THE EFFECT OF PULSED ELECTRIC FIELD AND WINE AGING ON TOTAL PHENOL CONTENT AND COLOUR OF RED WINES

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Abstract: *Pulsed Electric Field (PEF) is a non-thermal emerging technology that is applied as pre-treatment to the extraction of bioactive compounds from grapes in order to obtain high quality wine. In the present work, we applied PEF treatment (with different parameters) to two varieties of red grapes (Pinot Noir and Merlot) and recorded the total phenols and chromatic properties (RGB, CIE L*a*b*, CIE L*C*h*, wine colour index (CI), and wine tint (Tint)) of fresh (November 2016) and aged wines (6 months, April 2017). Our results showed that the efficiency of PEF treatment depends on the variety of grapes. The best extractivity of total phenols was obtained for Pinot Noir grapes, compared to Merlot (2.5 times, and 1.4 times, respectively, compared to the untreated sample). All PEF treatments, for both Pinot Noir and Merlot wines, produce an increase in wine quality by positive higher levels of chromatic properties, wine colour properties. These properties are changing with the aging of wine, but differently for Pinot Noir and Merlot wines. Our results show the PEF treatment can be used in the winemaking process in order to increase the phenol content and consequently colour intensity in the red wine.*

Keywords: Pulsed Electric Field, red wine aging, total phenols, colour

INTRODUCTION

The studies presented in the scientific literature show that the use of various non-thermal methods in the food industry resulted in the inactivation of harmful microorganisms by destroying the cell membranes. For this reason, these methods have been used for preserving food. In recent years, non-thermal methods (especially pulsed electric field, PEF) are used in the wine industry to extract phenolic compounds (anthocyanins) as efficiently as possible in order to obtain high quality wines (López, et.al., 2008).

The use of the pulsed electric field (PEF) contributes to the increase in the extraction of bioactive compounds. This method involves low temperatures, low power consumption, and reduced processing times. Boussetta, et al., (2009), Grimi, et. al., (2009), and Puértolas, et. al. (2010) used pulsed electric field (PEF) treatment to loosen the cell membrane in vegetables and fruits. PEF treatment involves the application of short-lasting electrical field pulses at high intensity to liquid or semi-liquid foods, usually located in a room containing two electrodes (Clodoveo et al., 2016).

PEF treatment used for phenol extraction during maceration and fermentation of red grapes has been also studied by Donsi, et.al. (2010), Delsart et.al. (2012, 2014), Luengo et al. (2014), and Leong, et.al. (2016). They analyzed the extraction of anthocyanins and tannins obtained by this method. They showed that an increase in the pulsed electric field (PEF) from 1 to 7 kV/cm causes an increase in the anthocyanin and total phenol extraction rate for the three varieties investigated, namely Merlot, Syrah, and Cabernet Sauvignon.

From the polyphenol class, flavonoids are the most abundant in wine, representing over 85% of total phenols (Garcia-Martin and Sun, 2013). The flavonoid class includes colourless flavan-3-ols (catechin, epicatechin, and their procyanidins and condensed tannins). Along with these compounds, in red wines are also present anthocyanins and flavonols.

Anthocyanins are the compounds responsible for the color of grapes and wines,

being extracted from the skin of grapes along with tannins at the beginning of fermentation. During maturation, aging, and storage of wine, phenols play an important role in the colour and taste of the wine. During storage, phenols are subject to a number of reactions, resulting in colour and sensory changes. Generally, anthocyanins react with flavan-3-ols, undergo polymerization reactions and reactions that lead to the formation of pyranoanthocyanins (Ivanova et al., 2012; Remy et al, 2000).
Besides the role of phenolic compounds in viticulture and oenology, they present a broad spectrum of beneficial effects including anti-inflammatory, antimicrobial, anti-carcinogenic, antioxidant etc. (Xia et al., 2010).

The aim this of study was to highlight the influence of applying PEF treatment to two varieties of red grapes (Pinot Noir and Merlot) on total phenols and chromatic properties (RGB, CIE L*a*b*, CIE L*C*h*, wine colour index (CI) and wine tint (Tint)) of fresh (November 2016) and aged wines (6 months, April 2017).

MATERIALS AND METHODS

Samples and the process of obtaining wine

The study was performed on two varieties of red grapes: Merlot (MT) and Pinot Noir (PN) harvested in the Crişana-Sântimreu vineyard (47°14′55″N 22°2′42″E47°14′55″N 22°2′42″E) in Romania, in 2016. After declustering and crushing the grapes, the samples were PEF-treated (PN_PEF and MT_PEF). The originality of this study consists in the use of a two-conductor drum system that rotates in opposite directions through which the sample is applied.

A sample of each variety, representing the control sample (PN_CTRL and MT_CTRL), was not PEF-treated. The steps taken to obtain the wine are shown in Figure 1. The parameters used for PEF treatments and sample coding are shown in Table 1.

The method applied in our study involves the use of a voltage of 7 kV and 8 kV, respectively, a frequency of 178 Hz and 344Hz, respectively, and a processing time between 150 and 300 s (Table 1).

Table1. Parameters used for PEF-treatment

Sample no.	**Distance between the drums [mm]**	**Voltage[kV]**	**Pulse Duration[s]**	**Frequency[Hz]**
PN_CTRL & MT_CTRL	-	-	-	-
PN_PEF_13 & MT-PEF_23	2.5	7	150	178
PN_PEF_14 & MT-PEF_24	2.5	8	300	344

The untreated mash and PEF treated mash were maintained at room temperature for some days, followed by the pressing process to obtain the must and seeding with selected yeasts (*Sacharomyces cerevisiae*, EC1118) in order to reach the fermentation process. The residual sugar concentration was monitored daily and fermentation was considered finalized when the concentration of sugar was under 3g/L. At the end of the fermentation, the yeast was separated by open decanting, obtaining the clear wines, that were placed into glass bottles of 750 ml and kept in a refrigerator at 4 °C. The total phenol content, and colour parameters were determined in fresh wine and wine aged at 6 months.

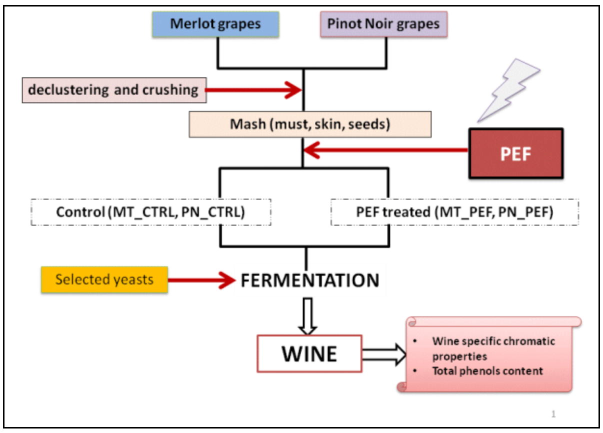

Figure 1. Flow chart of the wine-making process, after treatment with PEF of two varieties of red grapes (Pinot Noir and Merlot)

Total phenol analysis (TPh)

Total phenolic compounds from both wines (young and 6 months aged) were evaluated by the Folin-Ciocalteu method (Singleton, et al., 1999). The total polyphenol content of the samples was expressed as mg gallic acid equivalents (GAE)/L.

Chromatic analysis of wines

The chromatic properties of wine samples were determined by UV-VIS absorbance spectrum measurement using a Shimadzu PharmaSpec UV-1700 (Shimadzu Corporation, Kyoto 604-8511, Japan) with a 10 mm path-length quartz cuvette.

Wine colour intensity index (CI) was calculated by summation of the absorbance at 420nm, 520 nm, and 620 nm. Tint or shade was calculated as the percentage ratio between the 420 nm and 520 nm absorbance. Yellow colour (%Yellow), red colour (%Red), and blue colour (%Blue) percentages were determined as ratio between 420 nm, 520 nm, and 620 nm, respectively, and colour intensity [Puértolas, et al., 2011]. The evaluation of the chromatic parameters starts from the visible transmittance spectrum of wine extract between 380 and 780 nm.

The spectrum is convoluted with the colour matching functions specific for the human eyes. The convoluted functions are integrated and the results are the XYZ standard chromatic coordinates.

The transformation of XYZ coordinates in RGB coordinates allows us to optically display the rendered colour. The CIEL*a*b*and CIE L*C*h* parameters (L*, a*, b*, C*, and h*) were also calculated from XYZ chromatic coordinates.

The colour differences were generated from CIE L*a*b* coordinates: $\Delta E_{12} = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{1/2}$, where L* is lightness, **a*** is the chromatic axis from green(-) to red(+), **b*** is the chromatic axis from blue(-) to yellow(+) and the numeral indices are provided from two different colours.

Statistical analysis

The obtained results represent the mean value ± standard deviation and were processed by one-way analysis of variance (ANOVA). Mean value differences were analyzed with Tukey-s test ($p \leq 0.05$), using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

RESULTS AND DISCUSSIONS

Effect of PEF treatments on total phenol content of young and 6-month old wines

In this study, PEF treatment was used to extract as effectively as possible phenolic compounds from the skin of two varieties of grapes. The results regarding the extraction of phenols present in wine are shown in Figure 2.

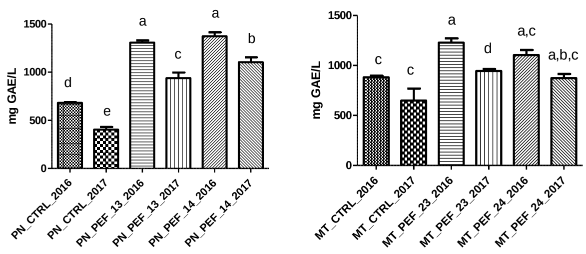

Figure 2. The effect of PEF on total phenol content (mg GAE/L) of young (2016) and 6-month old wines (2017) (Pinot Noir, PN and Merlot, MT). Different letters prescribe statistical significant differences between the samples ($p \leq 0.05$).

PEF treatment, regardless of the parameters used (voltage, frequency, and pulse duration) resulted in a significantly higher extraction compared to the control sample. PEF treatment led to an extraction of more effective total phenols in the case of PN grapes compared to MT (2.5 times and 1.4, respectively, compared with the control sample).

Puértolas et al. (2010) following red grapes treatment with PEF identified increased concentrations of (+) -catechins and (-)-epicatechins. El Darra et al., (2016) have determined that the most appropriate stage for PEF treatment to obtain red wines is during cold maceration.

Effect of PEF treatments on chromatic properties of young and 6-month old wines

Fresh and 6-month old wines obtained by treating PN and MT grapes with PEF have also been analyzed from the point of view of chromatic properties. The results obtained are presented in Tables 2 and 3.

Table 2. Chromatic properties of young Pinot Noir and Merlot wines (2016). Untreated samples (PN_CTRL, MT_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14, MT_PEF_23, MT_PEF_24)

2016	R	G	B	L*	a*	b*	C*	h*	Colour
PN_CTRL	246	139	112	68.91	37.84	32.06	49.59	40.27	
PN_PEF_13	198	59	46	45.96	54.21	39.52	67.08	36.10	
PN_PEF_14	194	81	53	49.03	43.60	38.64	58.26	41.55	

2016	R	G	B	L*	a*	b*	C*	h*	Colour
MT_CTRL	239	95	87	59.12	55.11	33.44	64.47	31.25	
MT_PEF_23	186	1	17	38.65	63.19	46.66	78.55	36.44	
MT_PEF_24	183	5	9	38.29	62.06	49.18	79.18	38.40	

Table 3. Chromatic properties of 6-month old Pinot Noir and Merlot wines (2017). Untreated samples (PN_CTRL, MT_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14, MT_PEF_23, MT_PEF_24)

2017	R	G	B	L*	a*	b*	C*	h*	Colour
PN_CTRL	248	132	102	67.61	41.66	36.09	55.12	40.90	
PN_PEF_13	206	50	32	46.27	59.33	47.18	75.80	38.49	
PN_PEF_14	225	93	57	56.31	49.34	45.71	67.26	42.81	

2017	R	G	B	L*	a*	b*	C*	h*	Colour
MT_CTRL	240	74	64	55.88	62.55	42.51	75.63	34.20	
MT_PEF_23	189	0	1	38.47	65.89	52.74	84.39	38.67	
MT_PEF_24	190	0	0	39.07	65.68	55.56	86.03	40.23	

At first the colour differences with the control sample as reference for each wine age was calculated (Figure 3 and 4).

All the investigated Pinot Noir PEF treatments for both fresh and aged wines present positive colour differences (ΔE). The PEF treatment variant 13 produces the highest colour difference. Also this treatment generates the highest content of anthocyanins. Thus, the PEF treatment variant 13 has the best electroporation efficiency compared with the other treatment for both young and 6-month old Pinot Noir wines.

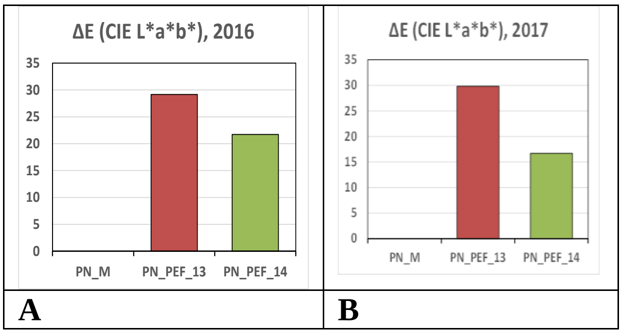

Figure 3. The colour differences with the control sample as reference for young, 2016 (A) and 6 months aged, 2017 (B) Pinot Noir wines. Untreated sample (PN_M) and PEF treated samples (PN_PEF_13, PN_PEF_14)

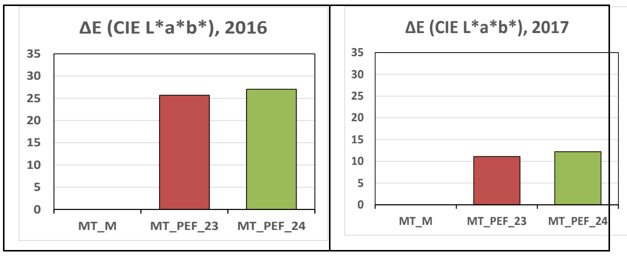

Figure 4. The colour differences with the control sample as reference for young, 2016 (A) and 6 months aged, 2017 (B) Merlot wines. Untreated sample (MT_M) and PEF treated samples (MT_PEF_23, MT_PEF_24)

The comparisons between the same treatments but for different wine ages show that the colour differences are higher for PEF treated wine samples (Figure 5). This fact suggest that, after 6 months, the Pinot Noir PEF wines present higher biochemical "activity" and are not as stable as the untreated wine sample.

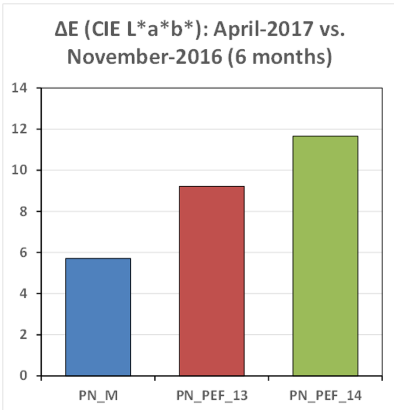

Figure 5. The colour differences between the same treatment but for different ages of Pinot Noir wines. Untreated sample (PN_M) and PEF treated samples (PN_PEF_13, PN_PEF_14)

All the investigated PEF treatments for both Merlot wine ages present positive colour differences (ΔE). Both PEF treatment variants 23 and 24 produce the higher colour difference compared with the untreated wine sample. This fact is the effect of the PEF electroporation treatment.

In order to compare the wine chromatic properties and MAP (monomeric anthocyanin pigment) content, we calculated the relative differences expressed in percentage with the control wine sample as reference (Figure 6).

MAP and CI show an increase with PEF treatment. The highest gains for MAP and CI are present for PEF variant 13, as prescribed by colour differences. This result is valid in both Pinot Noir studied wine ages.

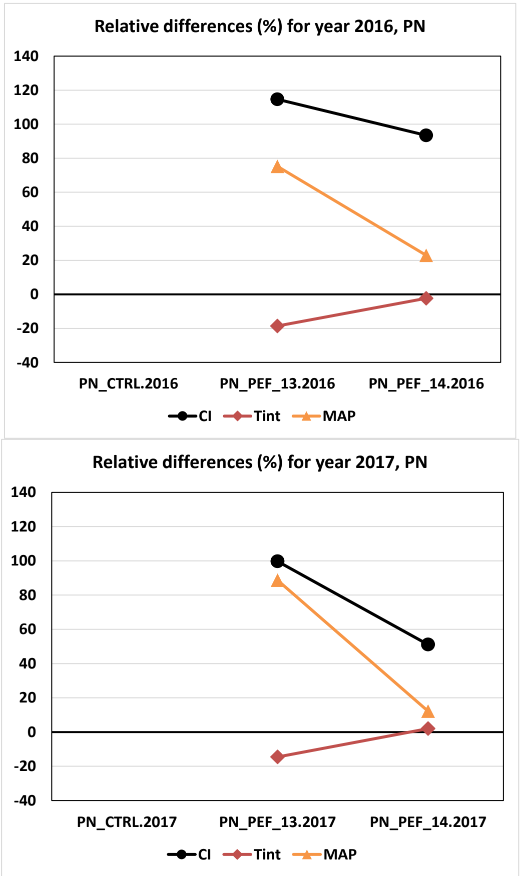

Figure 6. Relative differences of CI, Tint, and MAP expressed in percentage with the control Pinot Noir wine sample as reference. Untreated sample (PN_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14)

Relative differences between the same PEF but different Pinot Noir wine ages show that for this wine the PEF treatment comes with biochemical instability over the aging process.

The MAP and CI parameters show positive increases with PEF treatment for the Merlot wines (Figure 7). Due to PEF electroporation, both PEF variants 23 and 24 produces higher levels of MAP and CI, compared with the untreated wine sample.

Due to relative constant relative differences between the same PEF but different Merlot wine ages, show that for this wine, the PEF treatment comes with good biochemical stability over the aging process.

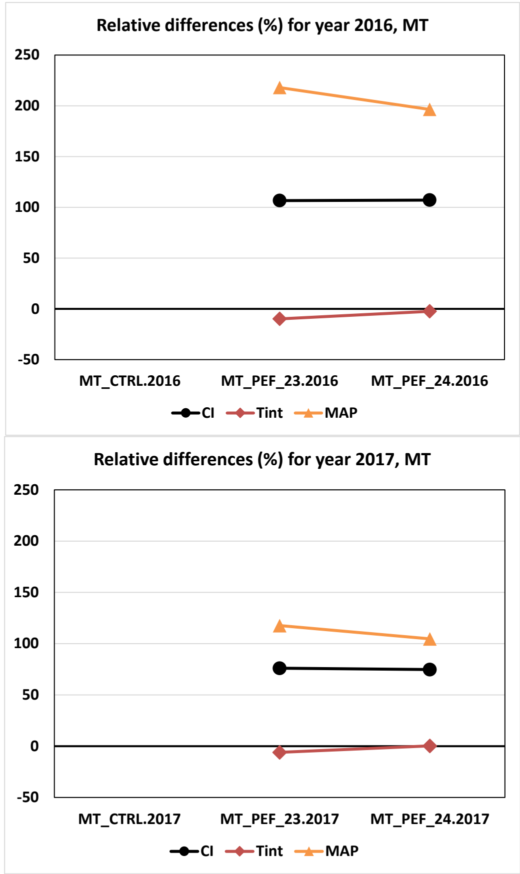

Figure 7. Relative differences of CI, Tint, and MAP expressed in percentage with the control Merlot wine sample as reference. Untreated sample (MT_CTRL) and PEF treated samples (MT_PEF_23, MT_PEF_24)

CONCLUSIONS

All PEF treatments, for both Pinot Noir and Merlot wines, produce an increase in wine quality by positive higher levels of chromatic properties, wine colour properties, and total phenols and MAP content. These properties are changing with the aging of wine, but differently for Pinot Noir and Merlot wines.

For the PEF-treated Pinot Noir wines there is biochemical instability (higher property differences between the aged PEF compared with the control wines). For the PEF Merlot wines there is better biochemical stability (lower property differences between the aged PEF compared with the control wines). Despite the wine aging process, the PEF electroporation effect is still the same after 6 months and the wines quality is the same, higher than that of the untreated wines.

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