JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 2351–2357 2351
DOI:10.1021/jf904035v

Effect of Pulsed Electric Field Processing of Red Grapes on Wine Chromatic and Phenolic Characteristics during Aging in Oak Barrels

E. Puértolas, G. Saldaña, I. Álvarez, and J. Raso*

Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, c/ Miguel Servet, 177, 50013, Spain

The influence of a pulsed electric field (PEF) treatment of grape berries at pilot-plant scale on the evolution of the chromatic and phenolic characteristics of Cabernet Sauvignon red wines during aging in American oak barrels and subsequent storage in bottle has been studied. Results obtained in this investigation confirm that the better chromatic characteristics and higher phenolic content obtained due to the PEF treatment after the fermentation process remain or even increase during aging in oxidative conditions in American oak barrels and their subsequent storage in bottle. No sensory differences in color and bouquet were detected after 8 months of aging in bottle by triangle tests. According to the results, PEF is a promising enological technology to obtain wines with the high phenolic content necessary for the production of high quality oak aged red wines.

KEYWORDS: Aging; Cabernet Sauvignon; color; oak barrels; polyphenols; pulsed electric fields; red wine

INTRODUCTION

An oxidative aging in wood barrels followed by a reductive aging in bottle is a traditional practice in the production of high quality red wines (*1*). During the contact of the wine with the wood, different physical, chemical and physicochemical reactions take place that implicate important changes in the wine composition (*2*−*4*). Moreover, simultaneously with these reactions, an extraction of a great variety of aromatic compounds and phenolic substances from wood occurs, adding complexity to the wine (*5*). All these processes cause the stabilization of the wine color, a gradual softening of the taste and the consequent loss of astringency, and also an increase in the aromatic richness of the wines (*6*, *7*). Throughout the subsequent reductive aging in bottle, wine continues evolving. New compounds may be formed, such as copigments between anthocyanins and other phenolic compounds, and the concentration of the compounds originally presented may increase or decrease (*1*). These changes play an important role in the roundness of the sensorial characteristics of wine.

In recent years, a decrease in wine consumption and a growth of wine production in emerging countries have occurred. In order to maintain their competitiveness, these facts have led to the wineries of traditional wine production zones as Europe to introduce new production technologies in an attempt to obtain high quality wines. The use of novel technologies such as pulsed electric fields (PEF) that are able to enhance polyphenol extraction during red winemaking may represent an important alternative to traditional techniques.

*Corresponding author. Postal address: Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, c/Miguel Servet, 177, 50013, Zaragoza, Spain. Tel: +34976762675. Fax: +34976761590. E-mail: jraso@unizar.es.

PEF technology has generated increasing interest in recent years for liquid food pasteurization and for improving mass transfer operations in the food industry (*8*−*10*). The process is based on the application of short-duration high-intensity electric field strengths that induce the electroporation of cell membranes (*11*). The pore formation provokes microbial inactivation and enhances the diffusion of solutes through cell membranes. The potential advantages in winemaking of PEF as an antimicrobial treatment on yeast and bacteria and to improve polyphenol extraction have been previously investigated (*12*−*15*).

An increase in the extraction of phenolic compounds during the vinification of different grape varieties treated by PEF has been observed (*14*). In these promising studies, the PEF treatment was applied in batch systems of low capacity and the positive effects were only evaluated during the fermentation−maceration step and in the freshly fermented wine. Recently, the feasibility of processing red grapes by PEF at pilot-plant scale has been demonstrated (*16*). It has been observed that the differences in phenolic composition between PEF and control wines obtained at the end of the alcoholic fermentation remain during the storage in bottle (*17*). Therefore, PEF technology has an unquestionable potential to be applied in the wineries in a midterm to reduce the maceration time during winemaking or to obtain wines with higher phenolic content. However, at the moment there are no data in the literature about the changes that wine produced from PEF treated grapes undergoes during aging in wood barrels.

In this investigation, the effect of the application of a PEF treatment at pilot-plant scale to the grape berries on the chromatic characteristics and the phenolic composition of Cabernet Sauvignon red wines during aging in barrels of American oak (*Quercus alba*) and the subsequent storage in bottle has been investigated.

Published on Web 01/22/2010

MATERIALS AND METHODS

Samples. Wine was produced from grapes of *Vitis vinifera* L. var. Cabernet Sauvignon grown in a vineyard located in "Somontano" Designation of Origin (northeast of Spain) and harvested in the 2007 vintage. Grapes were manually harvested at optimum ripening stage (23.5 °Brix, titratable acidity: 5.8 g of tartaric acid/L) and transported to the Food Science and Technology Pilot-Plant of the University of Zaragoza.

Pilot-Plant PEF Equipment. PEF equipment used in this investigation (Modulator PG, ScandiNova, Uppsala, Sweden) was previously described (*16*). The apparatus generates square waveform pulses of a width of 3 μs with a frequency up to 300 Hz. The maximum output voltage and current were 30 kV and 200 A, respectively.

The collinear treatment chamber has been also previously described (*16*). The treatment chamber has two treatment zones of 2 cm between the electrodes with an inner diameter of 2 cm. Using this design, the applied electric field strength in the treatment zones is not uniform. In order to know its distribution, the electric field strength was numerically simulated by the finite element method, using the Comsol Multiphysics software (Comsol Inc., Stockholm, Sweden). To standardize the treatment, the electric field strength used to characterize the PEF treatments corresponded to the electric field strength in the mid position of the central axis of the treatment zone (*18*).

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

PEF Treatments. After crushing and destemming, grapes were PEF treated. PEF treatment consisted of an average of 50 pulses of 3 μs at an electric field strength of 5 kV/cm (total specific energy: 3.67 kJ/kg) at a frequency of 122 Hz. Previous experiments showed that more intense treatments did not increase the extraction of phenolic compounds in Cabernet Sauvignon grapes (*15*).

The temperature was measured both at the inlet and outlet of the treatment chamber. Experiments were conducted at room temperature, and the temperature increment due to the treatment never exceeded 2 °C.

Winemaking. After PEF treatment, potassium metabisulfite (30 ppm) was added and two sets of 100 kg of grapes were fermented in stainless steel tanks. Two additional sets of untreated grapes were used as control. Fermentations were performed by selected yeast of *Saccharomyces cerevisiae* (EC1118, Lalvin, Ontario, Canada). Fermentation temperature was kept at 25 ± 1 °C. The duration of the skin maceration was 96 h for the samples treated by PEF and 144 h for the controls. This duration was decided in function of the phenolic extraction verified during the vinifications. Thus, the skins were removed when a nonincrement in total polyphenol index was observed in two consecutive days (data not shown). During the fermentation process, temperature and must density were monitored daily and the cap was punched down twice a day. The concentration of residual sugars at the end of the fermentation (8 days) was always lower than 3 g/L. Malolactic fermentation was conducted by inoculation with *Oenococcus oeni* (Enoferm Beta, Lallemand, Ontario, Canada), concluding approximately after two weeks. Malolactic fermentation was monitored by the degradation of malic acid, determined by a commercial enzymatic method (R-Biopharm, Darmstadt, Germany). In all experiments, the malic acid concentration at the end of malolactic fermentation was less than 0.2 g/L. Then, the wines were racked and aged afterward in new 20 L midtoasted barrels of American oak (*Quercus alba*). After 6 months of aging in barrels, wines were racked again, bottled and stored in a condition room kept at 18 ± 1 °C during 8 months.

Chemical Analysis. Chromatic characteristics of the wines were determined by a direct measurement of the absorbance of the wines at 420, 520, and 620 nm using a Unicam UV500 spectrophotometer (Unicam Limited, Cambridge, U.K.) with a 1 mm path length quartz cuvette. Color intensity (CI) was calculated as the sum of absorbance at 420, 520, and 620 nm. Tint was determined as the proportion of the absorbance measured at 420 and 520 nm; and proportion of yellow color (%Ye), red color (%Rd) and blue color (%Bl) as the relation between 420, 520, and 620 nm respectively and color intensity (*19*, *20*). The CIELAB parameters (a^*, b^*, L^*, C^* and h^*) were calculated using the software MSCV (*21*). The color difference (ΔE^*) in CIELAB units between PEF and control wines was determined by the following equation (*22*):

$$\Delta E^*_{1,2} = \sqrt{(\Delta L^*_{1,2})^2 + (\Delta a^*_{1,2})^2 + (\Delta b^*_{1,2})^2}$$

HPLC Analysis of Phenolic Compounds. The HPLC analysis was performed according to the chromatographic conditions described by Puértolas et al. (*17*). A Varian ProStar high performance liquid chromatograph (Varian Inc., Walnut Creek, CA) comprising a ProStar 240 ternary pump, a ProStar 410 autosampler and a ProStar 335 photodiode array detector was used. The system was controlled with Star chromatography workstation v.6.41 (Varian). A column Microsorb-MV 100−5 C18 (25 × 0.46 cm; 5 μm particle size) with a precolumn (5 × 0.46 cm; 5 μm 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 solution (95:5) (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, column was washed with acetonitrile during 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) and then directly injected (10 μL) in the chromatograph. Chromatograms at 280 nm (flavan-3-ols), 320 nm (hydroxycinnamic acids and derivatives), 360 nm (flavonols) and 520 nm (anthocyanins) in the photodiode array detector were recorded.

The different phenolic compounds analyzed were tentatively identified according to the retention time and the UV−vis spectra of the pure standards when possible (quercetin-3-glucoside, myricetin and quercetin from Fluka (Buchs, SG, Switzerland); caffeic acid, *p*-coumaric acid, (+)-catechin and (−)-epicatechin from Sigma-Aldrich (St. Louis, MO); isorhamnetin-3-glucoside from Extrasynthèse (Genay, France)), to their order of elution and to the UV−vis spectral characteristics published in the literature (*23*−*25*).

For commercial compounds, the quantification was carried out with the calibration curves obtained using the concentrations normally present in wine. For the non commercially available compounds, quantification was made using the calibration curves of the most similar compound: malvidin chloride (Sigma) for monomeric anthocyanins, quercetin-3-glucoside for myricetin-3-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.

Sensory Analysis. After 8 months in bottle, the wines were sensorially evaluated with a panel made up of 19 members of the Analytical Chemistry Department of the University of Zaragoza. The panelists were well-trained judges, and previously they had participated in similar studies. Wines were evaluated by discriminative analysis (triangle tests), using a completely randomized design in two sessions. The objective of the analysis was to determine if the panel could distinguish the PEF oak aged wine from the control in function of their color and bouquet (significant $p < 0.05$).

Statistical Analysis. Two samples for each vinification ($n = 2$) were analyzed. The data present in tables and figures represent mean values ± 95% confidence level. The statistical analysis of the data was accomplished using the statistical software Minitab (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Evolution of Chromatic Characteristics. Figure 1 shows the values of Glories (*19*) and Sudraud (*20*) chromatic indexes (CI, tint, %Ye, %Rd, %Bl) during the oxidative aging in barrels and the subsequent reductive aging in bottle for PEF and control wines. During the complete aging process, the values of CI of both wines remained practically constant. A similar result was obtained by Sánchez-Iglesias et al. (*26*) during 12 months of aging in oak barrels of Tempranillo wines. Even though the maceration time was 48 h shorter for the PEF than for control wine, at the end of malolactic fermentation, just before starting the aging step, the CI of PEF wine was 29% higher. This difference initially detected remained constant throughout the 14 months of aging. The stabilization of color of wines during aging has been observed by other

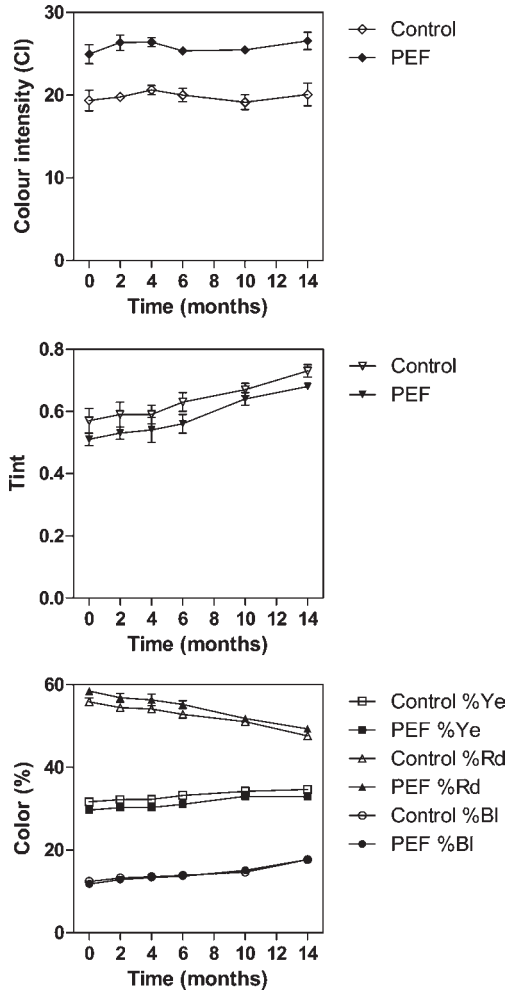

Figure 1. Evolution of color intensity (CI) (rhombuses), tint (inverted triangles), %Ye (squares), %Rd (triangles) and %Bl (circles) of PEF wine (closed symbols) and control wine (open symbols) during 6 months of aging in American oak barrels and the subsequent storage in bottle.

authors, and it has been attributed to the copigmentation reactions of anthocyanins with other phenolic compounds, especially flavan-3-ols (*27*, *28*).

Evolution of tint, %Ye, %Rd and %Bl during aging of PEF wines has not been reported. As it is shown in **Figure 1**, these indexes followed a similar pattern in both PEF and control wines. Whereas the %Rd decreased during aging, tint, %Ye and %Bl values increased progressively. This behavior is in accordance with the data published in the literature during aging of red wine (*26*, *29*). Although PEF wine presented higher %Rd and smaller %Ye than the control wine, these differences were always smaller than 5%. No differences were detected between wines on %Bl. On the other hand, the control wine presented superior tint value than the PEF wine for all the aging process. These differences decreased from an initial level of 12% to 7% after 14 months of aging. Tint value underlines the relative importance of yellow tones on the red color. The higher tint value of the control wine represented that the proportion of yellow tones to red tones is higher than in the PEF wine.

Figure 2 illustrates the change in CIELAB parameters (L^*, C^*, h^*, a^* and b^*) along the aging in barrels and bottle for PEF and control wines. Independently of the wine, a decrease in the values of the CIELAB parameters was observed. This decrease was mainly pronounced after aging in oak barrels, particularly in the last 4 months of aging in bottle. In spite of this similar evolution between both wines, differences in reached values of the CIELAB parameters were detected. L^* parameter is a measure of lightness, from completely opaque (0) to completely transparent (100). PEF wine showed lower L^* values than the control one, indicating that PEF wine was darker than the control. The detected differences increased from 23% to 73% after 14 months of aging. Whereas the b^* (yellowness) parameter was always higher in PEF wines, no differences in a^* (redness) and C^* (chroma) values were observed between wines. Finally, the h^* parameter (hue) was always superior in the PEF wine (from 64% to 31%). These general differences in CIELAB parameters due to the PEF treatment are similar to the differences previously published in the literature for freshly fermented Cabernet Sauvignon wines (*15*).

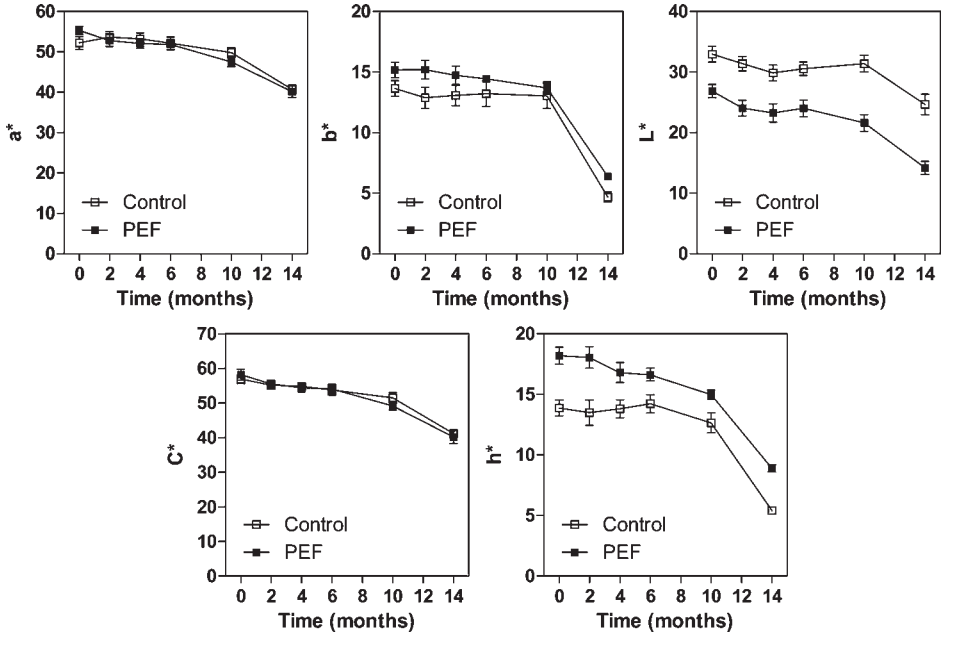

Figure 2. Evolution of CIELAB parameters (a^*, b^*, L^*, C^* and h^*) of PEF wine (closed squares) and control wine (open squares) during 6 months of aging in American oak barrels and the subsequent storage in bottle.

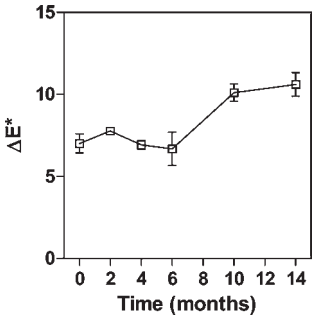

Figure 3. Evolution of color difference (ΔE^*) in CIELAB units between PEF and control wines during 6 months of aging in American oak barrels and the subsequent storage in bottle.

The ΔE^* parameter has been suggested to estimate in CIELAB units how two wines are different. ΔE^* values ≥3 CIELAB units indicate that the differences can be perceived by the human eye (*22*). **Figure 3** shows the evolution of the chromatic differences (ΔE^*) between the PEF and the control wines during the aging in barrels and the subsequent maturation in bottle. It can be observed that, at each sampling time, the ΔE^* was always greater than 6.7 CIELAB units. During the aging in barrels, the differences between both wines remained constant (from 6.7 to 7.0 CIELAB units). However, after bottling an important increase of the ΔE^* parameter was observed. After 8 months of aging in bottle (14 months of total aging) the chromatic difference between the wines reached a value of 10.6 CIELAB units.

Evolution of Phenolic Families. Relating to the phenolic compounds presented in the wines, the evolution of the total content of anthocyanins, flavan-3-ols, hydroxycinnamic acids and flavonols during the aging process is shown in **Figure 4**. The decreasing pattern of the phenolic families was similar independently of the wine, suggesting that the PEF treatment did not influence the evolution of the content of the principal phenolic families.

Monomeric anthocyanins are directly responsible for the color of red wine. In both wines, the total anthocyanic content considerably declined during aging. For example, in controls anthocyanins decreased from 384.73 to 53.69 mg/L, which was 86% of diminishing. This decrease was especially pronounced in the first 4 months of aging, in which the anthocyanic content decreased by 44% and 48% in the control and the PEF wine, respectively. The loss of anthocyanins has been traditionally explained due to either degradation reactions or polymerization reactions and the formation of new stable pigments with other compounds, especially flavan-3-ols (*30*). In this case, the constant CI during aging may indicate that the stabilization reactions had more importance than the degradation ones. Relating to the differences between wines, PEF wine presented higher total anthocyanic concentration than control during the first months of aging. However, these differences diminished progressively, and after 4 months of aging, they were always lower than 8%.

During aging, the content of flavan-3-ols declined linearly in both wines. The differences between the PEF and the control wines increased from 25% at the beginning of aging to 38% at the end. The decrease in free flavan-3-ols that took place simultaneously with the decrease of anthocyanins probably indicates that both compounds participated in the formation of new pigments that contributed to the maintenance of CI and to the addition of blue tones to the wine (*31*). In these polymerization and copigmentation reactions, the aging in barrels plays an important role. For example, phenolic aldheydes from oak wood get involved in the copigmentation reactions (*32*). Moreover, the wood characteristics, such as porosity or permeability, determine

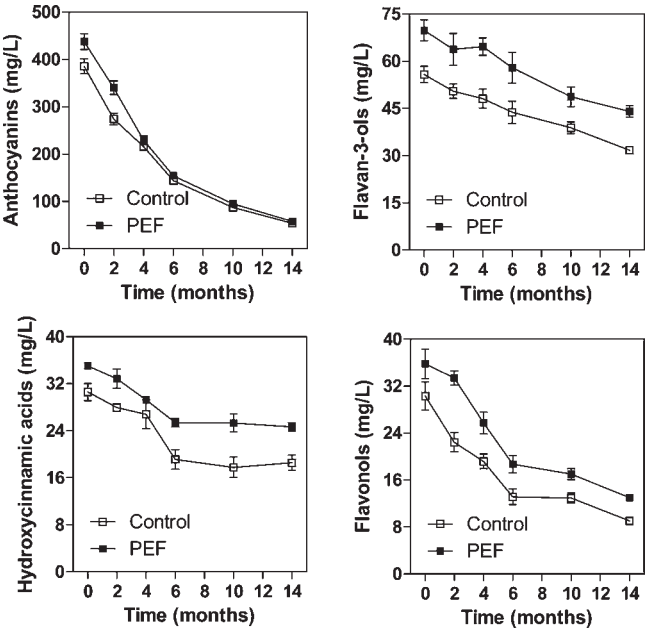

Figure 4. Evolution of the total content of anthocyanins, flavan-3-ols, hydroxycinnamic acids and flavonols of PEF wine (closed squares) and control wine (open squares) during 6 months of aging in American oak barrels and the subsequent storage in bottle.

the oxidative conditions that facilitate the development of these reactions.

In relation to the total hydroxycinnamic acids and derivatives, its concentration decreased during aging in barrels independently of the wine. However, after bottling the concentration of these compounds remained constant during the 8 months of storage in bottle. The evolution of flavonol concentration showed similar behavior, decreasing quickly during aging in barrels and then slightly diminishing during storage in bottle. Both phenolic families in conjunction with flavan-3-ols are involved in copigmentation reactions (*17*, *24*, *30*). For both hydroxycinnamic acids and flavonols, PEF wine presented higher concentration than the control one in the course of aging time. For example, after the 14 months of aging, PEF wine showed concentrations of hydroxycinnamic acids and flavonols 33% and 44% higher than the control wine, respectively.

Evolution of Individual Phenolic Compounds. Table 1 shows the concentration of individual anthocyanins, flavan-3-ols, flavonols and hydroxycinnamic acids and derivatives throughout aging in both PEF and control wine. In all cases, the main monomeric anthocyanins detected were malvidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-acetylglucoside + peonidin-3-acetylglucoside and malvidin-3-coumaroylglucoside. For both wines, the relative proportions of unacylated, acylated and coumarylated forms remained constant around 64, 29 and 7%, respectively, during aging in oak barrels. After 8 months of aging in bottle, the relative proportions slightly varied. The unacylated anthocyanins increased from 64% to 69%, the acylated forms decreased from 29% to 24% and the coumarylated forms remained constant. Similar behaviors have been described previously in the literature for Cabernet Sauvignon grape variety (*17*, *24*). In spite of the differences on the individual monomeric concentrations, no evidence of a particular effect of the PEF treatment on a specific anthocyanic compound was detected. This effect has been previously observed for other enological techniques to increase the phenolic extraction such as the use of macerating enzymes (*33*).

Table 1. Evolution of Individual Phenolic Compounds (mg/L) in PEF and Control Wines during 6 Months of Aging in American Oak Barrels and the Subsequent Storage in Bottle[a]

	control wine						PEF wine					
	0 months	2 months	4 months	6 months	10 months	14 months	0 months	2 months	4 months	6 months	10 months	14 months
Anthocyanins												
delphinidin-3G	14.15 ± 1.16	10.23 ± 0.74	8.07 ± 0.96	5.30 ± 0.35	3.73 ± 0.14	2.15 ± 0.20	18.74 ± 0.91	15.14 ± 0.50	8.71 ± 0.34	5.99 ± 0.24	3.68 ± 0.15	2.35 ± 0.14
cyanidin-3G	0.69 ± 0.15	1.02 ± 0.46	0.92 ± 0.39	0.87 ± 0.15	1.11 ± 0.62	0.51 ± 0.09	1.97 ± 0.15	1.31 ± 0.45	0.69 ± 0.12	0.61 ± 0.9	0.42 ± 0.05	0.34 ± 0.09
petunidin-3G	17.69 ± 1.16	13.38 ± 1.29	10.76 ± 0.58	7.75 ± 0.67	5.21 ± 1.09	3.17 ± 0.65	21.02 ± 1.48	16.22 ± 0.72	11.23 ± 0.65	7.45 ± 0.28	6.12 ± 0.99	3.34 ± 0.35
peonidin-3G	6.12 ± 1.25	4.64 ± 0.66	3.91 ± 0.40	2.94 ± 0.09	2.16 ± 0.59	1.29 ± 0.28	7.87 ± 1.10	6.47 ± 0.75	4.77 ± 0.43	3.23 ± 0.35	1.87 ± 0.08	1.39 ± 0.04
malvidin-3G	204.95 ± 7.44	148.58 ± 3.23	114.96 ± 2.23	76.66 ± 0.98	44.13 ± 0.78	29.39 ± 1.35	221.26 ± 4.06	174.34 ± 3.32	119.28 ± 1.65	83.62 ± 1.45	55.21 ± 1.99	32.02 ± 1.19
delphinidin-3G-Ac	4.57 ± 0.68	3.18 ± 0.21	2.62 ± 0.31	1.58 ± 0.12	0.88 ± 0.09	0.51 ± 0.06	5.75 ± 0.76	5.23 ± 0.56	2.78 ± 0.23	1.66 ± 0.34	0.99 ± 0.11	1.06 ± 0.30
cyanidin-3G-Ac	1.14 ± 0.09	1.01 ± 0.19	1.17 ± 0.17	0.83 ± 0.59	0.51 ± 0.07	0.37 ± 0.02	3.10 ± 0.18	2.18 ± 0.40	1.75 ± 0.48	1.26 ± 0.15	0.87 ± 0.42	0.48 ± 0.14
petunidin-3G-Ac	7.08 ± 0.12	3.88 ± 0.21	3.73 ± 0.15	2.05 ± 0.28	0.84 ± 0.03	0.58 ± 0.08	7.19 ± 0.87	6.04 ± 0.76	4.14 ± 0.85	2.39 ± 0.69	1.92 ± 0.57	0.79 ± 0.05
malvidin-3G-Ac + peonidin-3G-Ac	99.73 ± 1.98	69.86 ± 3.45	54.97 ± 1.29	35.66 ± 1.82	23.22 ± 0.84	12.33 ± 0.25	116.44 ± 1.76	89.19 ± 1.23	59.99 ± 1.01	37.79 ± 0.48	17.06 ± 0.96	10.69 ± 0.64
delphinidin-3G-Cm	5.49 ± 1.02	3.43 ± 0.19	2.82 ± 0.21	1.82 ± 0.26	0.99 ± 0.32	0.60 ± 0.15	5.54 ± 0.29	3.99 ± 0.58	2.66 ± 0.32	1.99 ± 0.13	1.20 ± 0.09	0.73 ± 0.06
cyanidin-3G-Cm	0.93 ± 0.09	0.63 ± 0.52	0.56 ± 0.08	0.56 ± 0.17	0.41 ± 0.09	0.35 ± 0.05	1.16 ± 0.09	0.87 ± 0.08	0.66 ± 0.09	0.59 ± 0.6	0.66 ± 0.14	0.60 ± 0.26
petunidin-3G-Cm	0.96 ± 0.10	0.72 ± 0.07	0.70 ± 0.09	0.60 ± 0.07	0.52 ± 0.05	0.45 ± 0.04	1.85 ± 0.07	1.37 ± 0.06	1.21 ± 0.09	0.81 ± 0.10	0.85 ± 0.04	0.64 ± 0.13
peonidin-3G-Cm	1.52 ± 0.08	1.06 ± 0.09	0.96 ± 0.10	0.87 ± 0.03	0.67 ± 0.02	0.63 ± 0.12	1.92 ± 0.10	1.62 ± 0.10	1.29 ± 0.10	0.76 ± 0.08	0.77 ± 0.07	0.68 ± 0.02
malvidin-3G-Cm	19.71 ± 1.01	12.05 ± 0.53	9.27 ± 0.73	5.72 ± 0.29	2.49 ± 0.08	1.36 ± 0.26	23.96 ± 1.01	16.35 ± 0.89	10.10 ± 0.72	6.28 ± 0.31	3.52 ± 0.09	1.82 ± 0.06
Hydroxycinnamic Acids												
t-caftaric acid	22.38 ± 2.59	20.38 ± 0.71	18.50 ± 3.23	11.34 ± 1.67	7.24 ± 0.88	6.51 ± 0.88	26.34 ± 0.65	24.11 ± 1.78	19.54 ± 0.71	15.66 ± 0.65	11.97 ± 0.65	9.64 ± 0.19
t-coutaric acid	7.39 ± 0.78	6.76 ± 0.36	6.38 ± 0.98	3.71 ± 0.87	2.34 ± 0.44	1.97 ± 0.38	7.89 ± 0.34	7.72 ± 0.56	7.31 ± 0.22	4.64 ± 0.12	3.66 ± 0.21	2.97 ± 0.15
caffeic acid	0.53 ± 0.10	0.55 ± 0.02	1.57 ± 0.27	3.27 ± 0.72	6.80 ± 1.12	8.46 ± 0.78	0.45 ± 0.02	0.57 ± 0.12	1.87 ± 0.12	4.07 ± 0.34	7.84 ± 0.78	10.12 ± 0.62
p-coumaric acid	0.27 ± 0.13	0.24 ± 0.02	0.33 ± 0.29	0.77 ± 0.34	1.38 ± 0.42	1.61 ± 0.23	0.32 ± 0.02	0.45 ± 0.08	0.53 ± 0.05	1.01 ± 0.23	1.84 ± 0.24	1.91 ± 0.14
Flavonols												
myricetin-3G	12.69 ± 3.21	9.89 ± 1.82	7.92 ± 1.01	4.68 ± 0.98	4.29 ± 0.45	2.32 ± 0.32	14.70 ± 3.76	12.92 ± 1.04	8.75 ± 1.35	5.87 ± 0.57	4.23 ± 0.34	2.61 ± 0.23
isorhamnetin-3G	3.81 ± 0.65	3.14 ± 0.32	2.99 ± 0.23	2.19 ± 0.33	2.31 ± 0.12	1.70 ± 0.11	4.42 ± 0.56	4.54 ± 0.32	3.93 ± 0.23	2.86 ± 0.21	2.86 ± 0.19	2.34 ± 0.08
quercetin-3G	11.68 ± 2.63	8.84 ± 1.08	8.28 ± 0.78	6.28 ± 0.89	6.53 ± 0.34	5.01 ± 0.24	14.26 ± 3.10	14.84 ± 0.65	12.24 ± 1.65	9.15 ± 1.23	9.31 ± 0.45	8.07 ± 0.31
myricetin	1.43 ± 0.32	0.57 ± 0.08	ND	ND	ND	ND	1.78 ± 0.22	1.09 ± 0.09	0.82 ± 0.09	0.74 ± 0.08	0.60 ± 0.06	ND
quercetin	0.69 ± 0.12	ND	ND	ND	ND	ND	0.65 ± 0.13	ND	ND	ND	ND	ND
Flavan-3-ols												
(+)-catechin	33.67 ± 4.76	27.68 ± 3.12	27.16 ± 5.53	24.50 ± 4.97	20.19 ± 4354	16.20 ± 1.98	41.89 ± 5.01	38.57 ± 6.89	41.11 ± 4.23	34.33 ± 4.87	27.21 ± 3.57	23.47 ± 2.09
(−)-epicatechin	22.12 ± 2.84	22.79 ± 1.78	20.95 ± 2.23	19.26 ± 3.41	18.69 ± 1.81	15.54 ± 1.10	27.96 ± 2.31	25.19 ± 3.78	23.54 ± 2.12	23.59 ± 2.22	21.51 ± 2.09	20.59 ± 1.43

[a] ND: not detected. G: glucoside. Ac: acylated. Cm: coumarylated.

Concerning flavan-3-ols, (+)-catechin and (−)-epicatechin concentrations were determined. In both wines, the concentration of (+)-catechin was always higher than the concentration of (−)-epicatechin. However, the relative proportion of these compounds changed during aging. In both wines, while (+)-catechin proportion decreased from 60% to 52%, the (−)-epicatechin proportion increased from 40% to 48%.

The evolution in the concentration of the main hydroxycinnamic acids, caffeic acid and *p*-coumaric acid, and their respective tartaric derivatives, *t*-caftaric acid and *t*-coutaric acid, was also studied in this investigation. Changes in the concentrations of these compounds were similar in both wines. At the same time as the content of *t*-caftaric and *t*-coutaric acids decreased, the content of caffeic and *p*-coumaric acids increased, mainly after the first 4 months of aging in barrels. These increments of the free acid forms have been attributed to the hydrolysis of the corresponding tartaric esters and other polymeric compounds, such as coumarylated anthocyanins (*25*). In both wines, *t*-caftaric acid was initially the main hydroxycinnamic acid presented. However after the 6 months of aging in barrels and the consequent 8 months of storage in bottle, the concentration of caffeic acid was the highest.

Finally, changes on individual flavonols (myricetin-3-glucoside, isorhamnetin-3-glucoside, quercetin-3-glucoside, myricetin and quercetin) have been also studied. In spite of their low concentration, these compounds play an important role in the stabilization of wine due to their participation in the copigmentation reactions (*28*). As occurred with the rest of phenolic families, PEF wine presented a higher concentration of individual flavonols than control, and no specific effect on a determined compound was detected. The glucoside forms were the predominant flavonols present in the wines. The concentration of all flavonols studied decreased during aging. This reduction was especially marked on the myricetin-3-glucoside content. Although this compound was the main flavonol detected at the beginning, during aging its concentration decreased faster than the concentration of quercetin-3-glucoside. For this reason, quercetin-3-glucoside resulted as the most detected one after 2 months and 4 months of aging in PEF and control wines, respectively.

Sensory Analysis. After 8 months of storage in bottle, PEF and control wines were subjected to a discriminative analysis by triangle tests, in order to determine if there were sensorial differences between wines on their color and bouquet. In spite of the fact that the ΔE^* value (10.6 CIELAB units) was higher than the theoretical limit of perception for the human eye, judges did not detect any differences between wines ($p > 0.05$). This lack of sensorial appreciable differences could be explained due to the high CI of wines, 20.08 and 26.56 for control and PEF wines respectively. Neither were significant differences in bouquet ($p > 0.05$) detected by judges, indicating that barrel aging affected similarly the wine aroma of control and PEF wine.

Feasibility of PEF Technology. In this investigation, it has been demonstrated that the application of a PEF treatment of an electric field strength of 5 kV/cm and a specific energy of 3.67 kJ/kg to the grapes of Cabernet Sauvignon increased the wine phenolic content, and this effect remained after aging in barrel. The cost of this treatment, using a current price of 0.1 €/(kW h), could be situated around 0.1 €/ton (*34*). Due to this low treatment cost, actually the major concern for implementation of PEF technology in the food industry is the generation of high voltage pulses with sufficient peak power for processing large quantities of product. Currently, pilot-plant scale equipments for permeabilization purposes are available in different laboratories, and prototype equipment at industrial size is being developed. The cost of this kind of generators is situated around 100−200 k€. In this paper, the possibility of applying PEF treatments at a continuous flow of 118 kg/h has been demonstrated. Preliminary studies conducted in our lab with the same generator have shown its capability to apply PEF treatments of intensity up to 4.3 kV/cm to the grape mass at flow rates near to the wineries' requirements (1.6 ton/h).

Therefore, major hurdles for the application of PEF in the wineries for improving phenolic extraction have been overcome. However, it is necessary to carry out studies at wineries to confirm the results obtained at pilot-plant scale.

ACKNOWLEDGMENT

Authors appreciatively acknowledge Frank Hall for the review of the English language and Purificación Hernández for the collaboration on the sensory analysis.

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Received for review November 17, 2009. Revised manuscript received January 12, 2010. Accepted January 12, 2010. E.P. and G.S. gratefully acknowledge the financial support for their doctoral studies from the "Ministerio de Ciencia e Innovación".