield of juice production of over 49%.

Effect of PEF on the oenological parameters of red wines

Table 2 shows the oenological parameters of red wines measured for control samples (MT_M and PN_M) and PEF wine samples. PEF treatments of the samples did not significantly alter the alcohol content and total acidity compared to the control sample. Regarding the concentration in malic acid, this differs depending on the variety of grapes, and the PEF treatment applied.

Garde-Cerdan *et al*. (2013) applied four PEF treatments for three varieties of grapes ('Graciano', 'Tempranillo', and 'Grenache') and observed that in the must of these grapes, PEF treatment improved mass transfer, which led to a better extraction of organic acids in the grape matrix. Instead, the differences in the concentration of organic acids in the three grape varieties are due to the different distribution of acids in the cells but also to the type of skin that is characteristic of each grape variety.

However, there are other studies in which the PEF treatment of the samples did not affect some oenological parameters of the grape must samples (López-Giral *et al*., 2015). Clodoveo *et al*. (2016) describe the mechanism by which PEF treatment destroys cell membranes of grapes, this treatment having no effect on alcohol content, total acidity, pH, concentration in reducing sugars and volatile acidity.

Table 2. Oenological parameters of untreated and PEF treated wine samples

Wine Samples	Alcohol (% vol)	Density (g/cm^3)	Total acidity (g tartric acid/L)	Volatile acidity (g acetic acid /L)	pH	Malic acid (g/L)	Glucose + Fructose (g/L)
MT_M	15.26 ± 0.03 b	0.99 ± 0.00 f	6.10 ±0.02 a	0.46 ± 0.02 bc	3.49 ± 0.01 fg	1.40 ± 0.00 abc	2.97 ± 0.06 bc
MT_PEF_1	14.41 ± 0.02 f	0.99 ± 0.00 cde	5.79 ±0.02 c	0.46 ± 0.01 bc	3.58 ± 0.01 d	1.40 ± 0.00 abc	2.40 ± 0.10 d
MT_PEF_2	14.99 ± 0.02 cd	0.99 ± 0.00 de	5.76 ±0.02 c	0.46 ± 0.01 b	3.54 ± 0.01 e	1.23 ± 0.06 d	2.97 ± 0.06 bc
MT_PEF_3	15.04 ± 0.02 cd	0.99 ± 0.00 bc	6.02 ±0.02 b	0.46 ± 0.01 bc	3.49 ± 0.01 fg	1.33 ± 0.06 bcd	3.50 ± 0.10 a
MT_PEF_4	14.70 ± 0.05 e	0.99 ± 0.00 bcd	6.00 ±0.02 b	0.44 ± 0.01 bc	3.51 ± 0.01 f	1.30 ± 0.00 cd	1.80 ± 0.00 e
MT_PEF_5	14.97 ± 0.03 cd	0.99 ± 0.00 f	5.81 ±0.01 c	0.43 ± 0.01 c	3.47 ± 0.01 g	1.30 ± 0.00 cd	1.60 ± 0.00 e
PN_M	15.26 ± 0.05 b	0.99 ± 0.00 ef	5.33 ±0.04 e	0.38 ± 0.01 d	3.63b ± 0.01	1.50 ± 0.00 a	2.77 ± 0.06 c
PN_PEF_1	15.27 ± 0.03 b	0.99 ± 0.00 cde	5.18 ±0.02 f	0.39 ± 0.01 d	3.75 ± 0.01 a	1.50 ± 0.00 a	2.27 ± 0.06 d
PN_PEF_2	15.46 ± 0.05 a	0.99 ± 0.00 cde	5.18 ±0.02 f	0.52 ± 0.01 a	3.74 ± 0.01 a	1.50 ± 0.00 a	3.07 ± 0.15 b
PN_PEF_3	15.06 ± 0.01 c	0.99 ± 0.00 b	5.42 ±0.01 d	0.45 ± 0.01 bc	3.59 ± 0.01 cd	1.33 ± 0.06 bcd	2.23 ± 0.15 d
PN_PEF_4	14.74 ± 0.02 e	0.99 ± 0.00 a	5.40 ±0.02 de	0.47 ± 0.02 b	3.62 ± 0.02 bc	1.37 ± 0.06 bc	1.77 ± 0.06 e
PN_PEF_5	14.95 ± 0.04 d	0.99 ± 0.00 cde	5.37 ±0.02 de	0.38 ± 0.01 d	3.59 ± 0.02 cd	1.43 ± 0.06 ab	2.27 ± 0.06 d

Note: The parameter values, displayed as mean ± SD. Three samples from each wine variety and treatment were individually analysed in duplicate (N = 6). For each column (i.e. parameter), different letters prescribe statistical significant differences between the samples (P = 0.05).

Effect of PEF on bioactive compounds content and antioxidant capacity of red wines

Table 3 presents the bioactive compound content and the antioxidant capacity of wine samples obtained from two varieties of grapes, 'Pinot Noir' and 'Merlot', treated and untreated in PEF. The highest content of total phenols and monomeric anthocyanins presents the 'Merlot' wine (MT_M, control sample) compared to the 'Pinot Noir' control sample (PN_M). PEF treatment resulted in significant increases in total phenol content except for treatment 1. The most efficient PEF treatment was obtained when the distance between the drums of the PEF device was 2.5 mm, 8 kV, 300 s, and a frequency of 344 HZ (treatment 4). In terms of total flavonoid content, the highest amount was recorded for treatment 4, where a 81.46% increase in PN wine was obtained.

From Table 3 it appears that PEF treatment results in a more efficient extraction of phenolic compounds and flavonoids, whereas anthocyanic pigment extraction has a different trajectory depending on the type of grapes. In the case of wine obtained from 'Merlot' grapes treated in PEF, the content of anthocyanin pigments was significantly lower compared to the control sample. Instead, in the case of wine from 'Pinot Noir' grapes, there was an increase in the content of anthocyanin pigments, treatment 4 being the most effective one for extracting these pigments.

The DPPH and FRAP methods were applied to evaluate the antioxidant capacity of MT and PN wines obtained from different PEF treatments. The results obtained are shown in Table 3. The antioxidant capacity, measured by the DPPH method, ranged from 0.04 to 0.1 mmol TE/L and the value of FRAP method varied between 7.10 to 9.23 mmol TE/L in both wine samples. For the evaluation of antioxidant capacity of different plant matrices there are many methods that differ in terms of their principles and experimental conditions. Because, in a complex matrix there are a lot of compounds, each antioxidant has varying contributions to the total antioxidant potential (Cao and Prior, 1998).

The highest antioxidant capacity was obtained for PN wine (determined by the DPPH method), especially for PEF treatments 3 and 4. The FRAP method is based on reducing Fe^{3+} -TPTZ ions to the blue complex Fe^{2+} -TPTZ by wine samples, changes which are monitored by increasing the absorbance to 595 nm. In the case of 'Merlot' wines, the FRAP test shows not significant effects between the PEF treated wines and the control sample. Instead, in the PN wines, were recorded significant increases of FRAP values in the case of treatments 4 and 5.

The antioxidant capacity of the wines depends on the grape processing technology, the variety of grapes, and the year of harvest. Hosu *et al.* (2011) studied the antioxidant capacity of wines obtained from three varieties of grapes ('Cabernet Sauvignon', 'Pinot Noir', and 'Merlot') produced in different years and obtained from different vineyards. Their results demonstrate that the antioxidant capacity of wines depends on the combined effect of the grape variety and the vineyard.

Table 3. The content of bioactive compounds (TPh, MAP, TFlav) and the antioxidant capacity of untreated and PEF treated wine samples

Wine Samples	TPh (mg GAE/L)	MAP (mg/L)	TFlav (mg QE/L)	DPPH	FRAP
				mmol TE/L	
MT_M	886.97 ±9.07 g	79.00 ± 2.73 a	1339.24 ± 24.16 h	0.04 ± 0.01 e	9.00 ± 0.45 ab
MT_PEF_1	857.21 ± 8.22 h	64.23 ± 4.11 c	1403.67 ± 16.53 g	0.05 ± 0.00 de	9.33 ± 0.57 a
MT_PEF_2	914.32 ± 7.10 f	70.71 ± 3.24 bc	1460.10 ± 27.85 f	0.05 ± 0.01 de	9.10 ± 0.36 ab
MT_PEF_3	1240.39 ± 7.25 cd	66.48 ± 4.34 c	1784.15 ± 27.98 d	0.09 ± 0.01 ab	9.23 ± 0.50 ab
MT_PEF_4	1254.41 ± 7.35 c	66.94 ± 3.65 c	1860.42 ± 19.00 c	0.07 ± 0.01 bc	9.18 ± 0.38 ab
MT_PEF_5	1235.54 ± 11.36 d	76.92 ± 4.07 ab	1700.97 ± 20.77 e	0.08 ± 0.01 b	8.89 ± 0.41 ab
PN_M	680.04 ± 7.50 j	44.17 ± 4.02 e	1361.11 ± 24.02 gh	0.03 ± 0.01 e	7.48 ± 0.54 cd
PN_PEF_1	642.34 ± 9.28 k	50.20 ± 3.87 de	1292.78 ± 26.04 i	0.04 ± 0.01 e	7.10 ± 0.63 d
PN_PEF_2	758.15 ± 7.08 i	45.83 ± 4.71 e	1361.32 ± 13.89 gh	0.03 ± 0.01 e	7.69 ± 0.42 cd
PN_PEF_3	1306.43 ± 7.91 b	54.53 ± 3.93 d	2222.55 ± 24.13 b	0.10 ± 0.01 a	8.20 ± 0.89 bc
PN_PEF_4	1378.50 ± 8.65 a	81.15 ± 3.14 a	2469.97 ± 18.12 a	0.10 ± 0.01 a	8.89 ± 0.30 ab
PN_PEF_5	1165.37 ± 8.60 e	65.56 ± 3.64 c	1737.16 ± 8.58 e	0.06 ± 0.00 cd	9.21 ± 0.65 ab

Note: The parameter values, displayed as mean ± SD. Three samples from each wine variety and treatment, were individually analysed in duplicate (N=6). For each column (i.e. parameter), different letters prescribe statistical significant differences between the wine samples (P = 0.05).

Effect of PEF on chromatic parameters of red wines

The effects of the PEF treatments performed on MT and PN wines and the CIE chromatic parameter values are presented in Table 4, while the chromatic parameters of the wines are shown in Table 5. Colour differences (ΔE) calculated based on CIE L*, a*, and b* are presented in Fig. 1.

Table 4 shows that PEF-treated 'Merlot' and 'Pinot Noir' wines have a low L parameter value compared to the control samples, indicating that treated wines are darker in colour than the control samples. The lowest L parameter value was obtained in the case of treatment 4 for 'Merlot' wine and treatment 3 for 'Pinot Noir' wine.

The CI parameter value is generally higher for PEF-treated samples compared to the control samples, and the differences are significant. Only for treatments 3 and 4 for 'Pinot Noir' wine were registered lower CI values compared to the control sample. Similar results were obtained by Puertolas *et al.* (2010) who showed that PEF-treated wine has a higher CI value than the control sample. Tint value underlines the relative importance of the yellow and red colours. This value is significantly higher for control samples compared to PEF samples from 'Merlot' wine. In contrast, tint value for 'Pinot Noir' wines is statistically significantly higher for PEF-treated 'Pinot Noir' samples compared to the control sample.

Multivariate data analysis by principal component analysis

In recent years, principal component analysis (PCA) has been used to discriminate the effects of various treatments on volatile compounds (Garde-Cerdán *et al.*, 2013), bioactive compounds, and their biological effects from different matrices (Medina-Meza and Barbosa-Cánovas, 2015). In this paper, the PCA plot was used to observe similarities and differences between control (PN_M and MT_M) and PEF-treated samples, in order to highlight which treatment is considered the most effective for the increase in bioactive compounds, such as total phenols, flavonoids, and anthocyanins in wines (Fig. 2). Using PCA, Medina-Meza and Barbosa-Cánovas (2015) revealed that PEF treatment using a chamber of a larger diameter (25 mm) was the most effective in extracting bioactive compounds and antioxidant capacity, as it allows applying a higher number of pulses, being suitable for solid-liquid extraction processes.

Treatments 3 and 4 applied to Merlot grapes (MT_PEF_4 and MT_PEF_3) have the highest abundance and levels of the first and second variable groups, i.e. a*, C*, TPh, TFlav, DPPH, b* and h* (Fig. 2A). Furthermore, the wine samples MT_PEF_1 and MT_PEF_2 presents the highest abundance and levels of the third variable group, i.e. CI, FRAP and %Red. The 'Merlot' sample MT_PEF_5 has the highest abundance of MAP and Tint. The 'Merlot' untreated wine sample, MT_M, has the highest level of lightness, L*. Due to these facts, the 'Merlot' wine samples to which treatments 3 and 4 were applied (MT_PEF_3 and MT_PEF_4) provide the highest antioxidant capacity and bioactive compounds, as well as the highest levels of a*, b*, C*, %Blue and h* chromatic parameters - i.e. the wine sample colours shifted to a dark and red-blue tinted one.

In the same way as in the 'Merlot' wine case, from Fig.

Table 4. The CIE chromatic parameters of untreated and PEF treated wine samples

Wine Samples	L*	a*	b*	C*	h*
MT_M	60.22 ± 2.16 b	57.61 ± 3.10 b	36.72 ± 3.21 cd	68.84 ± 3.12 bcd	35.21 ± 2.51 d
MT_PEF_1	51.96 ± 2.79 c	59.89 ± 3.28 ab	41.81 ± 3.04 bc	74.57 ± 2.45 bc	37.13 ± 3.48 cd
MT_PEF_2	46.06 ± 2.90 d	60.84 ± 2.59 ab	41.07 ± 2.81 bc	75.78 ± 2.50 ab	38.73 ± 4.51 bcd
MT_PEF_3	40.35 ± 2.30 e	64.37 ± 3.46 a	46.93 ± 3.48 ab	83.28 ± 3.74 a	43.14 ± 4.99 bc
MT_PEF_4	38.51 ± 2.34 e	59.47 ± 4.63 ab	50.98 ± 4.01 a	83.02 ± 3.14 a	42.22 ± 3.70 bcd
MT_PEF_5	52.91 ± 2.37 c	58.68 ± 3.30 ab	41.03 ± 4.48 bc	68.22 ± 1.56 cd	40.58 ± 4.88 bcd
PN_M	68.76 ± 2.25 a	38.21 ± 2.29 de	39.47 ±4.17 c	56.89 ± 5.25 fg	45.18 ± 4.11 ab
PN_PEF_1	70.29 ± 3.52 a	33.34 ± 2.83 ef	25.98 ±4.94 e	51.40 ± 5.35 fg	44.56 ± 3.23 bc
PN_PEF_2	73.12 ± 2.85 a	28.28 ± 4.09 f	34.79±4.04 cd	50.30 ± 3.01 g	52.62 ± 2.94 a
PN_PEF_3	46.15 ± 2.92 d	55.86 ± 2.03 b	39.42 ±2.66 cd	73.35 ± 4.71 bc	41.36 ± 3.83 bcd
PN_PEF_4	52.48 ±1.94 c	49.03 ± 3.50 c	41.88 ±4.55 bc	64.74 ± 4.63 de	45.39 ± 2.95 ab
PN_PEF_5	60.99 ± 2.81 b	43.74 ± 2.83 cd	32.01 ±3.10 de	58.41 ± 4.42 ef	39.80 ± 3.47 bcd

Note: The parameter values, displayed as mean ± SD. Three samples from each wine variety and treatment were individually analysed in duplicate (N = 6). For each column (i.e. parameter), different letters prescribe statistical significant differences between the samples (P = 0.05).

Table 5. The wine specific chromatic parameters of untreated and PEF treated wine samples. The coding of samples is shown in Table 1

Wine Samples	CI	%Red	%Blue	Tint
MT_M	2.19 ± 0.02 f	51.20 ± 0.58 d	6.76 ± 0.04 j	0.80 ± 0.01 g
MT_PEF_1	4.56 ± 0.02 a	54.18 ± 0.29 a	7.98 ± 0.07 h	0.69 ± 0.00 i
MT_PEF_2	4.60 ± 0.04 a	52.57 ± 0.47 c	8.06 ± 0.15 gh	0.76 ± 0.01 h
MT_PEF_3	3.23 ± 0.04 bc	53.67 ± 0.37 ab	8.37 ± 0.12 f	0.72 ± 0.00 i
MT_PEF_4	3.27 ± 0.04 b	53.07 ± 0.31 bc	7.51± 0.0 9 i	0.74 ± 0.01 h
MT_PEF_5	2.62 ± 0.04 e	45.83 ± 1.26 e	8.26 ± 0.14 fg	0.88 ± 0.03 f
PN_M	1.52 ± 0.02 h	43.38 ± 0.29 f	8.39 ± 0.06 f	1.11 ± 0.01 c
PN_PEF_1	3.19 ± 0.02 c	45.58 ± 0.36 e	9.83 ± 0.11 c	0.93 ± 0.01 e
PN_PEF_2	2.91 ± 0.01 d	40.60 ± 0.50 h	11.62 ± 0.15 a	1.14 ± 0.01 b
PN_PEF_3	1.39 ± 0.03 i	36.91 ± 0.54 i	8.89 ± 0.06 d	1.42 ± 0.01 a
PN_PEF_4	1.32 ± 0.03 j	41.86 ± 0.34 g	8.64 ± 0.07 e	1.13 ± 0.02 bc
PN_PEF_5	2.05 ± 0.01 g	43.91 ± 0.27 f	10.44 ± 0.09 b	0.99 ± 0.02 d

Note: The parameter values, displayed as mean ± SD. Three samples from each wine variety and treatment were individually analysed in duplicate (N = 6). For each column (i.e. parameter), different letters prescribe statistical significant differences between the samples

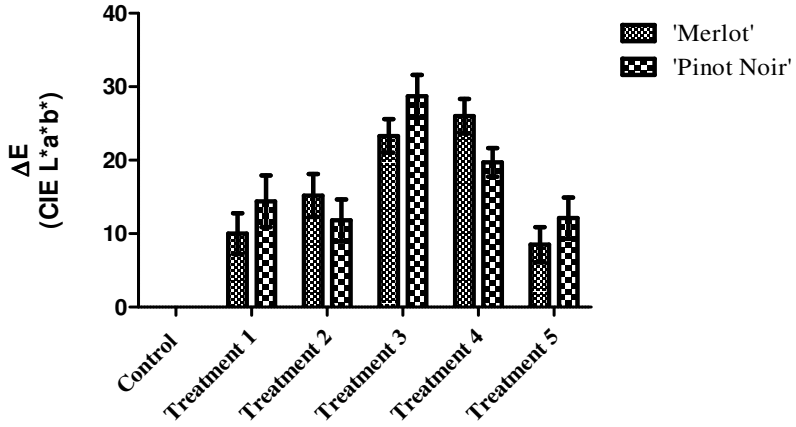
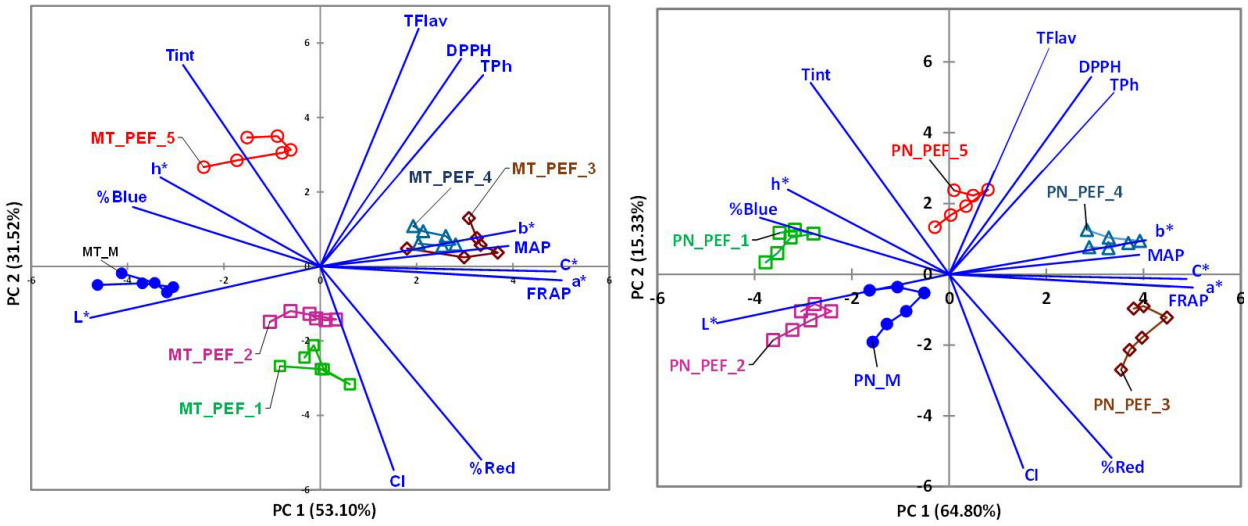


Fig. 1. Colour differences, ΔE, pairwise evaluated between control samples and PEF wine samples for each wine ('Merlot' and 'Pinot Noir')

2B, the 'Pinot Noir' PEF sample (PN_PEF_4) after applying treatment 4, provides the highest antioxidant capacity (DPPH and FRAP) and bioactive compounds (TPh, TFlav and MAP) content and a* level, as well as the second highest levels of b*, C* and tint chromatic parameters. This means that the colour of wine sample PN_PEF_4 shifted to a dark and red tinted one. Furthermore, the 'Pinot Noir' PEF sample PN_PEF_3 provides the highest levels of b*, C*, and tint chromatic parameters, as well as the second highest antioxidant capacity (DPPH and FRAP), biochemical compounds (TPh, TFlav and MAP) content, and a* level. This means that the colour of wine sample PN_PEF_3 shifted to a dark and blue tinted one.



A. Merlot B. Pinot Noir

Fig. 2. Biplot graphical representations of 'Merlot' (left side, A) and 'Pinot Noir' (right side, B) wine samples and parameters (as vectors) by means of the first two main coordinates

Conclusions

This paper investigated the content of bioactive compounds, antioxidant capacity, and chromatic changes in the final wine products after five PEF treatments performed on 'Merlot' and 'Pinot Noir' grapes. We performed five PEF treatments for each variety of grapes. Treatments 3 and 4 (distance between the drums 2.5 mm, voltage 7 and 8 KV, respectively, pulse duration 150 and 300 s, respectively, and frequency 178 and 344 Hz, respectively) for 'Merlot' wine show significant increases in TPh, TFlav, and DPPH. PEF treatments for 'Merlot' grapes did not lead to an increase in MAP content, while for 'Pinot Noir' grapes there are significant increases. PEF-treated 'Merlot' samples became dark-coloured and red-blue tinted. PEF-treated 'Pinot Noir' samples also became dark-coloured but red tinted. Applying PCA has highlighted that PEF treatments have led to high quality red wines in terms of their content in bioactive compounds, antioxidant capacity, and colour. In conclusion, the 'Pinot Noir' and 'Merlot' varieties of grapes can undergo PEF treatment to obtain high quality wine with beneficial effects on consumers.

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