Journal Pre-proof

PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc and Grenache grapes

Marcos Andrés Maza, Pereira Carolina, Juan Manuel Martínez, Camargo Alejandra, Ignacio Álvarez, Javier Raso

PII: S1466-8564(20)30321-0

DOI: https://doi.org/10.1016/j.ifset.2020.102375

Reference: INNFOO 102375

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 13 November 2019

Revised date: 29 March 2020

Accepted date: 9 April 2020

Please cite this article as: M.A. Maza, P. Carolina, J.M. Martínez, et al., PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc and Grenache grapes, *Innovative Food Science and Emerging Technologies* (2020), https://doi.org/10.1016/j.ifset.2020.102375

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

Journal Pre-proof

PEF treatments of high specific energy permit the reduction of maceration time during vinification of *Caladoc* and *Grenache* grapes

Marcos Andrés Maza[1,2], Pereira Carolina[1], Juan Manuel Martínez[2], Camargo Alejandra[1], Ignacio Álvarez[2] and Javier Raso[2]*

[1]Universidad Nacional de Cuyo, Facultad de Ciencias Agrarias, Departamento de Ciencias Enológicas y Agroalimentarias, Mendoza, Argentina.
[2]Food Technology, Facultad de Veterinaria, Instituto Agroalimentario de Aragón-IA2, (Universidad de Zaragoza-CITA), Zaragoza, Spain.

Journal Pre-proof

*__Corresponding author__: Dr. Javier Raso.
Postal address: Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, c/Miguel Servet, 177, 50013, Zaragoza, Spain.
Tel No.: +34976762675
Fax No.: +34976761590
E-mail: jraso@unizar.es

Abstract

Phenolic compounds extracted from the solid parts of the grapes during the maceration-fermentation stage define many of the sensory attributes of red wine such as color, bitterness or astringency.

The effect of moderate a PEF treatment (M-PEF) (5 $kV.cm^{-1}$, 8.8 $kJ.kg^{-1}$) and an intense PEF treatment (I-PEF) (5 $kV.cm^{-1}$, 52.9 $kJ.kg^{-1}$) on the reduction of maceration time during vinification of *Caladoc* and *Grenache* grapes was investigated.

In both grape varieties, M-PEF treatment combined with 4 days of maceration was the most effective treatment in achieving high anthocyanin content, color intensity and total phenol index at the end of fermentation. The I-PEF treatment promoted a rapid release of anthocyanins and phenolic compounds, along with a fast increment in the color intensity of the must after 24 h of maceration. Although the color intensity and anthocyanin content decreased significantly throughout fermentation when grape pomace was removed after 24 h, these parameters were similar, after 3 months of bottling, in the case of *Caladoc* and slightly lower in *Grenache* than the control wine, for which maceration was extended for 10 days.

Therefore, results obtained in this investigation are the first to demonstrate the potential of I-PEF for the reduction of maceration time to 24 hours in red winemaking.

Keywords: Red wine; polyphenol compound; high intensity PEF; maceration

Journal Pre-proof

1. Introduction

Over the last decades, considerable research efforts have been devoted to the development of non-thermal processing technologies (Zhang, Barbosa-Cánovas, & Balasubramaniam, 2011). These technologies permit improving different unit operations in the food industry such as extraction providing more sustainable and eco-friendly processes (Chemat et al. 2017).

PEF technology is regarded as a promising alternative to thermal processing with the purpose of improving microbial inactivation (Wang et al. 2018), mass transfer (Puértolas, Luengo, Álvarez, & Raso, 2012), and structure modification (Oey, Faridnia, Leong, Burritt, & Liu, 2017). The treatment generates a high intensity electric field between two electrodes by applying pulses of high voltage and short duration. The effects of PEF on foods are attributed to a presumed structural rearrangement of the cell membranes called electroporation, which consists in the formation of local defects or pores (Kotnik, Rems, Tarek, & Miklavčič, 2019). The electroporation of grape skin cells with the purpose of improving the extraction of phenolic compounds during the maceration-fermentation step in red winemaking is one of the most widely investigated applications of PEF in recent years (Ricci, Parpinello, & Versari, 2018). Maceration is one of the most critical stages in red winemaking. During maceration, phenolic compounds that define many of the sensory attributes of red wine such as color, bitterness or astringency are transferred from the skins and seeds into the must (Bautista-Ortín, Busse-Valverde, López-Roca, Gil-Muñoz, & Gómez-Plaza, 2014; Busse-Valverde et al., 2010). Obtaining a wine with enough polyphenol content required that the solid parts of the grapes remain in contact with the fermenting must between 7-10 days. In order to increase its production capacity, wineries are interested in shortening the maceration time without affecting wine quality.

Different studies conducted in the laboratory, but also at pilot plant and semi-industrial scale, have demonstrated that PEF treatments can allow winemakers to reduce maceration time and/or obtain a wine with a greater amount of phenolic compounds (Puértolas, López, Condón, Álvarez, & Raso, 20). In view of such effects, PEF could become an alternative to techniques such as thermovinification or flash release, currently used in wineries to improve polyphenol extraction based on the heating of grapes. Whereas thermovinification consists in heating grapes at temperatures between 70 and 75º C for a period ranging from 30 min to 24 hours (Sacchi, Bisson, & Adams, 2005), the process known as "flash release" consists in a rapid heating of grapes (85-95º C) with direct steam injection, after which grapes are exposed to a vacuum that induces instant vaporization of the water they contain, thereby cooling them and weakening their skin cell envelopes (Moutounet and Escudier 2000). After application of these techniques, solid parts of the grapes are removed after few hours of maceration and fermentation is conducted in liquid phase. The benefits of fermenting in liquid phase include a better use of the effective volume of the tanks, an improved control of fermentation temperature, and savings in labor as well as in the energy consumption required to periodically pump the wine over the skin mass that rises to the top of the fermentation tanks.

Although it has been demonstrated that electroporation of grape skins by PEF significantly improves the extraction of polyphenols such as anthocyanins and tannins, a certain maceration time is required to obtain wines with a sufficient amount of these compounds (López et al. 2009). Typical maceration times reported by different authors for wines obtained with grapes treated by PEF range from 3 to 6 days (Maza et al. 2019).

In the present study, intense PEF treatments in terms of specific energy were applied to electroporate grape skins of two grape varieties (Caladoc and Grenache) in order to evaluate whether the maceration step could thereby be reduced to just a few hours.

2. Material and methods

2.1. Grape samples

Seven hundred kilograms of *Caladoc* (21.1°Brix, titratable acidity: 6.1 $g.L^{-1}$ tartaric acid) and *Grenache* (26.9° Brix, titratable acidity: 4.8 $g.L^{-1}$ tartaric acid) red grapes (Fuendejalón, Spain) were manually harvested in 2018. Harvesting was carried out in the first week of September for *Caladoc* grapes and in the first week of October for *Grenache* grapes. Prior to the PEF treatments, electrical conductivity was measured with a FYA641LFP1 conductivity probe (Ahlaborn, Holzkirchen, Germany) connected to an Almemo 2590 data logger (Ahlaborn, Holzkirchen, Germany).

2.2. PEF equipment and processing

An EPULSUS® PM1-10 PEF-generator (Energy Pulse Systems LDA, Lisbon, Portugal) was used. This apparatus, with an output voltage and current of 10 kV and 200 A, respectively, generates monopolar square waveform pulses of 2 to 200 µs with a frequency up to 200 Hz. The applied voltage was measured with a high voltage probe (Tektronix, P6015A, Wilsonville, Oregon, USA) connected to an oscilloscope (Tektronix, TBS 1102B-EDU, Wilsonville, Oregon, USA).

The treatment chamber consisted of three stainless steel cylindrical electrodes, separated by two methacrylate insulators based on a previous design by Toepfl et al. (2007). Whereas the central electrode is connected to the high voltage, the electrodes of the two extremes are grounded. Two cylindrical treatment zones of 2.0 cm between the electrodes and an inner diameter of 2.0 cm were defined as a colinear configuration. The

electric field strength used to characterize the PEF treatments corresponds to the field strength in the middle position of the treatment zone's central axis, which is almost equivalent to the field strength calculated by dividing the applied voltage and the gap between the electrodes (Toepfl et al. 2007). Mass flow was 140 $kg.h^{-1}$, providing a residence time of the medium in the treatment zone of 0.32 s. Temperature was measured before and after the PEF treatments by means of a type K thermocouple (Ahlborn, Holzkirchen, Germany) connected to an Almemo 2590 data logger (Ahlborn, Holzkirchen, Germany). The characteristics of the applied PEF treatments and the outlet temperature of the grape pomace are shown in Table 1.

Total specific energy (W_{spec}) was calculated according to equations (1) and (2) using the pulse number (n), the mass flow rate (m) and the energy delivered per pulse (W_{pulse}) that was calculated from the applied voltage (V), the current (I) and pulse width (τ).

$$W_{spec} = \frac{n\,W_{pulse}}{m} \qquad (1)$$

$$W_{pulse} = V\,I\,\tau \qquad (2)$$

2.3. Winemaking

The red grapes were weighed, crushed and destemmed with a Master E-10 destemmer (Enomundi, Zaragoza, Spain). Then the crushed grapes were pumped by a progressive cavity pump (Rotor-MT, Bominox, Gerona, Spain) to the colinear treatment chamber. After PEF treatment, the crushed grapes was distributed into fourteen stainless steel tanks (eight for *Caladoc* and six for *Grenache* grapes). Two additional batches of untreated grapes were used as control for each variety. In each tank, $K_2S_2O_5$ (10 $mg.kg^{-1}$)and 15 $g.hl^{-1}$ of a commercial suspension of the yeast *Saccharomyces cerevisiae* (OenoFrance La Marquise E491, Epernay, France) were added. All treatments were fermented in duplicate at 22±1º C. Maceration times depending on the intensity of the

applied PEF treatment were: 4 hours for *Caladoc* grapes treated with the intense PEF treatment (I-PEF), 24 hours for *Caladoc* and *Grenache* grapes treated with the I-PEF treatment, 4 days for *Caladoc* and *Grenache* grapes treated with moderate PEF treatment (M-PEF), and 10 days for untreated *Caladoc* and *Grenache* grapes. During the maceration- fermentation process, enological parameters, temperature and must density were monitored daily. Solid parts of the grapes were punched down once a day to maintain them in contact with the fermenting must. The concentration of residual sugars at the end of fermentation (13 days) was always lower than 3 $g.L^{-1}$. After fermentation, the wines were racked and stabilized for a period of one month at 2º C, and finally racked again, bottled, and stored in a conditioned room kept at 18 ± 1º C until analyzed.

2.4. General wine analysis

During fermentation, all wines were analyzed according to the methods prescribed by the OIV (Organization Internationale de la Vigne et du Vin, 2009). At the end of fermentation, alcohol content, total acidity, and pH were measured. The pH was determined with a Crison Basic20 pH-meter (Crison Instruments, SA, Barcelona).

2.4.1. Colorimetric index measurements

All samples were centrifuged in an Eppendorf AG centrifuge for 15 min at 3000 rpm (Eppendorf, Hamburg, Germany). The absorbance of the musts was measured at 420, 520, and 620 nm by a Biochrom LibraS12 spectrophotometer (Biochrom Limited, UK) with Hellma® Analytics QS Quartz SUPRASIL® 300 Precision cells (light path 1 mm) (Hellma Analytics, Müllheim, Germany). Color intensity (CI) was calculated as the sum of 420, 520, and 620 nm absorbance, and Hue was calculated as the proportion of the absorbance measured at 420 nm and 520 nm according to Glories (1984). Total polyphenol index (TPI) was determined by a direct reading of the absorbance at 280 nm

of diluted wine 1/100 ($v.v^{-1}$) with a Hellma® QS quartz SUPRASIL® 300 cuvette (light path 10 mm) (Hellma Analytics, Müllheim, Germany). TPI was calculated by multiplying the absorbance measured at 280 nm by 100. Total anthocyanins (AC) expressed in milligrams per liter of malvidin-3-glucoside were analyzed by determining the absorbance at 520 nm of diluted wine 1/100 ($v.v^{-1}$) with 1 % ($v.v^{-1}$) HCl (Ruiz-Hernández 2004).

2.4.2. Determination of condensed tannins

Condensed Tannins (TC) were determined according to Sarneckis et al. (2006). The determination was carried out by precipitation with methylcellulose. All values are reported in $mg.L^{-1}$ of epicatechin equivalents according to a calibration curve obtained from aqueous solutions of (-)-epicatechin (10, 25, 50, 75, 100, 150, and 200 $mg.L^{-1}$ of epicatechin).

2.4.3. High-Performance Liquid Chromatography (HPLC)

Anthocyanins were analyzed under the chromatographic conditions described by Puértolas, Saldaña, et al. (2010). An HPLC Varian ProStar high-performance liquid chromatograph (Varian Inc., Walnut Creek, CA) equipped with a ProStar 240 ternary pump, a ProStar 410 autosampler, and a ProStar 335 photodiode array detector were used. Separation was achieved on a reverse-phase column (LC Luna® 100 Å C18 250 x 4.6 mm; 5 µm particle size, Phenomenex) with a pre-column of the same material (LC Luna® 50 x 4.6 mm; 5 µm particle size, Phenomenex). Chromatograms at 520 nm were recorded. The analyzed phenolic compounds were identified according to the retention time and the UV-vis spectra of pure standards, and according to the UV-vis spectral characteristics published in the literature (Puértolas et al. 2011). The concentrations of all studied compounds were expressed in $mg.L^{-1}$.

2.5. Statistical analysis

The data presented in tables and figures represent mean values ± 95% confidence level. Analysis of variance (ANOVA) was carried out using InfoStat statistical software in the 2018 version. The graphics were carried out using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Effect of PEF treatments of different intensities on the extraction kinetics of color intensity, anthocyanins, and total phenolic compounds after different maceration times

The evolution of color intensity, anthocyanin content, and total phenolic compounds during the maceration-fermentation stage of *Caladoc* grapes treated by I-PEF after 4 and 24 hours of maceration are shown in Figure 1. The evolution of the same oenological indexes during maceration-fermentation of untreated and M-PEF treated *Caladoc* grapes after 10 and 4 days of maceration, respectively, is also shown in Figure 1 for comparison. Considerable differences were observed between vinifications conducted with PEF-electroporated grapes and with untreated grapes from the earliest moments of the maceration-fermentation stage onward. The I-PEF treatment led to a rapid release of anthocyanins and phenolic compounds, along with a rapid increment in the color intensity of the must from the onset of the maceration-fermentation step. After 4 hours of maceration, the color intensity, anthocyanin content, and total phenolic index of the must containing grapes treated with I-PEF were much higher than those of the fermenting must containing M-PEF-treated *Caladoc* grapes and control grapes, and the same difference could still be observed even after 24 hours of maceration. However, a pronounced decrease in anthocyanin content and color intensity was observed when the grape skins were removed after 4 hours of maceration. At the end of fermentation, as a

consequence of that tendency, wines obtained with grapes treated by I-PEF with a maceration of only 4 hours had the lowest value for the three indexes analyzed. Figure 1 also shows that color intensity, anthocyanins, and total phenolic index increased when maceration time for the grapes treated by I-PEF was extended to 24 h. Although these two indexes also declined after removing the grape pomace, the wine obtained at the end of fermentation had higher anthocyanin content and a similar color intensity and total phenol index to that of the control wine in which grape pomace remained in contact with fermenting must for 10 days.

Wine obtained with M-PEF-treated grapes after 4 days of maceration was the one with the highest anthocyanin content, color intensity, and total phenolic index at the end of fermentation. Although the values of anthocyanin content and color intensity of the fermenting must containing the grapes treated by M-PEF after 4 days of maceration were similar to those of the fermenting must containing *Caladoc* grapes treated by I-PEF after 24 hours of maceration, the decline in anthocyanin content and color intensity after the removal of grape pomace was less pronounced. The stabilization of these two indexes was probably related to the presence of a higher concentration of tannins in the wine after 4 days of maceration. It is well known that tannins are required to stabilize unstable anthocyanin, and that the presence of ethanol is necessary for the extraction of tannins in the seeds (Busse-Valverde et al. 2010; Hernández-Jiménez, Kennedy, Bautista-Ortín, & Gómez-Plaza, 2012). Since ethanol content in the first 24 hours of maceration-fermentation is too low, no presence of tannins in the seeds of wines obtained from such short maceration is expected.

The evolution of CI, AC and TPI during the maceration-fermentation stage of *Grenache* grapes treated by I-PEF after 24 hours of maceration is compared with the evolution of the same oenological indexes during maceration-fermentation of untreated and M-

PEF-treated *Grenache* grapes after 10 and 4 days of maceration in Figure 2, respectively. Since a considerably pronounced decline in color intensity and anthocyanin content in *Caladoc* was observed when the maceration time of the grapes treated by I-PEF was reduced to 4 hours, this combination was not evaluated when the study was conducted on *Grenache* grapes. Similarly to the case of *Caladoc* grapes, the application of an I-PEF treatment prior to vinification caused a rapid increment of the three indexes in the first 24 hours of maceration-fermentation. Anthocyanin content and color intensity obtained after only 24 hours of maceration were similar to the indexes obtained in control wine after 10 days of maceration. However, as in the case of *Caladoc*, the significant decrease observed in AC and CI after the removal of grape skins entailed that those indexes were lower at the end of fermentation than those of control wine. Although the TPI did not decrease significantly in the wine obtained with grapes treated with I-PEF after 24 hours of maceration, the value of that index in the wine after fermentation was lower than in control wine, due to the fact that polyphenol extraction was more elevated when maceration time was extended. In the case of *Grenache*, the low concentration of ethanol could also have been the reason for the lower total polyphenol index and the observed decrease in CI and AC when grape pomace was removed after 24 hours of maceration.

As in the case of *Caladoc*, the moderate PEF treatment combined with 4 days of contact of grape skins with the fermenting must was the most effective treatment in terms of AC, CI, and TPI at the end of fermentation.

3.2. Effect of PEF treatments of different intensities on oenological parameters of wine.

Table 2 compares the oenological parameters of the four *Caladoc* wines and the three *Grenache* wines after 3 months of bottling. As previously reported by other authors, pH,

alcoholic content, and total acidity of the wines obtained with grapes treated by PEF did not significantly differ from control wines even in those obtained with the most intense PEF treatments (Garde-Cerdán et al. 2013). The combination most effective in obtaining *Caladoc* wine with the highest CI, AC, and TPI consisted in the application of a moderate electric field prior to vinification with 4 days of maceration. The wine obtained with this approach displayed AC, CI, and TPI values that were 25, 81, and 26 % higher, respectively, than control wine with 10 days of maceration. Similar results have been reported in studies conducted with other grape varieties, which have demonstrated the benefit of the application of a PEF treatment for increasing polyphenol content or reducing maceration time (López et al. 2008; Maza et al. 2019; Puértolas, Saldaña, et al. 2010). The lower TPI and AC values obtained in the wines with only 4 hours of maceration significantly increased when maceration was extended to 24 hours. After prolonging the maceration time of the grapes treated by I-PEF for 24 hours, the obtained wine was not significantly different from control wine in terms of AC, TPI, and TC, whereby CI was slightly higher. As ethanol concentration after 24 hours of maceration is very low, tannins of the wine obtained after that short maceration period should proceed from the grape skins rather from seeds (Zamora 2003).

Similarly to *Caladoc*, the wine obtained with *Grenache* grapes treated with M-PEF after 4 days of maceration displayed the highest index values depending on polyphenol extraction. TPI, TC, and CI values of this wine were significantly higher compared to those of the other two wines. The wine obtained with I-PEF-treated grapes and short maceration (24 h) contained values that were lower than control for the 4 indexes associated with polyphenol extraction. However, the wine obtained with PEF-treated grapes displayed TPI and AC indexes similar to control (less than 10% lower).

The application of M-PEF treatments of different intensity to *Caladoc* and *Grenache* grapes prior to vinification did not significantly affect the %Ye, %Rd, and %Bl of the obtained wines. No statistically significant differences were found in these values for wines after three months of aging. Therefore, although the PEF treatments improved the extraction of those components of grapes responsible for the color of wine, the proportion in which these compounds were extracted was similar to that of the untreated grapes. In all cases, the values obtained in this study for the %Ye, %Rd, and %Bl were within a range considered as optimal (Glories 1984).

3.3. Effect of PEF treatments of different intensities on anthocyanin composition

Individual anthocyanins of the obtained wines were identified and quantified. It is well known that anthocyanins extracted from the skins of red grapes are the principal components responsible for the red wine color in young wines.

Table 3 compares the anthocyanin content of *Caladoc* and *Grenache* wines obtained from I-PEF treated grapes and short maceration time (4 and 24 hours) with the wines obtained from M-PEF treated grapes and longer maceration time (4 days), as well as with untreated grapes (10 days maceration). On general terms, similar anthocyanin profiles were observed for all wines obtained with each grape variety. Therefore, even when maceration time was reduced to 24 hours or even less, an M-PEF treatment did not produce a selective effect on any anthocyanin compound.

Table 3 shows that monoglucoside derivates of anthocyanins (Unacylated) predominated in all cases. Unacylated anthocyanins represented 70-80% and 85-95% of total anthocyanins for *Caladoc* and *Grenache* wines, respectively. These differences in the proportion of unacylated anthocyanins may be attributed to the grape variety, as has been ascertained by other authors (Puértolas, et al. 2011). In the wines from two

varieties obtained with different procedures, malvidin-3-glucoside was the most dominant monomeric anthocyanin; nevertheless, significant amounts of petudin-3-glucoside and delphinidin-3-glucoside were likewise found. Similar results have also been reported for wines obtained from other grape varieties. Regarding acylated and coumarylated compounds, conjugates of malvidin were the ones most detected in all the wines. These results agree with those reported by other authors concerning the composition of anthocyanin derivates in red wine (Cacho, Fernández, Ferreira, & Castells, 1992; Puértolas et al. 2011)

4. Discussion

Polyphenol extraction during the maceration-fermentation step is a diffusion process in which the diffusion rate and extraction yield are both highly dependent on the integrity of grape skins' cytoplasmic membrane (Cerpa-Calderón and Kennedy 2008; Pinelo et al. 2006). Several investigations have demonstrated that the application of PEF treatments of very low energy (<10 $kJ.kg^{-1}$) to grapes prior to the maceration-fermentation step can accelerate the extraction of polyphenols (Delsart et al. 2014; López et al. 2008; López-Giral et al. 2015). However, several days of maceration are required to obtain a sufficient amount of phenolic compounds in the final wine (Luengo et al. 2012; Puértolas et al. 2010).

This research investigated the potential of increasing the total specific energy delivered by PEF to the grapes for obtaining red wine with few hours of maceration for the first time. The rapid increment observed in the indexes that depend on polyphenol extraction may be attributed to an increment in the number and/or size of the pores created in the cytoplasmic membrane of the grape skin cells, or it could be associated with the increment in the number of electroporated cells in grape skin tissues (Weaver and Chizmadzhev 1996; Saulis. 2010). As compared with a parallel electrode treatment

chamber configuration, the colinear configuration used in this investigation has lower energetic requirements, thanks to its greater load resistance. However, inhomogeneity in the distribution of the electric field in this configuration could entail that a proportion of cells of the grape skins may have been unaffected or insufficiently affected by the electric field when treatments of low specific energy were applied (Huang, Yu, Gai, & Wang, 2013; van de Bosh 2007). An increment in specific energy delivered to the treatment chamber by increasing the number of the applied pulses could increase the proportion of cells affected by the critical electric field required for electroporation. This effect would be reflected in an increment in the amount of polyphenols released to the must within a shorter time period.

The intense PEF treatment applied here was especially effective in increasing color intensity in the first moments of maceration as consequence of the fast releasing of anthocyanins that are responsible in the initial color of red wine (Setford, Jeffery, Grbin, & Muhlack, 2019). However, similarly to the data reported on evolution in wines obtained with thermovinification or flash expansion techniques with or without very short maceration periods wines obtained with grapes treated by PEF and short macerations exhibited a considerable decrease in anthocyanin concentration when grape pomace was removed from the fermenting must (Gao, Girard, Mazza, & Reynolds, 1997).

Generally, a decrease in anthocyanin content during the first days of maceration is not observed. Then their concentration decreases when the rate of various reactions that undergoing anthocyanins (oxidation, copigmentation, adsorption by yeast) exceeds the extraction rate (Hermosín-Gutiérrez, Sánchez-Palomo Lorenzo, & Espinosa Vicario, 2005; Morata et al., 2003; Setford, Jeffery, Grbin, & Muhlack, 2017; Shenoy, 1993; Wesche-Ebeling & Montgomery, 1990).

One of the drawbacks associated with oenological techniques aiming to eliminate or reduce maceration time is that the wines thereby obtained have poor color stability due to their low tannin content, since the extraction of tannins from the berry seed requires the presence of ethanol (Alcalde-Eon et al. 2014). These molecules not only contribute to astringency and mouthfeel, but they also participate in condensation reactions with anthocyanins that ensure a stabilization of wine color after bottling. It is remarkable to note that the I-PEF treatment applied in this investigation also encouraged the extraction of tannins, even when the maceration period was shortened to 24 hours. Those tannins, therefore, helped maintain the color intensity of *Caladoc* and *Grenache* wines after three months of aging in bottle, and helped ensure that the CI values remained within the range of those reported for other young wines obtained with longer maceration periods.

5. Conclusions

In this investigation, the potential of the application of PEF for obtaining red wine with a maceration time of only 24 hours has been demonstrated for the first time. Although color intensity and anthocyanin content decreased significantly throughout fermentation when grape pomace was removed, oenological parameters of the wines after 3 months of bottling were similar and slightly lower than control wine in the case of *Caladoc* and *Grenache* wines, respectively.

Therefore, PEF could become an alternative to current techniques used in wineries to improve polyphenolic extraction and, as a consequence, to eliminate or reduce maceration time associated with the heating of grapes. PEF could solve several problems associated with thermal methods such as the loss of varietal aromas through temperature increment, the consumption of high quantities of energy, and space requirements.

6. Acknowledgments

M.M. gratefully acknowledges the Universidad Nacional de Cuyo, Argentina, for its financial support for his doctoral studies. Thanks likewise go to the European Regional Development Fund, to the Department of Innovation Research and University Education of the Aragon Government, and to the European Social Fund (ESF).

Journal Pre-proof

7. References

Alcalde-Eon, C., García-Estévez, I., Ferreras-Charro, R., Rivas-Gonzalo, J. C., Ferrer-Gallego, R., & Escribano-Bailón, M. T. (2014). Adding oenological tannin vs. overripe grapes: Effect on the phenolic composition of red wines. *Journal of Food Composition and Analysis*, *34*(1), 99–113. https://doi.org/10.1016/j.jfca.2014.01.004

Bautista-Ortín, A. B., Busse-Valverde, N., López-Roca, J. M., Gil-Muñoz, R., & Gómez-Plaza, E. (2014). Grape seed removal: effect on phenolics, chromatic and organoleptic characteristics of red wine. *International Journal of Food Science & Technology*, *49*(1), 34–41.

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I., & Bautista-Ortín, A. B. (2010). Effect of Different Enological Practices on Skin and Seed Proanthocyanidins in Three Varietal Wines. *Journal of Agricultural and Food Chemistry*, *58*(21), 11333–11339. https://doi.org/10.1021/jf102265c

Cacho, J., Fernández, P., Ferreira, V., & Castells, J. E. (1992). Evolution of Five Anthocyanidin-3-Glucosides in the Skin of the *Tempranillo*, *Moristel*, and *Garnacha* Grape Varieties and Influence of Climatological Variables. *American Journal of Enology and Viticulture*, *43*(3), 244–248.

Cerpa-Calderón, F. K., & Kennedy, J. A. (2008). Berry Integrity and Extraction of Skin and Seed Proanthocyanidins during Red Wine Fermentation. *Journal of Agricultural and Food Chemistry*, *56*(19), 9006–9014. https://doi.org/10.1021/jf801384v

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Review of Green Food Processing techniques.

Preservation, transformation, and extraction. *Innovative Food Science & Emerging Technologies*, *41*, 357–377. https://doi.org/10.1016/j.ifset.2017.04.016

Delsart, C., Cholet, C., Ghidossi, R., Grimi, N., Gontier, E., Gény, L., … Mietton-Peuchot, M. (2014). Effects of Pulsed Electric Fields on *Cabernet Sauvignon* Grape Berries and on the Characteristics of Wines. *Food and Bioprocess Technology*, *7*(2), 424–436. https://doi.org/10.1007/s11947-012-1039-7

Gao, L., Girard, B., Mazza, G., & Reynolds, A. G. (1997). Changes in Anthocyanins and Color Characteristics of *Pinot Noir* Wines during Different Vinification Processes. *Journal of Agricultural and Food Chemistry*, *45*(6), 2003–2008. https://doi.org/10.1021/jf960836e

Garde-Cerdán, T., González-Arenzana, L., López, N., López, R., Santamaría, P., & López-Alfaro, I. (2013). Effect of different pulsed electric field treatments on the volatile composition of *Graciano*, *Tempranillo* and *Grenache* grape varieties. *Innovative Food Science & Emerging Technologies*, *20*, 91–99. https://doi.org/10.1016/j.ifset.2013.08.008

Glories, Y. (1984). La couleur des vins rouges. *Connaissance de la vigne et du vin*, *18*(4), 253–274.

Hermosín-Gutiérrez, I., Sánchez-Palomo Lorenzo, E., & Espinosa Vicario, A. (2005). Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, *Cabernet Sauvignon*, *Cencibel*, and *Syrah*. *Food Chemistry*, *92*(2), 269–283. https://doi.org/10.1016/j.foodchem.2004.07.023

Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2012). Effect of Ethanol on Grape Seed Proanthocyanidin Extraction. *American*

Journal of Enology and Viticulture, *63*(1), 57–61. https://doi.org/10.5344/ajev.2011.11053

Huang, K., Yu, L., Gai, L., & Wang, J. (2013). Coupled Simulations in Colinear and Coaxial Continuous Pulsed Electric Field Treatment Chambers. *Transactions of the ASABE*, *56*(4), 1473–1484. https://doi.org/10.13031/trans.56.9167

Kotnik, T., Rems, L., Tarek, M., & Miklavčič, D. (2019). Membrane Electroporation and Electropermeabilization: Mechanisms and Models. *Annual Review of Biophysics*, *48*(1), 63–91. https://doi.org/10.1146/annurev-biophys-052118-115451

López, N., Puértolas, E., Condón, S., Álvarez, I., & Raso, J. (2008). Application of pulsed electric fields for improving the maceration process during vinification of red wine: influence of grape variety. *European Food Research and Technology*, *227*(4), 1099. https://doi.org/10.1007/s00217-008-0825-y

López, N., Puértolas, E., Hernández-Orte, P., Álvarez, I., & Raso, J. (2009). Effect of a pulsed electric field treatment on the anthocyanins composition and other quality parameters of *Cabernet Sauvignon* freshly fermented model wines obtained after different maceration times. *LWT - Food Science and Technology*, *42*(7), 1225–1231. https://doi.org/10.1016/j.lwt.2009.03.009

López-Giral, N., González-Arenzana, L., González-Ferrero, C., López, R., Santamaría, P., López-Alfaro, I., & Garde-Cerdán, T. (2015). Pulsed electric field treatment to improve the phenolic compound extraction from *Graciano, Tempranillo* and *Grenache* grape varieties during two vintages. *Innovative Food Science & Emerging Technologies*, *28*, 31–39. https://doi.org/10.1016/j.ifset.2015.01.003

Maza, M. A., Martínez, J. M., Hernández-Orte, P., Cebrián, G., Sánchez-Gimeno, A. C., Álvarez, I., & Raso, J. (2019). Influence of pulsed electric fields on aroma and

polyphenolic compounds of *Garnacha* wine. *Food and Bioproducts Processing*, *116*, 249–257. https://doi.org/10.1016/j.fbp.2019.06.005

Morata, A., Gómez-Cordovés, M. C., Suberviola, J., Bartolomé, B., Colomo, B., & Suárez, J. A. (2003). Adsorption of Anthocyanins by Yeast Cell Walls during the Fermentation of Red Wines. *Journal of Agricultural and Food Chemistry*, *51*(14), 4084–4088. https://doi.org/10.1021/jf021134u

Moutounet, M., & Escudier, J. L. (2000). Prétraitements des raisins par flash-détente sous vide; influence sur la qualité des vins. *Bull. OIV*, *73*, 827–828.

Oey, I., Faridnia, F., Leong, S. Y., Burritt, D. J., & Liu, T. (2017). Determination of Pulsed Electric Fields Effects on the Structure of Potato Tubers. In D. Miklavčič (Ed.), *Handbook of Electroporation* (pp. 1489–1507). https://doi.org/10.1007/978-3-319-32886-7_151

Organization Internationale de la Vigne et du Vin, (OIV). (2009). *Compendium of international Methods of Wine and Must Analysis, Paris France. 1*, 419.

Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology*, *17*(11), 579–590. https://doi.org/10.1016/j.tifs.2006.05.003

Puértolas, E., Álvarez, I., & Raso, J. (2011). Changes in Phenolic Compounds of Aragón Red Wines During Alcoholic Fermentation. *Food Science and Technology International*, *17*(2), 77–86. https://doi.org/10.1177/1082013210368555

Puértolas, E., López, N., Condón, S., Álvarez, I., & Raso, J. (2010). Potential applications of PEF to improve red wine quality. *Trends in Food Science & Technology*, *21*(5), 247–255. https://doi.org/10.1016/j.tifs.2010.02.002

Puértolas, E., Luengo, E., Álvarez, I., & Raso, J. (2012). Improving Mass Transfer to Soften Tissues by Pulsed Electric Fields: Fundamentals and Applications. *Annual Review of Food Science and Technology*, *3*(1), 263–282. https://doi.org/10.1146/annurev-food-022811-101208

Puértolas, E., Saldaña, G., Condón, S., Álvarez, I., & Raso, J. (2010). Evolution of polyphenolic compounds in red wine from *Cabernet Sauvignon* grapes processed by pulsed electric fields during aging in bottle. *Food Chemistry*, *119*(3), 1063–1070. https://doi.org/10.1016/j.foodchem.2009.08.018

Ricci, A., Parpinello, G. P., & Versari, A. (2018). Recent Advances and Applications of Pulsed Electric Fields (PEF) to Improve Polyphenol Extraction and Color Release during Red Winemaking. *Beverages*, *4*(1), 18. https://doi.org/10.3390/beverages4010018

Ruiz-Hernández, M. (2004). *Los compuestos fenólicos y el color del vino tinto. Tratado de vinificación en tinto* (first Ed.). Madrid: Mundi-Prensa.

Sacchi, K. L., Bisson, L. F., & Adams, D. O. (2005). *A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines*. 10.

Sarneckis, C. J., Dambergs, R. G., Jones, P., Mercurio, M., Herderich, M. J., & Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*, *12*(1), 39–49. https://doi.org/10.1111/j.1755-0238.2006.tb00042.x

Saulis, G. (2010). Electroporation of Cell Membranes: The Fundamental Effects of Pulsed Electric Fields in Food Processing. *Food Engineering Reviews*, *2*(2), 52–73. https://doi.org/10.1007/s12393-010-9023-3

Journal Pre-proof

Setford, P. C., Jeffery, D. W., Grbin, P. R., & Muhlack, R. A. (2017). Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process modelling. *Trends in Food Science & Technology*, *69*, 106–117. https://doi.org/10.1016/j.tifs.2017.09.005

Setford, P. C., Jeffery, D. W., Grbin, P. R., & Muhlack, R. A. (2019). Mathematical modelling of anthocyanin mass transfer to predict extraction in simulated red wine fermentation scenarios. *Food Research International*, *121*, 705–713. https://doi.org/10.1016/j.foodres.2018.12.044

Shenoy, V. (1993). Anthocyanins—Prospective food colours. Current Science, 64(8), 575-579. Retrieved June 21, 2019, from www.jstor.org/stable/24095573.

Toepfl, S., Heinz, V., & Knorr, D. (2007). High intensity pulsed electric fields applied for food preservation. *Chemical Engineering and Processing: Process Intensification*, *46*(6), 537–546. https://doi.org/10.1016/j.cep.2006.07.011

van den Bosch, H. F. M. (2007). 4 - Chamber design and process conditions for pulsed electric field treatment of food. In H. L. M. Lelieveld, S. Notermans, & S. W. H. de Haan (Eds.), *Food Preservation by Pulsed Electric Fields* (pp. 70–93). https://doi.org/10.1533/9781845693831.1.70

Wang, M.-S., Wang, L.-H., Bekhit, A. E.-D. A., Yang, J., Hou, Z.-P., Wang, Y.-Z., … Zeng, X.-A. (2018). A review of sublethal effects of pulsed electric field on cells in food processing. *Journal of Food Engineering*, *223*, 32–41. https://doi.org/10.1016/j.jfoodeng.2017.11.035

Weaver, J. C., & Chizmadzhev, Yu. A. (1996). Theory of electroporation: A review. *Bioelectrochemistry and Bioenergetics*, *41*(2), 135–160. https://doi.org/10.1016/S0302-4598(96)05062-3

Journal Pre-proof

Wesche-Ebeling, P., & Montgomery, M. W. (1990). Strawberry Polyphenoloxidase: Its Role in Anthocyanin Degradation. *Journal of Food Science*, *55*(3), 731–734. https://doi.org/10.1111/j.1365-2621.1990.tb05217.x

Zamora, F. (2003). *Elaboración y crianza del vino tinto : aspectos científicos y prácticos* (Edición: 1). Madrid: Antonio Madrid Vicente, Editor.

Zhang, H. Q., Barbosa-Cánovas, G. V., & Balasubramaniam, V. M. (Eds.). (2011). *Nonthermal processing technologies for food*. Chichester, West Sussex, UK ; Ames, Iowa: Wiley-Blackwell/IFT Press.

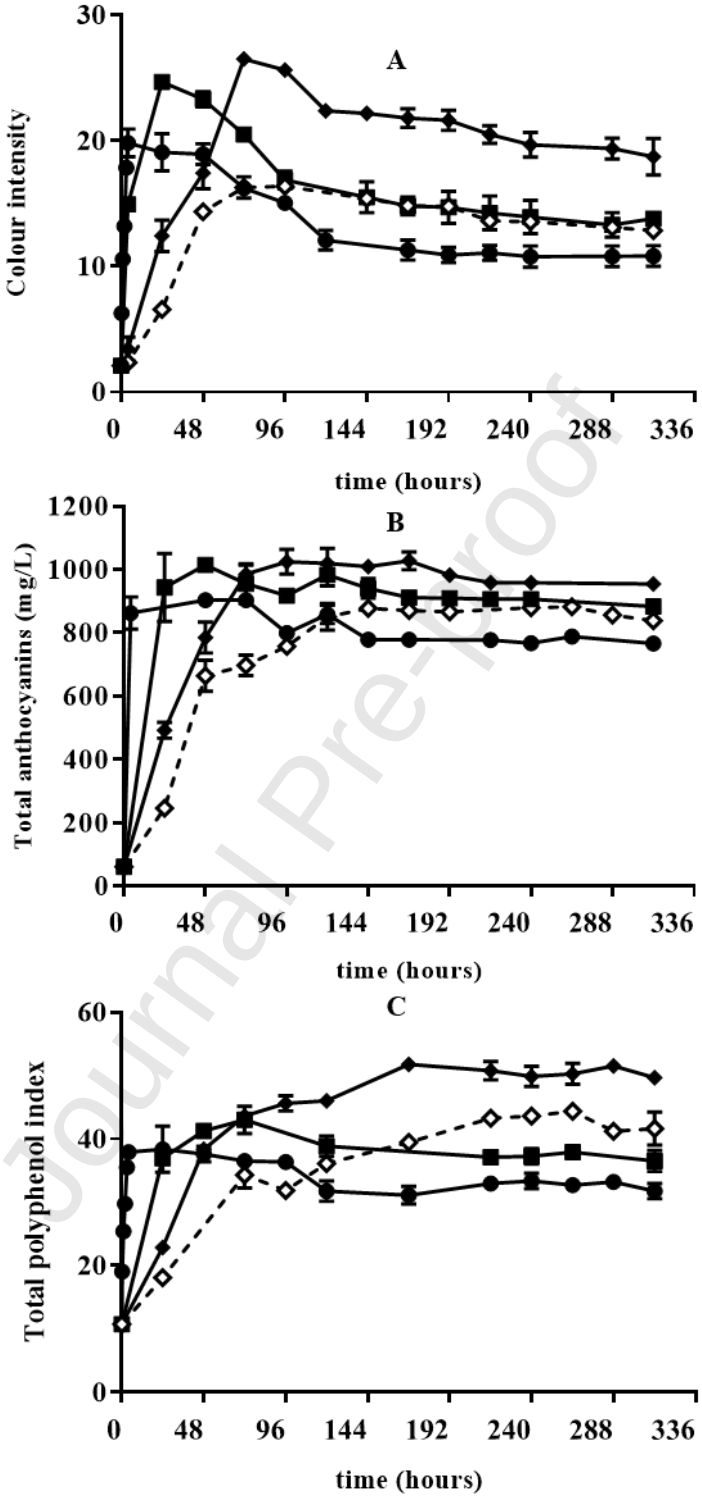
Journal Pre-proof

Figure 1: Evolution of color intensity (A), total anthocyanin content (B), and total polyphenol index (C) along maceration-fermentation of *Caladoc* grapes: (◊) untreated grapes after 240 hours of maceration, (♦) grapes treated by a moderate PEF treatment (M-PEF) (5 $kV.cm^{-1}$, 8.8 $kJ.kg^{-1}$) after 96 hours of maceration and grapes treated by an intense PEF treatment (I-PEF) (5 $kV.cm^{-1}$, 52.9 $kJ.kg^{-1}$) after (■) 24 hours of maceration and (●) 4 hours of maceration.

Figure 2: Evolution of color intensity (A), total anthocyanin content (B), and total polyphenol content (C), along maceration-fermentation of *Grenache* grapes: (◊) untreated grapes after 240 hours of maceration, (♦) grapes treated by a moderate PEF treatment (M-PEF) (5 $kV.cm^{-1}$, 8.8 $kJ.kg^{-1}$) after (■) grapes treated by an intense PEF treatment (I-PEF) (5 $kV.cm^{-1}$, 52.9 $kJ.kg^{-1}$) after 24 hours of maceration.

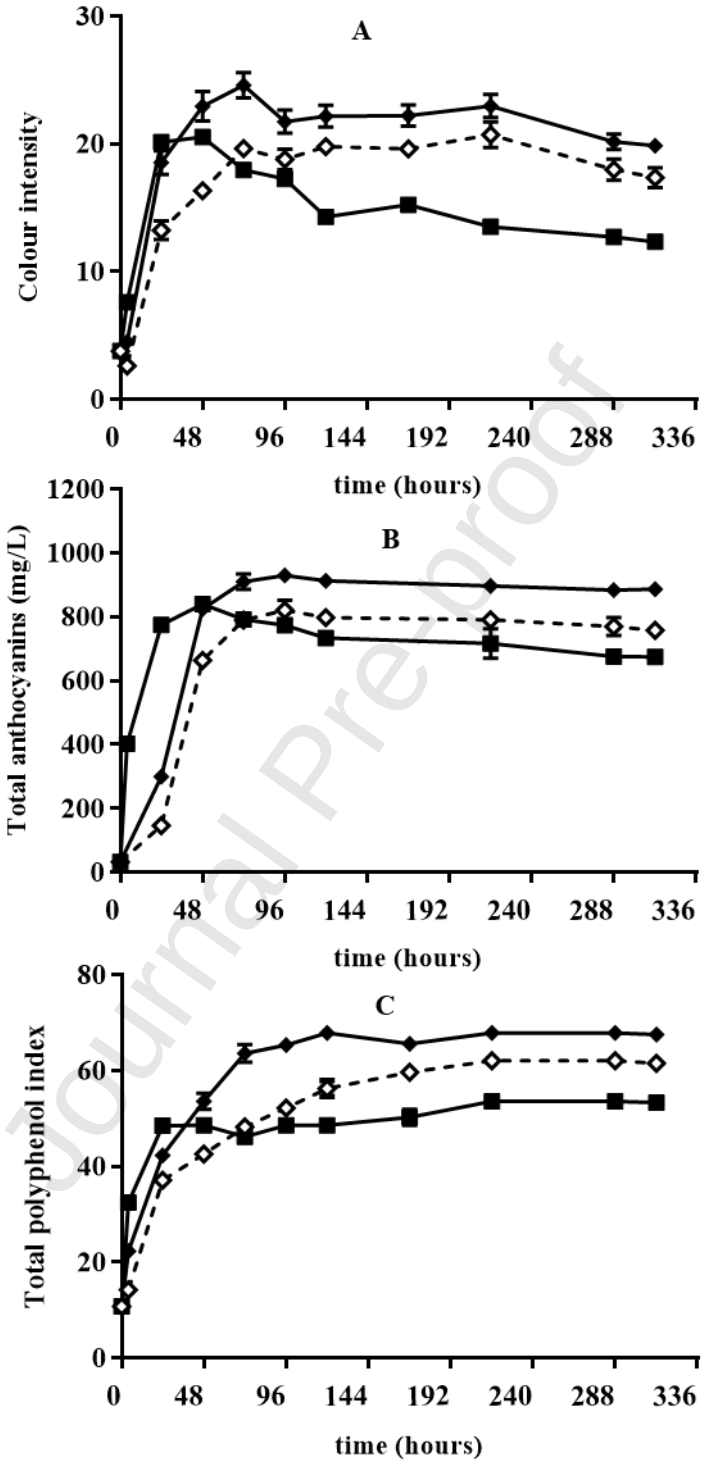
Journal Pre-proof

FIGURE 1



Journal Pre-proof

FIGURE 2



Journal Pre-proof

Table 1: PEF treatments applied to the grape mash

Treatment	Voltage (kV)	Electric field ($kV.cm^{-1}$)	Temp after treatment ºC	Number of pulses	Pulse width (µs)	Treatment time (µs)	Specific energy ($kJ.kg^{-1}$)
I-PEF	10.00	**5.00**	37.2±0.6	46.00	40.00	1840.00	**52.90**
M-PEF	10.00	**5.00**	22.1±0.5	8.00	40.00	320.00	**8.80**

Moderate PEF (M-PEF)
Intense PEF (I-PEF)

Journal Pre-proof

Table 2: Oenological parameters of *Caladoc* and *Grenache* wines after three months of bottling.

	Caladoc				*Grenache*		
	Control	**I-PEF (4 hours)**	**I-PEF (24 hours)**	**M-PEF (4 days)**	**Control**	**I-PEF (24 hours)**	**M-PEF (4 days)**
pH	3.36 ± 0.02a	3.34 ± 0.01a	3.37 ± 0.04a	3.37 ± 0.05a	3.28 ± 0.02ab	3.26 ± 0.02a	3.29 ± 0.01b
Alcohol	12.00 ± 0.14a	12.05 ± 0.07a	12.05 ± 0.21a	11.95 ± 0.07a	16.75 ± 0.07a	16.65 ± 0.21a	16.6 ± 0.28a
Total acidity ($g.L^{-1}$)*	5.91 ± 0.07a	5.82 ± 0.03a	5.84 ± 0.06a	5.80 ± 0.14a	4.37 ± 0.22a	4.32 ± 0.14a	4.21 ± 0.14a
IC (A.U.)	12.66 ± 0.44a	11.97 ± 0.66a	14.98 ± 0.71b	22.90 ± 0.47c	15.77 ± 0.52b	12.36 ± 0.85a	18.57 ± 0.74c
AC ($mg.L^{-1}$)**	766.26 ± 35.37a	746.61 ± 26.83a	799.26 ± 35.30a	954.38 ± 23.46b	837.25 ± 15.49b	749.00 ± 11.03a	883.74 ± 14.67c
Hue (420/520)	0.38 ± 0.02a	0.37 ± 0.01a	0.37 ± 0.01a	0.36 ± 0.01a	0.49 ± 0.01a	0.59 ± 0.05b	0.48 ± 0.01a
TPI (A.U.)	38.90 ± 0.28c	30.00 ± 0.85a	34.75 ± 1.77b	49.20 ± 1.13d	51.55 ± 1.34b	47.40 ± 1.41a	58.85 ± 0.49c
TC ($mg.L^{-1}$)***	1077.88 ± 27.53b	545.14 ± 170.21a	831.86 ± 25.03b	1472.57 ± 80.09c	1649.56 ± 240.29ab	1207.08 ± 100.12a	2015.93 ± 52.57b
(%Y)= (A_{420}/CI x 100)	24.19 ± 1.29a	24.76 ± 0.46a	24.82 ± 0.56a	24.34 ± 0.32a	29.66 ± 0.12a	32.99 ± 1.43b	29.27 ± 0.13a
(%R) = (A_{520}/CI x 100)	64.69 ± 0.45a	67.33 ± 1.20ab	67.46 ± 1.34ab	67.94 ± 0.91b	60.27 ± 0.08a	55.87 ± 2.64a	60.47 ± 0.12a
(%B) = (A_{620}/CI x 100)	11.13 ± 0.84b	7.92 ± 0.74a	7.73 ± 0.79a	7.74 ± 0.59a	10.07 ± 0.20a	11.14 ± 1.21a	10.26 ± 0.01a

M-PEF (Moderate PEF): 5 $kV.cm^{-1}$; 8.8 $kJ.kg^{-1}$.
I-PEF (Intense-PEF): 5 $kV.cm^{-1}$; 52.9 $kJ.kg^{-1}$
Values represent means with their standard deviation (n=2)
Different letters within the same line and grape variety indicate significant differences ($p \leq 0.05$).
TPI: total polyphenol index; CI: color intensity; AC: total anthocyanin content; TC: tannins condensed; %Ye, %Rd, %Bl: percentages of yellow, red, and blue colors respectively; A.U: absorbance units.
[a] Expressed as tartaric acid
[b] Expressed as malvidin-3-glucoside.
[c] Expressed as epicatechin.

Journal Pre-proof

Table 3: Individual anthocyanin content (mg.L^{-1}) of *Caladoc* and *Grenache* wines after three months of bottling.

	Caladoc				*Grenache*		
	Control	**I-PEF (4 hours)**	**I-PEF (24 hours)**	**M-PEF (4 days)**	**Control**	**I-PEF (24 hours)**	**M-PEF (4 days)**
Delphinidin-3G	28.22 ± 1.41 ab	19.53 ± 1.73 a	34.28 ± 8.42 b	52.71 ± 3.93 c	37.76 ± 0.95 b	27.00 ± 4.29 a	48.00 ± 2.83 c
Cyanidin-3G	3.93 ± 5.25 a	1.45 ± 0.27 a	3.63 ± 2.63 a	6.31 ± 2.23 a	5.87 ± 4.91 a	1.45 ± 0.66 a	7.45 ± 0.70 a
Petunidin-3G	47.01 ± 16.57 ab	37.33 ± 60 ab	20.92 ± 15.25 a	57.83 ± 7.28 b	47.26 ± 7.86 ab	37.49 ± 5.78 a	60.00 ± 1.41 b
Peonidin-3G	10.84 ± 1.52 a	7.81 ± 0.21 a	11.22 ± 2.88 a	18.19 ± 1.13 b	41.16 ± 0.30 b	24.96 ± 0.70 a	49.11 ± 1.87 c
Malvidin-3G	501.19 ± 14.16 b	387.47 ± 8.65 a	489.00 ± 25.34 b	682.66 ± 7.30 c	547.36 ± 3.39 b	436.66 ± 32.85 a	603.09 ± 3.95 b
Delphinidin-3G-Ac	3.94 ± 1.64 ab	2.59 ± 0.16 a	4.30 ± 0.59 ab	6.44 ± 0.38 b	5.50 ± 1.41 ab	4.00 ± 0.71 a	7.30 ± 0.71 b
Cyanidin-3G-Ac	4.25 ± 2.07 a	1.48 ± 1.33 a	4.45 ± 0.73 a	3.88 ± 0.65 a	1.75 ± 0.78 a	2.35 ± 0.71 a	2.60 ± 0.57 a
Petunidin-3G-Ac	7.28 ± 1.96 a	5.73 ± 0.95 a	6.11 ± 0.72 a	8.26 ± 0.29 a	4.95 ± 0.64 ab	0.87 ± 0.56 a	6.70 ± 2.40 b
Malvidin-3G-Ac + peonidin-3G-Ac	54.11 ± 5.98 a	54.20 ± 5.89 a	72.04 ± 3.17 b	74.04 ± 3.12 b	11.97 ± 1.19 b	1.71 ± 0.18 a	11.75 ± 2.47 b
Delphinidin-3G-Cm	6.64 ± 7.15 a	1.86 ± 0.52 a	1.43 ± 0.44 a	2.71 ± 0.37 a	3.15 ± 0.07 a	1.83 ± 0.01 a	3.80 ± 0.99 a
Cyanidin-3G-Cm	1.99 ± 1.43 a	1.05 ± 0.21 a	1.96 ± 0.48 a	0.27 ± 0.13 a	0.41 ± 0.01 b	nd	1.75 ± 0.35 c
Petunidin-3G-Cm	11.83 ± 0.70 b	3.65 ± 0.04 a	5.22 ± 3.46 a	8.53 ± 0.67 ab	5.45 ± 1.34 b	0.32 ± 0.16 a	5.55 ± 0.07 b
Peonidin-3G-Cm	7.05 ± 0.65 bc	1.89 ± 0.37 a	4.89 ± 1.68 b	8.29 ± 1.12 c	6.36 ± 0.34 b	0.39 ± 0.09 a	7.95 ± 0.21 c
Malvidin-3G-Cm	24.13 ± 0.66 ab	16.22 ± 50 a	24.98 ± 0.66 b	33.75 ± 3.19 c	9.98 ± 1.36 a	7.80 ± 0.48 a	15.20 ± 1.16 b
Unacylated	**591.17 ± 10.58 b**	**453.57 ± 4.31 a**	**559.03 ± 24.03 b**	**817.7 ± 2.82 c**	**679.4 ± 8.12 b**	**527.55 ± 22.83 a**	**767.65 ± 5.62 c**
Acetylated	**69.57 ± 0.31 a**	**63.99 ± 5.35 a**	**86.9 ± 3.74 b**	**92.62 ± 2.57 b**	**24.17 ± 0.08 b**	**8.93 ± 0.74 a**	**28.35 ± 4.74 b**
Coumarylated	**51.63 ± 7.73 bc**	**24.66 ± 4.37 a**	**38.47 ± 1.07 ab**	**53.53 ± 5.22 c**	**25.35 ± 3.13 b**	**9.41 ± 1.85 a**	**34.25 ± 0.33 c**
Total anthocyanins	**727.09 ± 25.25**	**549.98 ± 2.33**	**801.47 ± 22.89**	**1109.26 ± 58.76**	**759.35 ± 14.31**	**549.3 ± 25.22**	**897.93 ± 36.44**

M-PEF(Moderate PEF): 5 kV.cm^{-1}; 8.8 kJ.kg^{-1}.
I-PEF (Intense-PEF): 5 kV.cm^{-1}; 52.9 kJ.kg^{-1}
Values represent means with their standard deviation (n=2)
Different letters within the same line and grape variety indicate significant differences ($p \leq 0.05$)
Anthocyanins: mean±SD, gr.L^{-1} as malvidin-3-O-glucoside
nd: not detected. G: glucoside, Ac: acetylated, Cm: coumarylated

Journal Pre-proof

CRediT authorship contribution statement

MAM carried out the experiments, interpreted the results, and wrote the first draft of the manuscript. CP and JMM carry out the experiments and conducted analysis. AC provided help for interpretation of the results. IA and JR were involved in the design of the study, interpretation of the results, and final writing of the manuscript.

Journal Pre-proof

Conflicts of Interest

Authors declare no conflict of interest.

Journal Pre-proof

Highlights

- PEF of high specific energy permits reducing red winemaking maceration to 24 hours
- A fast increment of wine color intensity was promoted after few hours of maceration
- PEF could result an alternative to maceration techniques based on grape heating

Journal Pre-proof

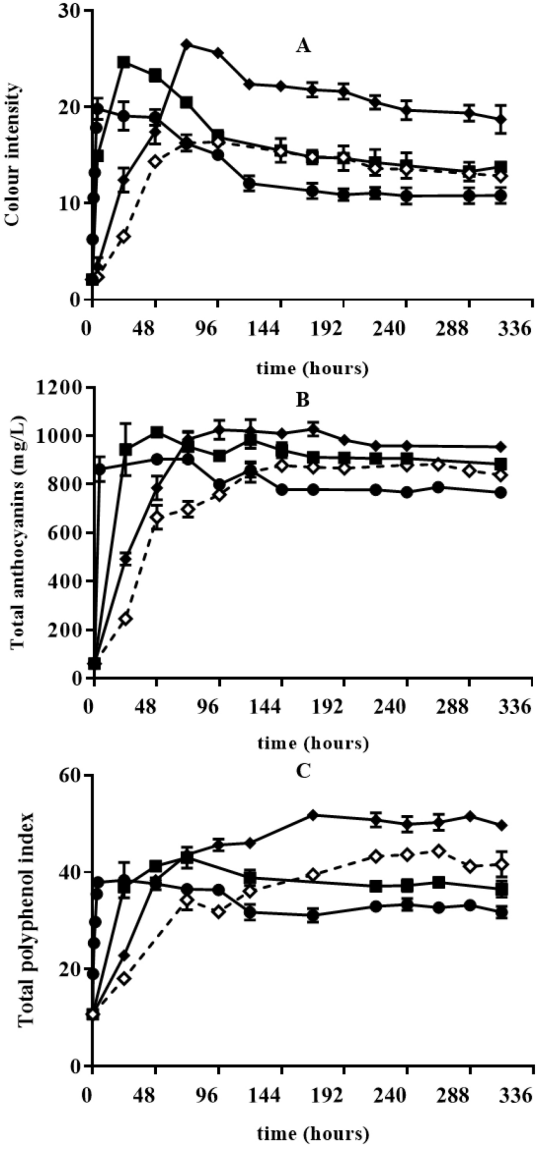

A
Colour intensity
30
20
10
0
0
48
96
144
192
240
288
336
time (hours)
B
Total anthocyanins (mg/L)
1200
1000
800
600
400
200
0
0
48
96
144
192
240
288
336
time (hours)
C
Total polyphenol index
60
40
20
0
0
48
96
144
192
240
288
336
time (hours)

Figure 1

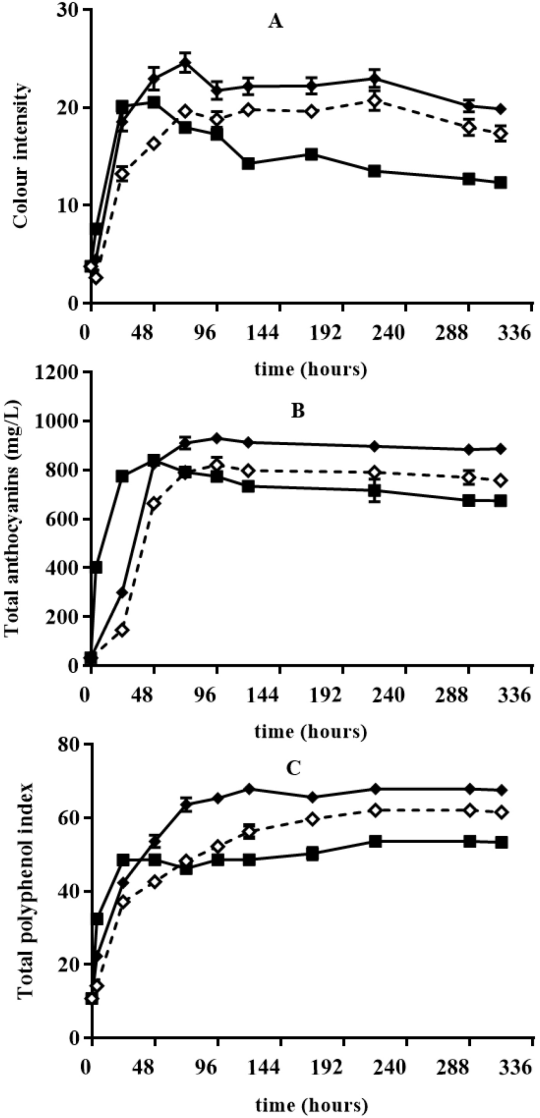

A
Colour intensity
30
20
10
0
0
48
96
144
192
240
288
336
time (hours)
B
Total anthocyanins (mg/L)
1200
1000
800
600
400
200
0
time (hours)
C
Total polyphenol index
80
60
40
20
0
time (hours)

Figure 2