

## Evolution of polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed electric fields during aging in bottle

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### ARTICLE INFO

#### Article history:

Received 6 May 2009

Received in revised form 6 July 2009

Accepted 11 August 2009

#### Keywords:

Polyphenols

Anthocyanins

Red wine

HPLC

Pulsed electric fields

Cabernet Sauvignon

Aging

### ABSTRACT

The evolution of the Folin–Ciocalteu index, colour intensity and the principal individual polyphenols throughout 12 months of aging in bottles of red wine from Cabernet Sauvignon grapes treated and untreated by pulsed electric fields (PEF) have been compared. PEF technology allowed bottling of wines with higher Folin–Ciocalteu index, colour intensity and individual polyphenols concentrations using a shorter maceration time. For both wines, aging did not affect the colour intensity, whereas the Folin–Ciocalteu index decreased. The evolution of the principal individual polyphenols during the 12 months of storage followed a similar pattern in both wines. At the end of aging, there were no significant differences observed in the content of monomeric anthocyanins between both wines, however, the content of flavan-3-ols, flavonols and hydroxycinnamic acids and derivatives was higher in PEF treated wine. These results indicate that PEF could be a suitable technology for obtaining wines with higher phenolic content or reducing the maceration time during vinification.

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### 1. Introduction

Phenolic compounds play a very important role in the quality of red wine, owing to their contribution to the wine sensory properties, mainly colour, astringency and bitterness (Boulton, 2001; Ribichaud & Noble, 1990). The chemical structure of polyphenols enables them to act as antioxidants, scavenging and neutralising free radicals. As a consequence, polyphenols of wine have been extensively studied in relation to their positive role in human health due to their antioxidant and anti-inflammatory activity (Gabor, 1986; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

Wine phenolics can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols). Anthocyanins are the main phenolic compounds involved in the colour of red wines (Boulton, 2001). Flavan-3-ols (monomeric forms and proanthocyanidins) are a large family of polyphenolic compounds which are mainly responsible for the astringency, bitterness and structure of wines, and also play an important role in the stabilisation of colour during aging (Boulton, 2001; Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999). Flavonols, which also contribute to bitterness, display antioxidant activity and affect red wine colour acting as copigments of anthocyanins (Ribichaud & Noble, 1990; Scalbert et al., 2005). Relating to non-flavonoid com-

pounds, hydroxycinnamic and hydroxybenzoic acids also act as copigments of anthocyanins and stilbenes have been recognised as compounds with antioxidant and anti-inflammatory properties (Eiro & Heinonen, 2002; Scalbert et al., 2005).

Red wines have a very complex phenolic composition that depends on the grape variety used, but also it is strongly affected by winemaking techniques and oenological practices (Baiano, Terracone, Gambacorta, & La Notte, 2009; Cantos, Espín, & Tomás-Barberán, 2002; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001).

Winemaking techniques play an important role in the extraction of polyphenols from the grapes and consequently in the further stability of wine properties. In the course of winemaking and aging, changes in polyphenolic composition occur due to the participation of these compounds in numerous reactions such as copigmentation, cycloaddition, polymerisation and oxidation. (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006; Cheynier & Ricardo da Silva, 1991; Eiro & Heinonen, 2002; Monagas, Gómez-Cordovés, & Bartolomé, 2005). These reactions start after grape crushing and continue throughout fermentation and aging, having an essential contribution to the sensory properties of wine, especially colour and astringency. The stabilisation of wine colour intensity is mainly associated with anthocyanin copigmentation and polymerisation, resulting in more stable structures and in a colour change from the red-bluish of young wines, to the reddish-brown colour of mature wines (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002). On the other hand, the decrease in

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astriogeneity of wine with aging is attributed to the participation of flavan-3-ols, particularly proanthocyanidins, in the copigmentation reactions (Atanasova et al., 2002).

Due to the large influence of polyphenols on sensory and nutritive quality of red wine, numerous studies have been focused on techniques able to influence the release of these compounds from grape during vinification (Pinelo, Arnous, & Meyer, 2006; Sacchi, Bisson, & Adams, 2005; Sun et al., 2001; Wightman, Price, Watson, & Wrolstad, 1997). López, Puértolas, Condón, Álvarez, and Raso (2008a,2008b) observed that the application of pulsed electric fields (PEF) to the red grape after crushing increases the extraction of phenolic compounds during the maceration–fermentation step. The process is based on the application of external electric fields that induce the electroporation of cell membranes, enhancing the diffusion of solutes. These first studies were conducted in static conditions. Recently, it has also been verified the feasibility of processing grapes by continuous PEF treatments at pilot-plant scale. Therefore, PEF technology has an unquestionable potential to obtain wines with higher polyphenolic content or to reduce the maceration time required to obtain a given polyphenolic concentration.

The occurrence of phenolic reactions during winemaking and aging depends on many factors. The polyphenolic concentration together with the relationship between the different individual polyphenols is one of them. As the extraction of polyphenols during the maceration–fermentation step is higher when grapes are previously treated by PEF, the changes in polyphenolic composition that occur during winemaking and aging could be different from those that occur with untreated grapes.

The objective of this investigation was to study the evolution of polyphenols in red wine from Cabernet Sauvignon grapes treated by PEF throughout the aging in bottle, and to compare it with the evolution of polyphenols in red wine from untreated grapes.

## 2. Materials and methods

### 2.1. Vinification process

Monovarietal red wines from grapes of *Vitis vinifera* var. Cabernet Sauvignon of the certification of origin Somontano (Aragón, northeast Spain) were elaborated at the Food Science and Technology pilot-plant of the University of Zaragoza. Grapes were harvested in 2007 during their optimal ripening stage (23.5° Brix, total acidity: 5.8 g/l) and transported to the pilot-plant. After destemming and crushing, sodium metabisulphite was added (30 mg/kg).

### 2.2. Pilot-plant PEF equipment

The apparatus used in this investigation (Modulator PG, Scandina, Uppsala, Sweden) generates square waveform pulses of a width of 3  $\mu$ s with a frequency up to 300 Hz. The maximum output voltage and current were 30 kV and 200 A, respectively. The actual voltage and the current intensity applied in the treatment chamber were measured with a high voltage probe (Tektronix, P6015A, Wilsonville, OR, USA) and a current probe respectively (Stangenes Industries Inc. Palo Alto, California, USA) connected to an oscilloscope (Tektronix, TDS 220, Wilsonville, OR, USA).

The colinear treatment chamber used in this investigation consists of two treatment zones of 2 cm between the electrodes with an inner diameter of 2 cm. In order to know the electric field strength distribution in the treatment zones, the field strength was numerically simulated by finite elements method, using Comsol Metaphysics software (Comsol Inc., Stockholm, Sweden). To standardise the results, the electric field strength used to charac-

terise the PEF treatments corresponds to the field strength in the mid position of the central axis of the treatment zone (Toepfl, Heinz, & Knorr, 2007).

A progressive cavity pump (Rotor-MT, Bominox, Gerona, Spain) was used to pump the grape mass to the colinear treatment chamber. The mass flow rate was 118 kg/h. This flow corresponds with a medium residence time in the treatment zone of 0.41 s.

### 2.3. PEF treatments

PEF treatment consisted in fifty pulses at a frequency of 122 Hz at electric field strength of 5 kV/cm (total specific energy: 3.67 kJ/kg). Previous experiments showed that longer or more intense treatments did not increase the extraction of phenolic compounds (López, Puértolas, Hernández-Orte, Álvarez, & Raso, 2009). The temperature was measured both at the entrance and at the exit of the treatment chamber. The initial temperature of the mass was 15 °C. In all experiments, the increment of the temperature due to the treatment never exceeded 2 °C.

### 2.4. Winemaking

After PEF treatment, two batches of grapes (100 kg each) were fermented in stainless steel tanks. As control two batches of untreated grapes were used. Fermentations were performed by selected yeast of *Saccharomyces bayanus* (EC1118, Lalvin, Ontario, Canada). Fermentation temperature was kept at 25  $\pm$  1 °C. The duration of the skin maceration was 96 h in PEF samples and 144 h in the control ones. This duration was decided in function of the polyphenolic concentration extracted verified during the vinifications. Thus, the skins were removed when similar total polyphenol index was obtained in two consecutive days (data not shown). During the fermentation process, temperature and must density were monitored daily and the cap was punched twice a day. The concentration of residual sugars at the end of the fermentation (8 days) was always lower than 3 g/l. Then, wines were racked and stabilised for a period of one month at –2 °C and finally raked again, bottled and stored in a condition room kept at 18  $\pm$  1 °C.

To study the evolution of main phenolic compounds, samples (two bottles of each experiment) were taken after 0, 2, 4, 6, 8, 10 and 12 months of bottling.

### 2.5. Colour intensity

Colour intensity (CI) of wine samples was calculated as the sum of the absorbances at 420 nm, 520 nm and 620 nm (Glories, 1984), using a Unicam UV500 spectrophotometer (Unicam Limited, Cambridge, UK) with a 1 mm quartz cuvette.

### 2.6. Phenolic analysis

Total phenolic compounds were evaluated by the Folin–Ciocalteu index (FCI), using the Folin–Ciocalteu reagent (Singleton & Rossi, 1965).

Individual polyphenolic compounds determination were performed on a Varian ProStar high performance liquid chromatograph (Varian Inc., Walnut Creek, CA, USA), comprising of a ProStar 240 ternary pump, a ProStar 410 autosampler and a ProStar 335 photodiode array detector. The system was controlled with Star chromatography workstation v.6.41 (Varian). A reversed-phase column Microsorb-MV 100-5 C18 (25  $\times$  0.46 cm; 5  $\mu$ m particle size) with a precolumn (5  $\times$  0.46 cm; 5  $\mu$ m particle size) of the same material was used. The temperature of the column and precolumn was maintained at 40 °C.

An elution gradient consisting of water–formic acid 95/5 v/v (solvent A) and acetonitrile (solvent B) was applied at a flow rate of 1 ml/min as follows: 2–6% of solvent B in 25 min, 6–15% of solvent B in 15 min, 15–20% of solvent B in 12 min and 20–40% of solvent B in 18 min. Before injection of the next sample, the column was washed with acetonitrile for 10 min and re-equilibrated with the zero-time solvent mixture during 20 min.

Wine samples were filtered (0.2 µm sterile syringe filter of cellulose acetate, VWR, West Chester, PA, USA) and then directly injected (10 µl) into the chromatograph. Chromatograms at 280 nm (flavan-3-ols), 320 nm (hydroxycinnamic acids and derivatives), 360 nm (flavonols) and 520 nm (anthocyanins) were recorded.

The different phenolic compounds analysed were tentatively identified according to the retention time and the UV–vis spectra of the pure standards when possible (quercetin 3-*O*-glucoside, myricetin, quercetin, kaempferol, and isorhamnetin from Fluka (Buchs, SG, Switzerland); caffeic acid, *p*-coumaric acid, (+)-catechin, and (–)-epicatechin from Sigma–Aldrich (St. Louis, MO, USA); kaempferol 3-*O*-glucoside and isorhamnetin 3-*O*-glucoside from Extrasynthèse (Genay, France)), and in base to the order of elution and the UV–vis spectral characteristics published in literature (Alcalde-Eon et al., 2006; de Villiers, Vanhoenacker, Majek, & Sandra, 2004; Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007; Hermosín-Gutiérrez, Sánchez-Palomo, & Vicario-Espinosa, 2005).

For commercial compounds, the quantification was carried out with the calibration curves obtained using the concentrations normally present in wine. For the non-commercial compounds, quantification was made using the calibration curves of the most similar compound: malvidin chloride (Sigma) for monomeric anthocyanins, quercetin 3-*O*-glucoside for myricetin 3-*O*-glucoside, caffeic acid for *t*-caftaric acid and *p*-coumaric acid for *t*-coutaric acid. The concentrations of the different studied compounds were expressed in mg/l.

### 2.7. Statistical data treatment

Significant differences between wines made from PEF treated grape and the control ones were assessed with analysis of variance (ANOVA), using the statistical software Minitab® (Minitab Inc., State College, PA, USA).

## 3. Results and discussion

### 3.1. Evolution of Folin–Ciocalteu index and colour intensity during aging

Evolution of Folin–Ciocalteu index (FCI) and colour intensity (CI) were analysed every two months during storage for 12 months

(Fig. 1A and B). At bottling, wine made from PEF treated grapes (PEF-wine) presented higher FCI and CI than wine obtained from untreated grapes (control wine). The application of a PEF treatment increased FCI from  $52.06 \pm 2.50$  to  $65.15 \pm 1.22$  and CI from  $21.52 \pm 1.22$  to  $28.22 \pm 1.09$ . These higher indexes for the PEF-wine were obtained even though the maceration time was shorter than for the control wine. Whereas, the maceration time for the control wines were 144 h, the PEF-wines time was only 96 h, meaning a reduction of a 33%.

Grape skin cell envelopes are a limiting barrier that prevents the release of polyphenols during the maceration step in winemaking process (Pinelo et al., 2006). It has been demonstrated that the application of PEF enhance the extraction of intracellular compounds of different fruits and vegetables (Ade-Omowaye, Angersbach, Eshtiaghi, & Knorr, 2000; Fincan, DeVito, & Dejmek, 2004; Grimi, Praporscic, Lebovka, & Vorobiev, 2007; Toepfl, Heinz, & Knorr, 2006). Electric field induces a trans-membrane potential difference across the cell membranes. When this potential difference reaches a critical value of 1 V, localised breakdown occurs, increasing cell membrane permeability (Pagán & Mañas, 2006). Therefore, the increment of the permeability of the grape skin cells by PEF processing seems to facilitate the extraction of polyphenols from the skins during maceration.

In order to know if the high levels of polyphenolic compounds presented in the PEF-wine at bottling could influence the wine evolution during the storage in bottle, wines were exposed to an aging process in bottle of 12 months. Wine aging in bottle prompted a decrease of FCI in both wines, being this reduction higher for PEF-wine. The FCI decreased 11.31 units in PEF-wine and only 6.15 units in control wine. However at the end of aging, the FCI was still a 17% higher in PEF-wine. CI values obtained before and after wine aging were non-significantly different ( $p > 0.05$ ) in both wines, meaning that the differences observed at bottling for the CI between both wines were maintained during storage. So after 12 months of aging in bottle the CI of PEF-wine was a 33% higher than of control wine.

### 3.2. Evolution of the total content of the phenolic families during aging

Fig. 2 shows the evolution of the content of total monomeric anthocyanins (TMA) (2A), and total flavan-3-ols, hydroxycinnamic acids and flavonols (2B) in PEF and control wines. Monomeric anthocyanins were the predominant polyphenols in both wines during aging, representing at bottling the 77% of the total phenolic content. At the beginning of the storage in bottle, the content of TMA was 17% higher in the PEF-wine than in the control wine. Throughout the aging process, the content of anthocyanins were considerably reduced. This reduction of concentration was faster during the first four months of aging, reaching a decrease in the

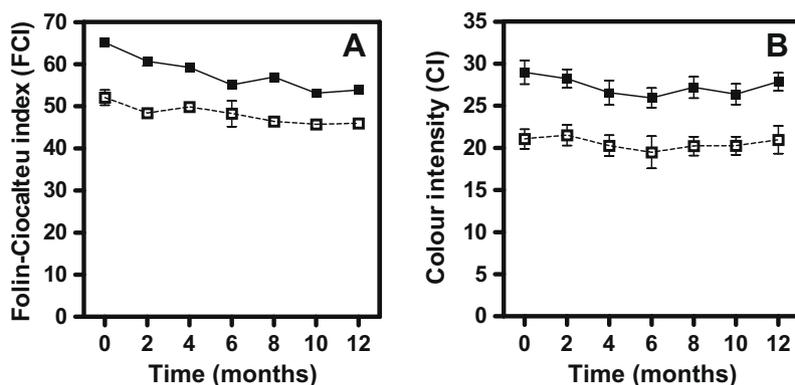


Fig. 1. Evolution of Folin–Ciocalteu index (FCI) (A) and colour intensity (CI) (B) of PEF-wine (■) and control (□) during 12 months of aging in bottle.

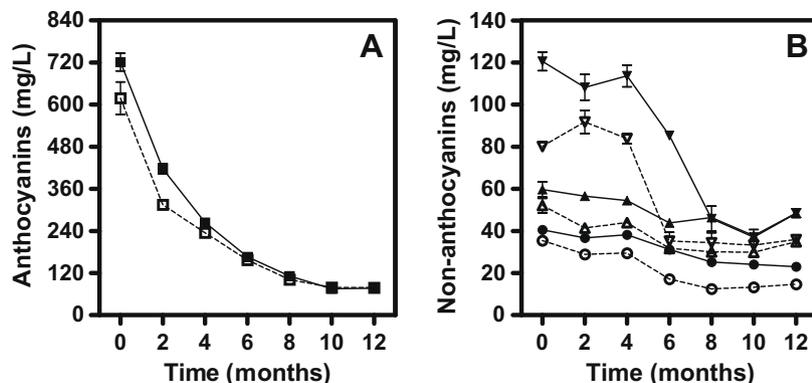


Fig. 2. Evolution of total anthocyanin content (TAC) (■, □) (A), and total flavan-3-ols (▼, ▽), hydroxycinnamic acids (●, ○) and flavonols (▲, △) (B) of PEF and control wines during 12 months of aging in bottle. Closed and opened symbols correspond to PEF-wine and control wine, respectively.

TMA content of a 63% in both PEF and control wines. Hermosín-Gutiérrez et al. (2005) also reported a fast decrease of anthocyanins during the first months of aging for Cabernet Sauvignon, and also for Syrah variety. However, the anthocyanin decrease in the first four months of aging did not coincide with a substantial decrease of non-anthocyanic polyphenols (see Fig. 2B). Therefore this initial reduction could be due to precipitation and oxidation reactions, and to the formation of anthocyanin–anthocyanin complexes rather than the formation of copigments with other phenolic fractions (Alcalde-Eon et al., 2006). From the fourth month of storage, the TMA content of both wines were non-significantly different ( $p > 0.05$ ), and the reduction of TMA concentration with aging was similar in both wines.

At the beginning of the storage in bottle, flavan-3-ols were the predominant non-anthocyanic polyphenols, followed by flavonols and hydroxycinnamic acids. At this time, the content of flavan-3-ols, hydroxycinnamic acids and flavonols in PEF-wine were 50%, 14%, and 15% higher than in control wine. Similarly to the evolution of TMA content, aging in bottle produced a reduction in the content of all non-anthocyanic groups of polyphenols in both wines. By contrast, the most important diminution on their concentration occurs from the fourth to the eighth month of storage, especially for flavan-3-ols. This reduction is mainly associated with copigmentation reactions with anthocyanins, which are responsible for the stabilisation of the red wine colour (Boulton, 2001). These stable pigments formed could explain why the CI were kept constant during aging of both wines (Fig. 1B), despite the important reduction observed on the TMA content.

The content of flavan-3-ols, flavonols and hydroxycinnamic acids throughout the aging in bottle decrease respectively 60%, 19% and 43% in PEF-wine and 55%, 33%, and 41% in control wine. After 12 months of storage, the content of flavan-3-ols, flavonols and hydroxycinnamic acids were significantly higher ( $p < 0.05$ ) in the PEF-wine than in the control, representing a difference of 35%, 39% and 57%, respectively.

### 3.3. Evolution of individual phenols during aging

Fig. 3 shows the evolution of the concentration of unacylated, acylated and coumarylated forms of monomeric anthocyanins during aging in bottle for PEF and control wines. As observed by other authors, storage in bottle caused a considerable decrease in the concentration of the three groups of anthocyanins, independently of the wine (Hermosín-Gutiérrez et al., 2005; Monagas, Gómez-Cordovés et al., 2005). Similarly to the evolution of TMA content, the concentration reduction was faster during the first four months of aging, reaching a decrease on the anthocyanic concentration of

approximately 61%, 63% and 69% for unacylated, acylated and coumarylated forms respectively in both wines. Although the evolution of the anthocyanins forms and their relative proportions were similar in both PEF and control wines, significant differences in their concentrations were detected during the first four months of aging ( $p < 0.05$ ). After this time, non-significant differences between the wines were detected ( $p > 0.05$ ).

Changes of the relative proportions of these compounds during aging in bottle were similar in both wines. The proportion of unacylated glycosides increased during aging approximately 8%, the proportion of acylated forms decreased 6% and the proportion of coumarylated forms remained practically constant or slightly decreased. Similar behaviours have been previously described in Cabernet Sauvignon red wines during aging in bottle (Hermosín-Gutiérrez, Sánchez-Palomo, & Vicario-Espinosa, 2005).

Fig. 4 shows the evolution of the principal hydroxycinnamic acids (caffeic acid and *p*-coumaric acid) and their main derivatives (*t*-caftaric acid and *t*-coutaric acid) during aging in bottle of red wines made from PEF treated and untreated grapes. The evolution of these compounds was similar in both wines. The results obtained in the control wine agree to those published for other varieties in the literature (Hermosín-Gutiérrez et al., 2005; Monagas, Bartolomé, & Gómez-Cordovés, 2005). A progressive decrease of *t*-caftaric and *t*-coutaric acids occurred throughout the storage process that was especially remarkable between the fourth and the eighth month of aging. This reduction probably was caused by the formation of copigments with anthocyanins as it has been already indicated (Darias-Martín et al., 2002). On the other hand, the concentrations of the free acids forms, caffeic and *p*-coumaric acids, remained low during the first four months of aging, and then their concentrations increased gradually. These increments have been generally attributed to the hydrolysis of the corresponding tartaric esters, caftaric acid and coutaric acid but also have been associated with the hydrolysis of other polymeric compounds, especially *p*-coumaric acid from coumarylated anthocyanins (Monagas, Bartolomé et al., 2005; Somers, Vérette, & Pocock, 1987; Wightman et al., 1997). Our results support this hypothesis. For example, in control wines the decrease of *t*-coutaric acid during the last two months of aging (0.05 mg/l) was lower than the increase of *p*-coumaric acid (0.38 mg/l).

The main hydroxycinnamic acid or derivative detected in both wines was *t*-caftaric acid, except after 12 months of aging, in which caffeic acid became the most detected one. PEF-wine showed a higher total concentration of hydroxycinnamic acids derivatives than the control one at bottling. The differences increased until the eighth month in bottle and then those decreased until the twelfth month of aging. For instance, the PEF-wine had a concen-

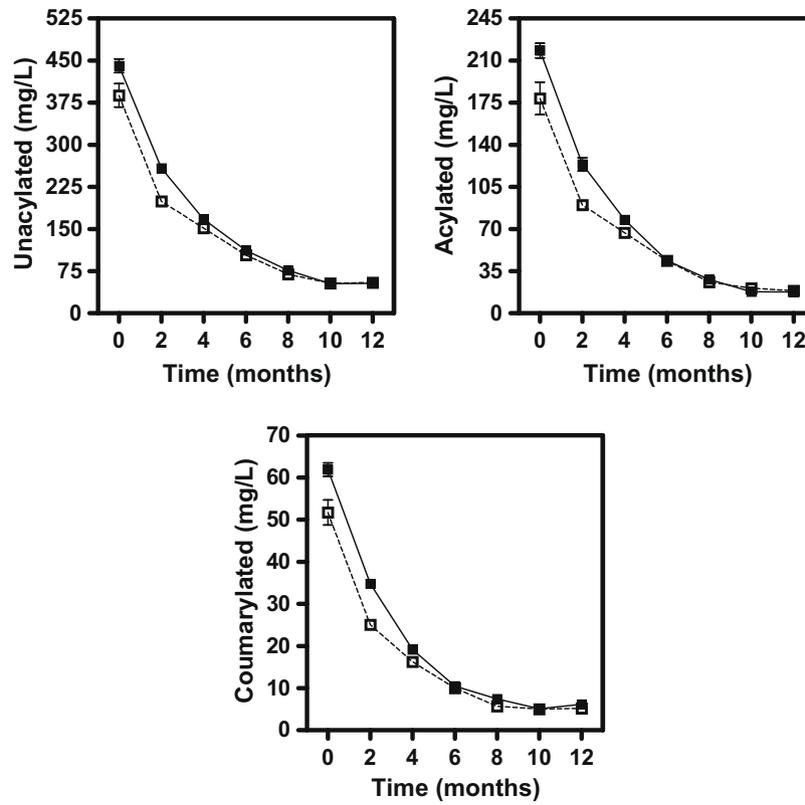


Fig. 3. Evolution of total content of unacylated, acylated and coumarylated anthocyanins of PEF-wine (■) and control (□) during 12 months of aging in bottle.

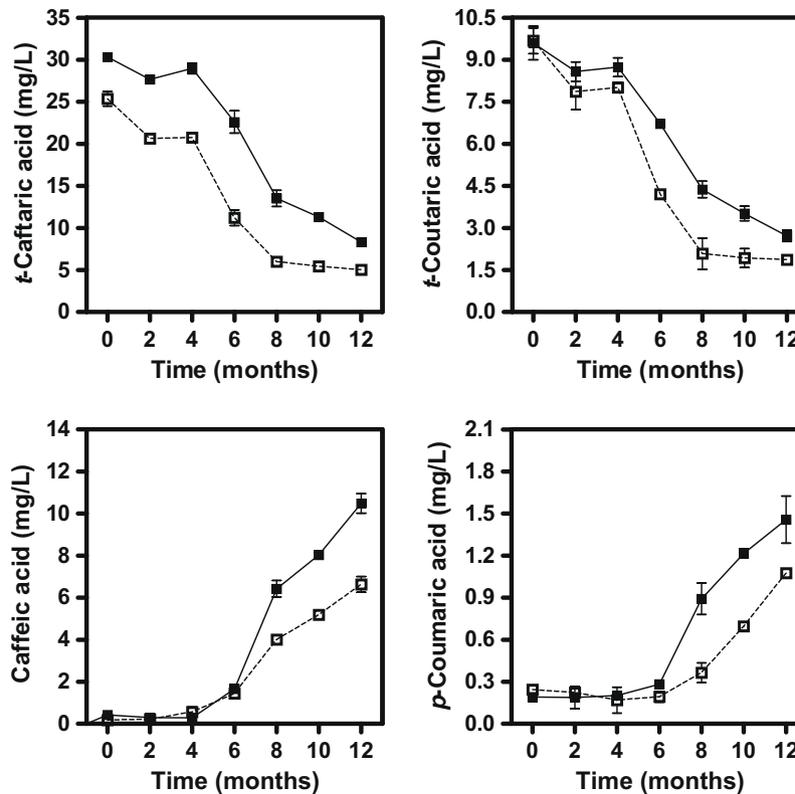


Fig. 4. Evolution of main hydroxycinnamic acids (caffeic and p-coumaric acids) and their derivatives (t-caftaric and t-coutaric acids) of PEF-wine (■) and control (□) during 12 months of aging in bottle.

tration of *t*-caftaric acid 20% higher than the control at bottling. After 6 months of storage, although the concentration of *t*-caftaric acid decreased in both wines, the concentration of this compound in PEF-wine was 102% higher than in control wine. After 12 months of aging, the difference in *t*-caftaric acid content between both wines decreased to a 66%. Concerning *t*-couteric, caffeic and *p*-coumaric acids, in first months of aging non-significant differences ( $p > 0.05$ ) were observed between the PEF and control wines. However, differences were detected after the sixth month of aging for *t*-couteric acid and after the eighth month for caffeic and *p*-coumaric acids.

The (+)-catechin and (–)-epicatechin evolutions during aging in bottle in PEF and control wines are presented in the Fig. 5. Similarly to the evolution of hydroxycinnamic acids derivatives, in the first four months the concentration of these compounds mainly re-

mained constant in both wines. Then the values decreased until the eighth month, remaining constant again until the twelfth month of storage. Hermosín-Gutiérrez et al. (2005) also reported similar evolution of these compounds in Cabernet Sauvignon variety. These authors described constant concentrations of monomeric flavan-3-ols during the first three months in bottle, diminishing these levels in the following months of aging. The decrease of these substances during aging is generally related to both oxidation and precipitation reactions and the participation of these compounds in several polymerisation reactions, implying the stabilisation of wine sensory properties, mainly colour (Boulton, 2001; Es-Safi et al., 1999).

(+)-Catechin and (–)-epicatechin content in both wines showed similar high values than those previously published in literature for Cabernet Sauvignon variety (Hermosín-Gutiérrez et al., 2005).

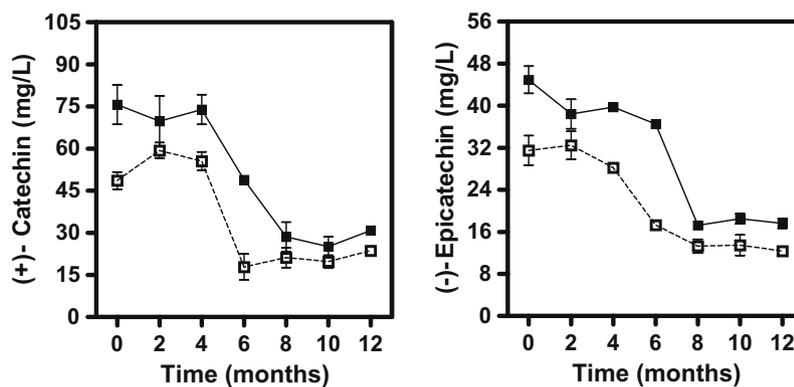


Fig. 5. Evolution of monomeric flavan-3-ols, (+)-catechin and (–)-epicatechin of PEF-wine (■) and control (□) during 12 months of aging in bottle.

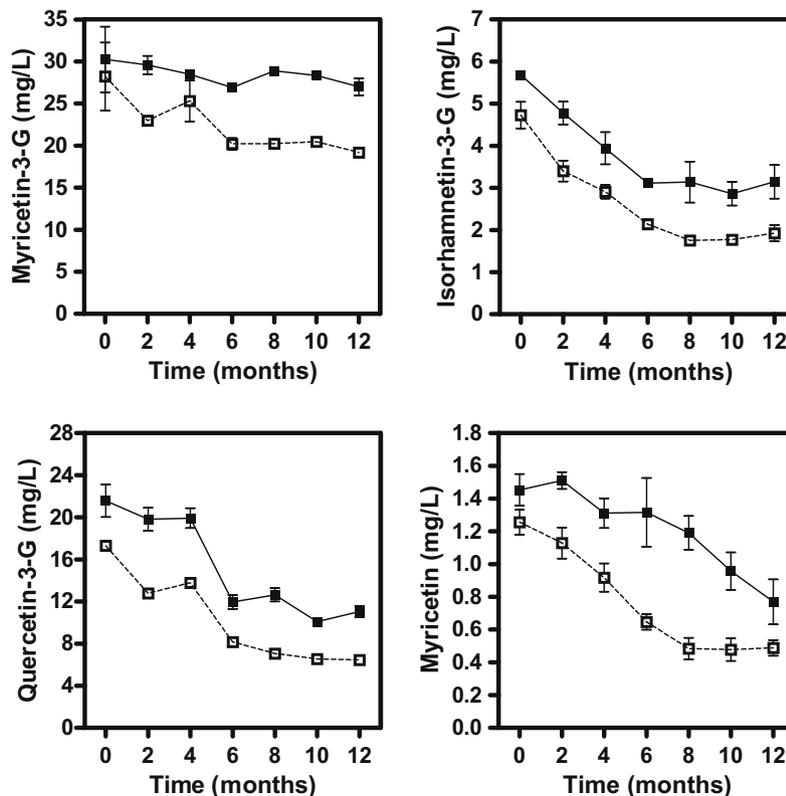


Fig. 6. Evolution of studied flavonols (myricetin 3-O-glucoside, quercetin 3-O-glucoside, isorhamnetin 3-O-glucoside and myricetin) of PEF-wine (■) and control (□) during 12 months of aging in bottle.

(+)-Catechin was the most abundant of them, independently of the wine or the aging time. This behaviour confirms the observations of other authors reporting that (+)-catechin is the most important flavan-3-ol detected in wines of different varieties such as Tempranillo or Monastrell (Gil-Muñoz, Gómez-Plaza, Martínez, & López-Roca, 1999; Gómez-Alonso et al., 2007; Monagas, Bartolomé et al., 2005).

PEF treated wines have shown higher concentrations of (+)-catechin and (–)-epicatechin than the control ones. The evolution of the differences in concentration of these compounds between the wines during aging was similar to the evolution in the hydroxycinnamic acids previously described. At the moment of bottling, the concentration of these compounds in PEF-wine was around 50% higher than in the controls. The highest difference between wines was observed after six months of aging (174% and a 111% for (+)-catechin and (–)-epicatechin respectively). Finally, after 12 months of aging, the concentrations of (+)-catechin and (–)-epicatechin detected in PEF-wine were respectively 53% and 39% higher than in the control ones.

Despite their low concentration, flavonols have an important role in the stabilisation of the wine colour, since they participate in the copigmentation reactions with anthocyanins. All the flavonols standards used were detected in the analysed samples, except quercetin, kaempferol, isorhamnetin and kaempferol 3-*O*-glucoside. Fig. 6 shows the concentration of myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside and myricetin throughout the aging process in bottle. In both PEF and control wines, a gradual reduction of the concentration of quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside and myricetin until the sixth month of aging was observed. Then, the concentration values remained constant or slightly decreased. Other authors reported this decreasing pattern for flavonol concentrations in other varieties such as Monastrell, Tempranillo or Graciano (Monagas, Bartolomé et al., 2005). These reductions were probably due to hydrolysis and copigmentation reactions (Ribichaud & Noble, 1990). On the contrary, the concentration of myricetin 3-*O*-glucoside slightly decreased, remaining high values during all the aging process.

In both wines, PEF treated and control, myricetin 3-*O*-glucoside was the most detected flavonol, independently of the aging time. Similarly to the hydroxycinnamic acids and flavan-3-ols, PEF-wine exhibited the highest flavonol concentrations. The differences observed between both wines also depended on the aging time, being higher in the first months of aging, and then decreasing until the end of the aging in bottle, for all the detected flavonols. At the end of the storage in bottle, the concentration of myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside and myricetin respectively was a 41%, 71%, 63% and 58% higher for PEF-wine than for control wine.

#### 4. Conclusions

According to the results obtained in this research, the application of a PEF treatment to grapes prior to the alcoholic fermentation, led to the production of a Cabernet Sauvignon wine after 12 month of aging in bottle with a higher polyphenolic concentration and colour intensity using a shorter maceration time than in control wines. The evolution of monomeric anthocyanins, hydroxycinnamic acids and derivatives, monomeric flavan-3-ols and flavonols during 12 months followed a similar pattern in the PEF-wine than in the control wine. At the end of storage for 12 months, there were no significant differences observed in the content of monomeric anthocyanins between both wines, however, the content of flavan-3-ols, flavonols and hydroxycinnamic acids and derivatives was higher in PEF-wine. These results sup-

port that PEF processing could be a suitable novel technology to be used in wineries to obtain red wines with a higher content in phenolic compounds or to reduce the maceration time.

#### Acknowledgements

E.P. and G.S. gratefully acknowledge the financial support for their doctoral studies from the “Ministerio de Ciencia e Investigación” of the Spanish Government. Authors appreciatively acknowledge Glenn Richardson the review of the English language.

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