Elsevier Editorial System(tm) for Food Chemistry

Manuscript Draft

Manuscript Number:

Title: Pulsed Electric Fields processing of white grapes cv. Garganega: effects on wine composition and volatile compounds

Article Type: Research Article (max 7,500 words)

Keywords: PEF; grape processing; white winemaking; extraction; varietal aroma

Corresponding Author: Dr. Piergiorgio Comuzzo, Ph.D.

Corresponding Author's Institution: Università degli Studi di Udine

First Author: Piergiorgio Comuzzo, Ph.D.

Order of Authors: Piergiorgio Comuzzo, Ph.D.; Marco Marconi; Gianmaria Zanella; Marco Querzè

Abstract: Pulsed electric fields (PEF) processing of grapes after crushing was studied on pilot-plant scale on the white cv. Garganega. The effects on must and wine composition, the modifications induced on wine color and predisposition to browning, the impact on wine aroma compounds and the extraction of aroma precursors from grapes were investigated. PEF pre-treatment of grapes did not change must and wine basic composition, neither it was able to modify the behavior of alcoholic fermentation. Contrary, PEF determined an increase of total dry extract, wine color and total phenolics. A treatment corresponding to a total specific energy of 22 kJ kg-1 allowed a more intense extraction of varietal aroma precursors, without provoking excessive color evolution and extraction of phenolic compounds, apparently increasing the stability of the wine towards oxidations. Due to the few papers available on this subject, PEF applications on white grapes should be optimized in further experiments.

Cover Letter

Pulsed Electric Fields processing (PEF) is an emerging technology, with several promising applications in food industry. In winemaking sector, PEF has been applied mainly on red varieties, with the purpose of increasing the extraction of color and phenolic compounds from the grapes. In the current research, PEF was tested on white grapes from the Italian variety Garganega, after crushing/destemming. As far as we know, this is one of the few papers reporting data on the use of this technology during white wine processing. Moreover, in the few publications available on the application of PEF to white cultivars, the effects of the treatment were characterized mainly by reporting simple analytical parameters, such as spectrophotometric measurements or turbidity. In this research, the effects of PEF processing on wine volatile composition and the ability of such technology to promote the release of varietal aroma precursors from the grapes have been also investigated, in addition to the other conventional parameters. To the best of our knowledge, these aspects have not been investigated yet, in the studies published since now about PEF technology in winemaking sector. For this reason, we think that this paper can give a significant contribution to the current knowledge about PEF application in wine industry.

Pulsed Electric Fields processing of white grapes cv. Garganega: effects on wine composition and volatile compounds

Piergiorgio Comuzzo [1]*, Marco Marconi [2], Gianmaria Zanella [3] and Marco Querzè [4]

[1] Università degli Studi di Udine – Dipartimento di Scienze Agroalimentari, Ambientali e Animali (Di4A), via Sondrio 2/A, 33100, Udine, Italy

[2] Juclas S.r.l. – Vason Group, via Mirandola 49/A, 37026, Settimo di Pescantina (VR), Italy

[3] Enologica Vason S.p.A. – Vason Group, via Nassar 37, 37029, San Pietro in Cariano (VR), Italy

[4] Alintel S.r.l., via Mascarino 12/N, 40066, Pieve di Cento (BO), Italy

* Corresponding Author

Piergiorgio Comuzzo

Tel: + 39 0432 55 8166

Fax: + 39 0432 55 8130

e-mail: piergiorgio.comuzzo@uniud.it

Abbreviated running title:

Pulsed Electric Fields on white grapes and effects on wine composition

Abstract

Pulsed electric fields (PEF) processing of grapes after crushing was studied on pilot-plant scale on the white cv. Garganega. The effects on must and wine composition, the modifications induced on wine color and predisposition to browning, the impact on wine aroma compounds and the extraction of aroma precursors from grapes were investigated. PEF pre-treatment of grapes did not change must and wine basic composition, neither it was able to modify the behavior of alcoholic fermentation. Contrary, PEF determined an increase of total dry extract, wine color and total phenolics. A treatment corresponding to a total specific energy of 22 kJ kg^{-1} allowed a more intense extraction of varietal aroma precursors, without provoking excessive color evolution and extraction of phenolic compounds, apparently increasing the stability of the wine towards oxidations. Due to the few papers available on this subject, PEF applications on white grapes should be optimized in further experiments.

KEYWORDS: PEF; grape processing; white winemaking; extraction; varietal aroma

1 Introduction

Pulsed Electric Fields (PEF) is a recent technological opportunity for food processing and preservation, based on the application of short pulses of high-voltage current to food products. The typical electric field intensity of a PEF treatment ranges from 10 to 80 kV cm^{-1}, with a pulse duration of micro to milliseconds (Maged & Amer Eissa, 2012).

When a high-voltage current is applied to food products, this may induce structural modifications of certain cell membrane constituents, such as some carrier proteins and phospholipid bilayers (Tsong, 1991). The dielectric polarization of phospholipids and their re-orientation, promoted by the electric field applied, provoke the formation of hydrophilic pores in the membrane itself (Tsong, 1991). This phenomenon is described as dielectric breakdown (Zimmermann, Pilwat & Riemann, 1974), electroporation (Tsong, 1991) or electropermeabilization (Teissie, Golzio & Rols, 2005), and may be reversible or irreversible, depending on the intensity of the electric field applied (Maged & Amer Eissa, 2012; Vega-Mercado, Góngora-Nieto, Barbosa-Cánovas & Swanson, 2007). This results in an increased permeability of the membrane itself to small molecules (Ortega-Rivas & Salmerón-Ochoa, 2014), swelling and cell breakdown (Vega-Mercado et al., 2007).

PEF technology has been introduced in food processing as a non-thermal treatment for the inactivation of microorganisms (Ortega-Rivas & Salmerón-Ochoa, 2014), with the purpose of achieving a better preservation of food color, texture, flavor and nutritional value, with respect to the traditional thermal processing methods (Barbosa-Cánovas, Góngora-Nieto, Pothakamury & Swanson, 1999; Maged & Amer Eissa, 2012). However, electroporation was also suggested for the extraction of bioactive compounds from plant materials (Vorobiev & Lebovka, 2012; Azmir et al., 2013), as well as for increasing the extraction yield during the processing of fruit juices (Schilling et al., 2007; Vorobiev & Lebovka, 2012), opening new perspectives for the use of PEF technology in food industry.

The interest of winemaking sector towards PEF is quite recent. PEF processing of grapes and wine is currently not included among the practices recommended by the International Organization of Vine and Wine (OIV) and for this reason, in Europe, the use of PEF is not allowed at winery scale (Regulation EC No 606, 2009).

Apart from some experiments related to the use of this technology for the microbiological stabilization of must and wine (Puértolas, López, Condón, Raso & Álvarez, 2009), the most of the papers published about PEF in winemaking, focus on the extraction of color and phenolic compounds from red grapes (López, Puértolas, Condón, Álvarez & Raso, J., 2008a; López, Puértolas, Condón, Álvarez & Raso, J., 2008b; Puértolas, López, Condón, Álvarez & Raso, 2010a; Puértolas, Hernández-Orte, Saldaña, Álvarez & Raso, 2010b; Puértolas, Saldaña, Álvarez & Raso, 2010c; Donsì, Ferrari, Fruilo & Pataro, 2011; El Darra, Grimi, Louka, Maroun & Vorobiev, 2012a; El Darra, Grimi, Maroun, Louka & Vorobiev, 2012b; Delsart et al., 2014). Recently, PEF was also found to accelerate the release of mannoproteins during yeast autolysis (Martínez, Cebrián, Álvarez & Raso, 2016). However, the use of this technology for the processing of white grape varieties and the effects on white wine composition were poorly investigated from the technological point of view and, to the best of our knowledge, there are currently very few publications dealing with these aspects (Praporscic, I., Lebovka, N., Vorobiev, E., & Mietton-Peuchot, M., 2007).

For this reason, the current work was aimed to investigate the application of PEF during the pilot-plant scale processing of white grapes from the variety Garganega, keeping into consideration the effects of the treatment on the concentration of varietal aroma precursors in the juice, the impact on the behavior of alcoholic fermentation, as well as the influence on wine color, total phenolics and volatile composition.

2 Materials and Methods

2.1 Reagents and materials

Sodium chloride, 30 % (w/w) hydrogen peroxide, 96 % (v/v) ethanol, ACS grade hydrochloric acid (37%), anhydrous sodium sulfate and citric acid were purchased from Carlo Erba Reagents (Milan, Italy). HPLC grade dichloromethane and *n*-pentane, HPLC grade methanol, ethyl heptanoate and 1-heptanol were from Sigma-Aldrich (St. Louis, MO, USA). Malt Extract Agar and bacteriological peptone were purchased from Oxoid (Basingstoke, UK). Milli Q grade water was produced by a Milli-Q Advantage A10 apparatus (Merck Millipore, Billerica, MA, USA). The active dry yeast strain (Flavor 2000), the pectolytic enzyme preparation (Flottozima® P), the yeast nutrient formulation (V-Starter Premium) and the potassium metabisulfite used for the vinification protocols were all supplied by Enologica Vason S.p.A. (S. Pietro in Cariano, VR, Italy). The glycosidase preparation (Rapidase Revelation Aroma) used for the determination of bound monoterpenes, was from Oenobrands SAS (Montpellier, France).

2.2 PEF treatments

Two hundred kg of Garganega grapes, harvested in the region of Valpolicella (Verona, Italy, harvest 2015), was supplied by a local winery, after destemming and crushing. The mash obtained was subjected to PEF processing on the pilot-plant described below.

PEF equipment consisted in a 8 kV, 30 A PEF generator (Model H.V.18kV_30A_Alintel Generator) and a 100 x 30 mm i.d. poly(methyl methacrylate) cylindrical cell provided with two toroidal stainless steel electrodes. Both the cell and the generator were supplied by Alintel S.r.l. (Pieve di Cento, BO, Itay). The mash was continuously pumped into the cell, by a single-screw volumetric pump (Model MXF30INCA, Liverani – Lugo, RA, Italy), at a flow rate of 200 l h^{-1}. PEF treatments were carried out, in three repetitions each, at an electric field strength of 1.5 kV cm^{-1}, with a duration of the single pulse of 0 μs (no pulse, Untreated), 8 μs (corresponding to a total specific energy of 11 kJ kg^{-1}) and 16 μs (corresponding to a total specific energy of 22 kJ kg^{-1}). For both the PEF treatments, PEF generator provided squared wave pulses, with a frequency of 600 Hz. Experiments were carried out at room temperature

(20 °C). The temperature increase of the mash, measured after the treatments, was lower than 5 °C for all the samples.

2.3 Winemaking protocols

After PEF processing, the mash (three repetitions for each treatment) was sulfited by the addition of 100 mg l^{-1} of potassium metabisulfite (corresponding approx. to 50 mg l^{-1} of sulfur dioxide) and immediately pressed with a water-press (Model W80, Grifo Marchetti, Piadena, CR, Italy). Pressing was standardized for all the samples, operating two pressing cycles, at a maximum pressure of 0.8 bar each.

The juice obtained was treated with 20 mg l^{-1} of pectolytic enzyme preparation and stored overnight at 8 °C for allowing static sedimentation. After racking, samples were supplemented with 200 mg l^{-1} of active dry yeasts, prepared on the basis of the supplier instructions, and 200 mg l^{-1} of yeast nutrient preparation. Alcoholic fermentation was carried out at 20 °C, monitoring daily the specific gravity of the fermenting must.

At the end of alcoholic fermentation, samples were racked in 0.75 l glass bottles, supplemented with 60 mg l^{-1} of potassium metabisulfite and sealed with crown cap closures. All the wines were stored at 20 °C until analysis.

2.4 Analytical determinations

2.4.1 Pressing yield

Juice extraction yield was evaluated as the percent ratio between the weight of the juice obtained and that of the mash before pressing, as suggested by Praporsic et al. (2007).

2.4.2 Microbiological analysis

In order to evaluate the effect of PEF treatment on the yeast populations naturally present on the crushed grapes, the mash was collected at the outlet of the PEF equipment, in 50 ml sterile

Falcon tubes. Samples were aseptically transferred in a stomacher bag and treated for 1 min in a Stomacher 400 homogenizer (Seward Ltd, Worthing, SXW, United Kingdom).

After homogenization, 1 ml of each sample was transferred in a 15 ml sterile tube and mixed with 9 ml of saline-peptone water (9 g l^{-1} sodium chloride and 1 g l^{-1} bacteriological peptone). After vortexing for 1.5 min in a VWR vortex mixer (International PBI, Milan, Italy), additional decimal dilutions were made in the same solution. The diluted samples were plated on Malt Extract Agar and incubated at 25 °C for 48-72 h, under aerobic conditions. Total yeast colonies were counted.

2.4.3 Alcoholic fermentation kinetics

The potential effects of PEF treatments on the fermentation kinetics was evaluated by measuring the behavior of the specific gravity of the samples, during fermentation itself. Measures were carried out daily, for the whole duration of alcoholic fermentation. Analyses were performed at 20 °C, by a DMA 4500 density-meter (Anton Paar, Graz, Austria). Samples (2 ml) were previously filtered on 0.45 μm nylon membranes (Albet-Hahnemühle, Barcelona, Spain), to eliminate the carbon dioxide dissolved.

2.4.4 FTIR analysis

Basic quality control parameters on musts and wines, were assessed by FTIR spectroscopy, by using a using a Winescan^{TM} FT-120 instrument (FOSS, Hillerød, Denmark); all the replicated samples were analyzed two times each, and the mean value of the two measurements was considered for data elaboration. For musts, the following parameters were considered: reducing sugars; pH; total acidity, malic acid, yeast assimilable nitrogen (YAN) and alcoholic strength. Wines were analyzed fifty days after the end of alcoholic fermentation; the data acquired were alcoholic strength, reducing sugars, total acidity, volatile acidity, pH, malic acid, lactic acid, tartaric acid, citric acid, total dry extract, glycerol, potassium, and ash.

2.4.5 Color and total phenolics

Wine color and Total Phenolic Index (TPI) were determined on the wines, fifty days after the end of alcoholic fermentation. Concerning color, analyses consisted in measuring the absorbance of the samples at 420 nm, in 10 mm optical path length quartz cuvettes (Hellma Analytics, Mülheim, Germany); readings were performed against distilled water. For TPI, the samples were previously diluted ten times with distilled water and absorbance was read at 280 nm in the same conditions. TPI was calculated multiplying by 10 the absorbance measured at 280 nm.

2.4.6 Browning assay

The predisposition of wines towards browning was determined by a modification of the POM-test, a browning assay previously described by Müller-Späth (1992). Five ml of wine were added up with 25 µl of a 3 % hydrogen peroxide solution and heated at 60 °C, for one hour. Browning was estimated as the percent increase of the absorbance at 420 nm. All analyses were carried out by a UV–vis spectrophotometer, model V-530 (Jasco Co. Ltd., Tokyo, Japan).

2.4.7 Aroma compounds

Aroma compounds were determined on the wines stored in bottles, fifty days after the end of alcoholic fermentation. Five ml of wine were mixed with 5 ml of a 30 % (w/v) sodium chloride solution and 200 µl of internal standard (ethyl heptanoate, 500 mg l^{-1} in 96 % v/v ethanol). The mixture was subjected to five extractions, with 2.5 ml of pentane: dichloromethane (2:1 v/v) each. The organic phase was collected in a Pyrex tube, dehydrated with anhydrous sodium sulfate and concentrated under nitrogen flow up to a final volume of about 1 ml. The samples obtained were subjected to GC-MS analysis, as detailed below.

2.4.8 Free and bound terpenes and norisoprenoids

The musts collected after pressing and prior to the addition of pectolytic enzymes, were analyzed to assess the effects of PEF processing on the release of free and bound terpenic molecules from the grapes. The procedure used was a modification of the method published by Dziadas & Jeleń (2010). An aliquot of juice was sampled after pressing and centrifuged at 3000 rpm for 10 min. Hundred ml of the limpid phase was added with 100 µl of internal standard (1-heptanol, 500 µg ml^{-1} in 96 % v/v ethanol) and loaded onto an Isolute® 500 mg, 6 ml, C18 SPE cartridge (Biotage, Uppsala, Sweden), previously conditioned with 25 ml of methanol and 25 ml of Milli Q grade water. Sample loading was followed by a washing step with 150 ml of Milli Q water. Free terpenes were then eluted with 25 ml of pentane: dichloromethane (2:1 v/v). The eluate was dehydrated with anhydrous sodium sulfate and stored at -20 °C until GC-MS analysis.

Bound terpenes were eluted from the same cartridge with 25 ml of HPLC grade methanol. The eluate was collected in conical tubes and evaporated in a vacuum centrifuge (Univapo 100 H - Uniequip, Planegg, Germany). The residue was resuspended in 5 ml of 0.2 M citrate buffer (pH 5.00) and added with 200 µl of glycosidase preparation (25 g l^{-1} in Milli Q grade water). The samples were stored at 40 °C for 20 hours, for allowing enzymatic hydrolysis, transferred in a 10 ml volumetric flask and supplemented with 100 µl of internal standard (1-heptanol). Bound terpenes and norisoprenoids were extracted five times with pentane: dichloromethane (2:1 v/v), by using the same procedure described in the Section 2.4.7. GC-MS analyses were carried out as follows.

2.4.9 GC-MS analyses

The system used for GC-MS analyses was a GC-17A gas chromatograph coupled with a QP-5000 mass spectrometer (both by Shimadzu, Kyoto, Japan). Volatile compounds were separated on a J&W DB-Wax capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) provided by Agilent Technologies Inc. (Santa Clara, CA, USA), under the following operating conditions: 40 °C for 1 min, then 4 °C min^{-1} up to 240 °C, held for 15 min. The injection (1 µl) was made in splitless mode, with a splitless time of 60 s. Injector and detector temperatures were both set at 240 °C. Carrier gas was helium at a linear flow rate of 35 cm s^{-1}. Electron impact mass spectra were recorded at 70 eV and volatile compounds were tentatively identified by comparison of their mass spectra and retention times with those of standard compounds, or by comparison of mass spectrum with those reported in the mass spectrum libraries Wiley 6 and NIST 107. Moreover, linear retention indices were calculated according to the retention times of *n*-alkanes, and compared with those reported in literature. Semi-quantitative analysis was based on the internal standard method, considering a response factor equal to 1.00.

2.5 Statistical analyses

Concerning chemical and microbiological analyses, the results were averages of three measurements obtained from three replicated experiments. One-way ANOVA was carried out on the values found for the different parameters analyzed. Means and standard deviations were calculated and significant differences were assessed by Tukey HSD Test at $p < 0.05$. All the elaborations were performed by the software Statistica for Windows (StatSoft, Tulsa, OK, USA), Version 8.0.

3 Results and Discussion

3.1 Effects of PEF processing on pressing yield and must composition

PEF treatment determined an appreciable increase of the pressing yield. The percent yield in juice for the Control sample (Untreated) was 78.0 % w/w (average value of the three repetitions analyzed). This value increased to 84.9 % w/w for the sample treated at 11 kJ kg^{-1} and to 81.4 % w/w for the one processed at 22 kJ kg^{-1}, with an average percent increase with respect to the yield of the Control of + 8.9 % and + 4.3 % respectively. These percentages are in agreement with those reviewed by Vorobiev & Lebovka (2012), who reported a 4 % increase of pressing yield (belt-press), after PEF processing of cider apple mash.

It is interesting to observe that the higher amount of juice recovered was obtained for the samples treated with the lowest specific energy (11 kJ kg^{-1}). In a lab-scale experiment, Praporscic and collegaues (Praporsic et al., 2007) observed an even higher increase of pressing yield (+ 24 %), operating with an electric field intensity of 0.75 kV cm^{-1}; PEF treatments were carried out for up to 30 trains of 100 pulses (100 µs each), in static conditions, corresponding to a total PEF time of 0.3 s. Based on these considerations, lower electric field intensities and specific energies during PEF processing, might represent a more suitable operating condition for achieving a higher juice extraction yield.

Praporscic et al. (2007) also observed that the PEF pre-treatment of the mash obtained from three grape varieties: Semillon, Sauvignon and Muscadelle, determined a decrease of must turbidity after pressing. Contrary, in the current experiment, a higher level of suspended solids was observed in PEF-processed juice and static sedimentation was more difficult in such musts than in the Untreated one. In particular, the higher was the specific energy applied, the greater was the amount of lees collected at the bottom of the containers, after static sedimentation (Supplementary Material, Fig. A). This different behavior with respect to literature results was probably due to the different operating conditions used in the two experiments, e.g. the characteristics of the grape variety, or the pressing machine used. In particular, in the winery practice, it is well known that different kind of machines and different levels of pressure applied may have a strong impact on the draining capacity of the cake formed during pressing and the turbidity of the juice obtained.

Concerning the effects of the treatments on must composition (Table 1), PEF processing did not affect neither the level of sugars in the juice, nor the YAN concentration. However, PEF provoked a slight variation of the acidic fraction. In particular, pH was significantly higher in the juice processed at 11 kJ kg^{-1}, with an average increase of + 0.08 units, with respect to the Untreated sample. This slight increase of the pH might be explained with a higher degree of salification of organic acids, due to an enhanced extraction of cations from the skins. The significant variations measured for malic acid and for titratable acidity are actually negligible from the practical point of view.

Finally, in the present experiment, none of the operating conditions tested, determined appreciable variations in the yeast populations counted in the mash after PEF treatments (Supplementary Material, Table A). A positive effect of PEF on the reduction of wild microorganisms in must and wine is reported in literature, but considerably higher specific energies (150-180 kJ kg^{-1}) are required for the inactivation of certain yeast or lactic acid bacteria strains (Luengo, Puértolas, López, Álvarez, & Raso, 2012).

3.2 Effects of PEF processing on fermentation behavior and wine composition

PEF treatments did not affect the kinetic of alcoholic fermentation. The behavior of specific gravity during fermentation itself (Fig. 1) was comparable for Untreated and PEF-processed samples. In all the cases, alcoholic fermentation was completed in seven days, with negligible levels of residual sugars (approx. 1 g l^{-1}). Basing on the values collected by FTIR analysis (Table 2), secondary or unwanted fermentations (e.g. malolactic) did not occur in the wines: malic and citric acid were preserved and volatile acidities were very low.

Concerning the differences among the wines obtained, the data reported in Table 2 confirms that wine basic quality control parameters were poorly affected by the PEF treatment of the mash. The small differences found for juice pH in Table 1, disappeared in the wines fifty days after the conclusion of alcoholic fermentation. The significant increase marked for glycerol content in the samples PEF 11 and PEF 22 is reasonably not relevant from the practical point of view, while slight variations due to PEF processing were found for total dry extract and, in minor amounts, for potassium and ash. Such variations are probably connected with the ability of PEF to increase the extraction of minerals from vegetal tissues (Gachovska, Ngadi,

& Raghavan, 2006) and phenolic compounds from grape skins (López et al, 2008a; López et al, 2008b; Puértolas et al., 2010a; Puértolas et al., 2010b; Puértolas et al., 2010c).

In effects, PEF was able to determine a more intense color and a higher level of total polyphenols in the wines analyzed (Table 3). Surprisingly, the lower was the specific energy, the more intense was the color development and the higher the TPI. This behavior is in opposition with the results published by Praporscic et al. (2007), who found that the PEF pre-treatment of the mash of three white grape varieties, led to an increased juice extraction yield, but to a lower color extraction. As mentioned in Section 3.1, the conditions of such experiment (0.75 kV cm^{-1}, for a total PEF time of 0.3 s) were different with respect to the present operating conditions. Moreover, according to Teissie and colleagues (Teissie et al., 2005), the mechanical stress induced by the electric field applied on biological membranes also depends on the composition of the medium, particularly for what concerns its ionic strength; for this reason, compositional aspects connected to varietal differences, might have played a significant role in determining the behaviors observed in the two experiments. In addition, the differences found might be ascribed also to the pressing method used. In fact, it is well known that different pressing machines can determine a different extraction of color and phenolic compounds, depending on the pressure applied.

What it is relevant in the current experiment is that the samples processed with the lowest specific energy are those for which the highest extraction yield was achieved (see Section 3.1), and those with the most intense color evolution (sample PEF 11, in Table 3). The size of the pores originated during the application of a PEF treatment depends on several factors, such as the intensity of the electric field applied (Zimmermann et al., 1974; Zimmermann, 1986) and the pulse duration (Saulis & Salulė, 2012). Probably, the lower specific energy transferred to the samples treated at 11 kJ kg^{-1} and the lower duration of the pulse, were able to promote mainly the release of water (higher pressing yield) and small phenolic molecules in the juice after pressing. Such small polyphenols might have been easily oxidized, provoking the intense browning measured in the wines after storage. Contrary, the presumably larger pore size originated by processing the mash at 22 kJ kg^{-1}, might have promoted the release of more complex and polymerized phenolic molecules, which might have contributed to achieve a greater stability of the phenolic fraction, potentially reducing the intensity of browning reactions. In fact, the reactivity of flavanols towards oxidation in aqueous phase (i.e. their antioxidant capacity) is reported to decrease with their complexity, e.g. from trimer to tetramer and with the glycosylation of the 3-hydroxyl group of the heterocycle (Plumb, De Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998). Despite PEF has been described as a technology able to inactivate polyphenol oxidase enzymes (e.g. tyrosinase), the conditions reported for such inactivation are greatly more intense in terms of electric field applied, with respect to those used in the current experiment (Yang, Li, & Zhang, 2004; Noci, Riener, Walkling-Ribeiro, Cronin, Morgan, & Lyng, 2008). For this reason, it seems unlikely that tyrosinase inactivation might be responsible for the lower color development in the samples processed at 22 kJ kg^{-1}.

Anyway, despite the reasons of such behaviors shall be further investigated in future experiments, the wines obtained by PEF processing with a total energy transfer of 22 kJ kg^{-1}, seemed to represent the best compromise between wine stability and the effects of PEF on the extraction of phenolic molecules. Such treatment led to a limited color development and a relatively small increment of total polyphenols in the wines, allowing the achievement of a potentially higher level of stability towards oxidations, as confirmed by the lower POM-test value detected for PEF 22 sample, with respect to the Untreated wine (Table 3).

3.3 Effect of PEF processing on wine aroma composition

Thirty-two volatile compounds were tentatively identified in the wines fifty days after the conclusion of alcoholic fermentation (Supplementary Material, Table B). Quantitative data are shown in Table 4. Alcohols, fatty acids, ethyl and acetic esters are the most represented compounds in terms of number.

Alcohols include compounds with both fermentative and pre-fermentative origin. Alcohols were poorly affected by PEF pre-treatment and the significant variations observed for 2-methyl-1-propanol, 1-hexanol and 2-phenylethanol seemed not relevant from the practical point of view. In the first two cases (2-methylpropanol and hexanol), the concentrations detected were lower than the odor threshold values reported for these two compounds in hydroalcoholic solution: 40 and 8 mg l^{-1} respectively (Guth, 1997). In the light of this, the slight increase determined for these two compounds in the wines obtained by PEF processing would seem to have a scarce potential impact on the sensory perception. The same considerations can be done regarding 2-phenylethanol. This alcohol is well known for its intense rose-like odor (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Phenylethanol slightly decreased in PEF-processed samples, but according to the concentrations reported for such alcohol in Table 4 and to the odor threshold reported for this compound (10 mg l^{-1} in hydroalcoholic solution – Guth, 1997), the variations found are poorly relevant concerning the sensory impact on wine.

The limited variations of the alcohols concentrations after PEF treatments might be connected with the scarce impact of this technology on the YAN levels detected on the juice (Table 1). In fact, as it is well known, fermentative alcohols are produced by the fermenting yeasts, starting from free amino acids, via the Ehrlich pathway (Ribéreau-Gayon et al., 2006).

It is interesting to observe that PEF pre-treatment did not significantly affect also the concentrations of C6 pre-fermentative alcohols. *Cis*- and *trans*-3-hexen-1-ol are both reported in Table 4. These compounds are characterized by green and herbaceous notes (Ribéreau-Gayon et al., 2006) and their presence in high concentration may compromise the sensory quality of the wine. The odor threshold value reported for the *cis*-isomer in wine-like solution is 400 µg l^{-1} (Guth, 1997); the concentrations reported for such alcohol in Table 4 remained below this value in all the samples analyzed, both for PEF-processed and Untreated wines.

PEF technology, in the conditions of the current experiment, seemed not able to determine appreciable increases of the concentrations of such compounds in the wines.

As discussed for alcohols, also the concentration of ethyl esters seemed poorly affected by PEF processing, except for ethyl 4-hydroxybutanoate and ethyl hexadecanoate. The concentration of the former decreased progressively as the specific energy of PEF treatments increased, while the latter was found in higher concentration in PEF 11 samples. Anyway, also in this case, the differences found among the samples seem not relevant from the practical point of view. The decrease detected for acetic esters in the wines obtained by PEF processing might be connected with the lower average concentration found for acetic acid in these samples, with respect to the Untreated wine.

Concerning fatty acids, significant variations were found only for octanoic, decanoic, butanoic and 3-methylbutanoic acids. Such volatiles were present in lower concentration in PEF-processed wines. Concerning the last three compounds, the concentrations reported in Table 4 are below the odor thresholds (15, 10 and 3 mg l^{-1} respectively) reported in wine-like solutions by Guth (1997). Contrary, the odor threshold of octanoic acid is reported to be 0.5 mg l^{-1}, and at high concentrations, it is connected with cheese-like, rancid and harsh off-flavors (Tao & Zhang, 2010). However, despite the opportunity to reduce the concentration of fatty acids by PEF application may appear an interesting perspective from the enological point of view, the diminutions observed in the current experiment seemed to be scarcely relevant in the practice. The reasons of such behavior remain unclear and the mechanism that lead to such diminution shall be further clarified in further experiments.

Besides fatty acids, also the concentration of some volatile phenols (4-vinylphenol and 4-vinylguaiacol) was significantly reduced by PEF processing. It is well known that the presence of such compounds in white wines comes from the enzymatic decarboxylation of cinnamic acids, operated by yeasts. Vinyl phenols are generally recognized as defects in wine, because of their carnation and pharmaceutical olfactory notes (Ribéreau-Gayon et al., 2006). What it is interesting in Table 4, concerning vinylphenols, is that the olfactory threshold of 4-vinylphenol and 4-vinylguaiacol is 180 µg l^{-1} (López, Aznar, Cacho, & Ferreira, 2002) and 40 µg l^{-1} (Guth, 1997; López et al., 2002), respectively. Vinyl-4-phenol is reported to the most unpleasant, with pharmaceutical and paint-like odor (Ribéreau-Gayon et al., 2006). PEF processing was able to decrease the concentration of such compound at a level which is below to the odor threshold reported, with a potential positive impact on the overall perception of the wines. The ability of PEF processing to potentially reduce the presence of vinylphenols in wine is probably connected with the reduction of the concentration of hydroxycinnamic acid precursors in the juice, by oxidation. In the case of the treatment at 11 kJ kg^{-1}, this hypothesis is supported by the significant color evolution observed for this set of samples (Table 3). Nevertheless, these findings need to be further investigated in the future.

No significant impact of PEF processing was found on diols and the other compounds reported in Table 4.

In the current experiment the effects of PEF processing on the release of varietal aroma precursors from the grapes was also investigated, analyzing the juice obtained after pressing and before the addition of pectolytic enzymes for fining. Fifteen terpenic and noisoprenoid molecules were tentatively identified in free or bound form in the juice analyzed ((Supplementary Material, Table C). Quantitative data for Untreated and PEF 22 samples are reported in Table 5. The most of the terpenols and norisprenoids were found in the juice in bound form. The most representative free terpenol is geraniol. PEF pre-treatment of the mash significantly increased the concentration of terpenic and norisoprenoid glycosides in the juice, for all the compounds analyzed. The most of them were detected at concentrations below the olfactory threshold (Garganega is not an aromatic variety), but in the case of geraniol, PEF processing allowed to reach a total concentration (free plus bound form) which is close to the odor threshold reported by Guth (1997) in wine-like solution (30 µg l^{-1}).

4 Conclusions

PEF technology is an interesting perspective for wine industry, not only for promoting the extraction of color and phenolic compounds from red grapes, but also for its application in white wine processing. The use of PEF on white varieties needs to be further optimized, due to the limited number of publications available in this field. Nevertheless, in suitable operating conditions, PEF pre-treatment of white grape mash after crushing, may allow a more intense extraction of varietal aroma precursors, without provoking an excessive extraction of phenolic compounds and with a limited impact on wine color and stability towards oxidations.

The most of the studies on PEF in winemaking were carried out on pilot-plant scale. In fact, as mentioned above (Section 1), the current European law does not allow the use of PEF technology at winery scale. Nevertheless, specific experimental protocols may be authorized by the single Member States, according to the rules and the procedures reported in the Regulation EC No 606 (2009). Due to the increasing interest of wine companies towards innovation and emerging technologies, the results achieved concerning PEF applications in winemaking shall be further investigated on pilot-plant, but the scale-up of such results with winery-scale experiments is a compulsory step for the eventual authorization of this technology in Europe.

References

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., & Omar, A.K.M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*, 426-436.

Barbosa-Cánovas, G.V., Góngora-Nieto, M.M., Pothakamury, U.R., & Swanson, B.G. (1999). *Preservation of foods with pulsed electric fields*. San Diego: Academic Press.

Commission Regulation (EC) No 606/2009 (2009). Laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. *Official Journal of the European Communities*, *L 193*, 1-59.

Delsart, C., Cholet, C., Ghidossi, R., Grimi, N., Gontier, E., Geny, L., Vorobiev, E., & 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*, 424-436.

Donsì, F., Ferrari, G., Fruilo, M., & Pataro, G. (2011). Pulsed Electric Fields – assisted vinification. *Procedia Food Science*, *1*, 780-785.

Dziadas, M., & Jeleń, H.H. (2010). Analysis of terpenes in white wines using SPE–SPME–GC/MS approach. *Analytica Chimica Acta*, *677*, 43-49.

El Darra, N., Grimi, N., Louka, N., Maroun, R.G., & Vorobiev, E. (2012a). Effects of pulsed electric field on prefermentative cold maceration of cabernet franc grapes. In *Proceeding of the International Conference Bio & Food Electrotechnologies – BFE 2012 Book of Full Papers* (pp. 121-124). 26-28 September 2012, Salerno, Italy.

El Darra, N., Grimi, N., Maroun, R.G., Louka, N., & Vorobiev, E. (2012b). Comparison of the ultrasound, pulsed electric field and thermal pretreatments prior to red fermentation. In *Proceedings of Proceeding of the International Conference Bio & Food Electrotechnologies – BFE 2012 Book of Full Papers* (pp. 93-96). 26-28 September 2012, Salerno, Italy.

Gachovska, T.K., Ngadi, M.O., & Raghavan, G.S.V. (2006). Pulsed electric field assisted juice extraction from alfalfa. *Canadian Biosystems Engineering*, *48*, 3.33-3.37.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*, *45*, 3027–3032.

López, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A*, *966*, 167-177.

López, N., Puértolas, E., Condón, S., Álvarez, I., & Raso, J. (2008a). 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*, 1099-1107.

López, N., Puértolas, E., Condón, S., Álvarez, I., & Raso, J. (2008b). Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. *Innovative Food Science and Emerging Technologies*, *9*, 477-482.

Luengo, E., Puértolas, E., López, N., Álvarez, I., & Raso, J. (2012). Potential applications of pulsed electric fields in wineries. *Stewart Postharvest Review*, *8*, 1-6.

Maged, E.A.M., & Amer Eissa, A.H. (2012). Pulsed electric fields for food processing technology. In A.H. Amer Eissa, *Structure and function of food engineering* (pp. 275-306). Rijeka: InTech Open Access. DOI: 10.5772/48678.

Martínez, J.M., Cebrián, G., Álvarez, I., & Raso, J. (2016). Release of Mannoproteins during *Saccharomyces cerevisiae* autolysis induced by pulsed electric field. *Frontiers in Microbiology*, *7*, 1-8.

Müller-Späth, H. (1992). Der POM-Test. *Deutscher Weinbau*, *23*, 1099-1100.

Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D.A., Morgan, D.J., & Lyng, J.G. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering*, *85*, 141-146.

Ortega-Rivas, E., & Salmerón-Ochoa, I. (2014). Nonthermal food processing alternatives and their effects on taste and flavor compounds of beverages. *Critical Reviews in Food Science and Nutrition*, *54*, 190-207.

Plumb, G.W., De Pascual-Teresa, S., Santos-Buelga, C., Cheynier, V., & Williamson, G. (1998). Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation galloylation and glycosylation. *Free Radical Research*, *29*, 351-358.

Praporscic, I., Lebovka, N., Vorobiev, E., & Mietton-Peuchot, M. (2007). Pulsed electric field enhanced expression and juice quality of white grapes. *Separation and Purification Technology, 52*, 520-526.

Puértolas, E., López, N., Condón, S., Raso, J., & Álvarez, I. (2009). Pulsed electric fields inactivation of wine spoilage yeast and bacteria. *International Journal of Food Microbiology*, *130*, 49-55.

Puértolas, E., López, N., Condón, S., Álvarez, I., & Raso, J. (2010a). Potential applications of PEF to improve red wine quality. *Trends in Food Science & Technology*, *21*, 247-255.

Puértolas, E., Hernández-Orte, P., Saldaña, G., Álvarez, I., & Raso, J. (2010b). Improvement of winemaking process using pulsed electric fields at pilot-plant scale. Evolution of chromatic parameters and phenolic content of Cabernet Sauvignon red wines. *Food Research International*, *43*, 761-766.

Puértolas, E., Saldaña, G., Álvarez, I., & Raso, J. (2010c). Effect of pulsed electric field processing of red grapes on wine chromatic and phenolic characteristics during aging in oak barrels. *Journal of Agricultural and Food Chemistry*, *58*, 2351-2357.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Handbook of Enology. The Chemistry of Wine Stabilization and Treatments. Vol. 2. New York: John Wiley & Sons.

Saulis, G., & Saule, R. (2012). Size of the pores created by an electric pulse: microsecond vs millisecond pulses. *Biochimica et Biophysica Acta*, *1818*, 3032–3039.

Schilling, S., Alber, T., Toepfl, S., Neidhart, S., Knorr, D., Schieber, A., & Carle, R. (2007). Effects of pulsed electric field treatment of apple mash on juice yield and quality attributes of apple juices. *Innovative Food Science and Emerging Technologies*, *8*, 127-134.

Tao, Y., & Zhang, L. (2010). Intensity prediction of typical aroma characters of cabernet sauvignon wine in Changli County (China). *LWT - Food Science and Technology, 43*, 1550-1556.

Teissie, J., Golzio, M., & Rols, M.P. (2005). Mechanisms of cell membrane electropermeabilization: A minireview of our present (lack of ?) knowledge. *Biochimica et Biophysica Acta*, *1724*, 270–280.

Tsong, T.Y. (1991). Electroporation of cell membranes. *Biophysical Journal*, *60*, 297-306.

Vega-Mercado, H., Góngora-Nieto, M.M., Barbosa-Cánovas, G.V., & Swanson, B.G. (2007). Pulsed electric fields in food preservation. In R.M. Shafiur, *Handbook of food preservation* (pp. 783-813). Boca Raton: CRC Press.

Vorobiev, E., & Lebovka, N.I. (2012). New and promising applications of pulsed electric field treatments in bio & food processing. In *Proceeding of the International Conference*

Bio & Food Electrotechnologies – BFE 2012, Book of Full Papers (pp. 88-92). 26-28 September 2012, Salerno, Italy.

Yang, R.-J., Li, S.-Q., & Zhang, Q.H. (2004). Effects of pulsed electric fields on the activity of enzymes in aqueous solution. *Journal of Food Science*, *69*, 241-248.

Zimmermann, U., Pilwat, G., & Riemann, F. (1974). Dielectric breakdown on cell membranes. *Biophysical Journal*, *14*, 881-889.

Zimmermann, U. (1986). Electrical breakdown, electropermeabilization and electrofusion. *Reviews of Physiology Biochemistry and Pharmacology*, *105*, 176-256.

Figure Captions

Fig. 1. Behavior of specific gravity (at 20 °C) during the alcoholic fermentation of Control (Untreated) and PEF-processed (PEF 11 and PEF 22) musts. Mean values of three repeated samples are reported; vertical bars represent standard deviations.

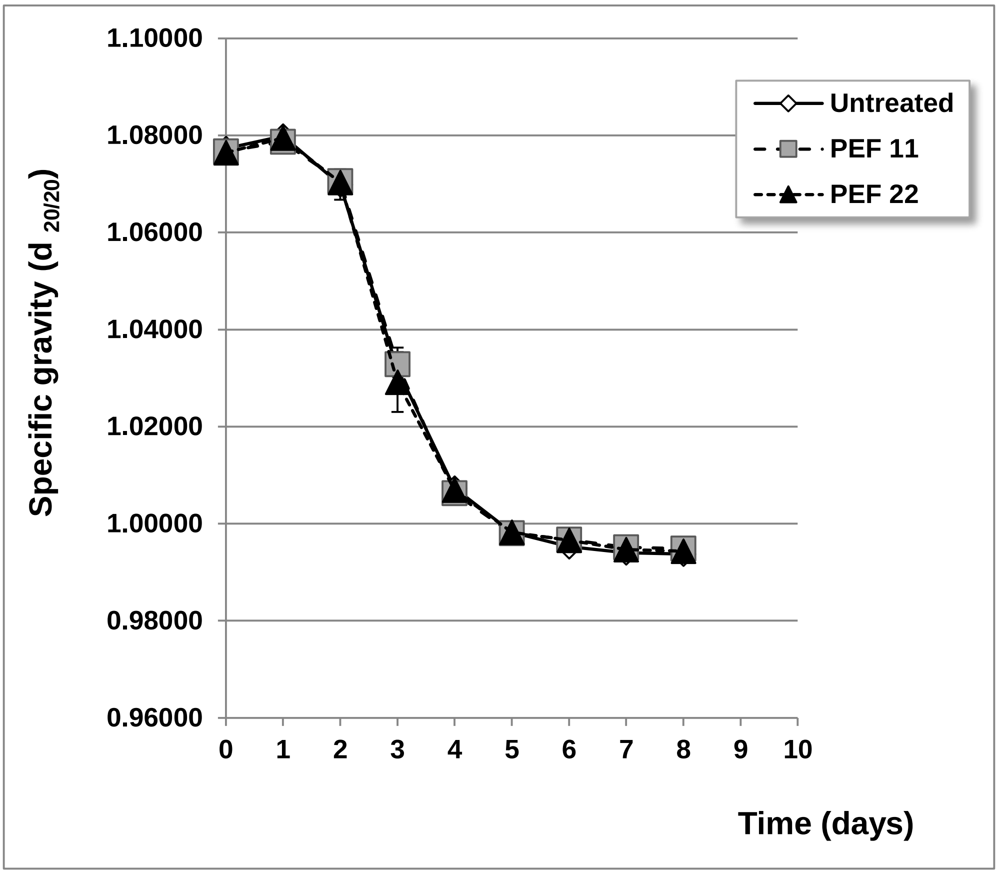
1.10000
1.08000
1.06000
1.04000
1.02000
1.00000
0.98000
0.96000
0
1
2
3
4
5
6
7
8
9
10
Specific gravity (d 20/20)
Time (days)
Untreated
PEF 11
PEF 22

Fig. 1

Table 1.

Analytical parameters (FTIR analysis) determined on Control (Untreated) and PEF-processed (PEF 11 and PEF 22) musts, after static sedimentation. Means and standard deviations (SD) of three repeated samples are reported. Different letters mark significant differences according to ANOVA and Tukey HSD Test, at $p < 0.05$

Sample	Reducing sugars ($g\ l^{-1}$)		pH		Total acidity ($g\ l^{-1}$)	
	Mean ± SD		Mean ± SD		Mean ± SD	
Untreated	182 ± 1	a	3.51 ± 0.01	a	5.01 ± 0.05	b
PEF 11	182 ± 2	a	3.59 ± 0.00	c	4.82 ± 0.03	a
PEF 22	179 ± 1	a	3.54 ± 0.01	b	4.95 ± 0.06	b

Sample	Malic acid ($g\ l^{-1}$)		YAN [a] ($mg\ l^{-1}$)		Alcoholic strength (% v/v)	
	Mean ± SD		Mean ± SD		Mean ± SD	
Untreated	2.24 ± 0.07	a	147 ± 6	a	0.12 ± 0.00	a
PEF 11	2.48 ± 0.06	b	155 ± 7	a	0.13 ± 0.00	a
PEF 22	2.39 ± 0.03	b	149 ± 6	a	0.12 ± 0.01	a

[a] Yeast Assimilable Nitrogen

Table 2.

Analytical parameters (FTIR analysis) determined on Control (Untreated) and PEF-processed (PEF 11 and PEF 22) wines, fifty days after the end of alcoholic fermentation. Means and standard deviations (SD) of three repeated samples are reported. Different letters mark significant differences according to ANOVA and Tukey HSD Test, at $p < 0.05$

Sample	Total acidity (g l^{-1}) Mean ± SD		Volatile acidity (g l^{-1}) Mean ± SD		pH Mean ± SD		Alcoholic strength (% v/v) Mean ± SD	
Untreated	5.08 ± 0.09	a	0.20 ± 0.03	a	3.47 ± 0.02	a	11.38 ± 0.03	b
PEF 11	5.13 ± 0.07	a	0.26 ± 0.00	b	3.49 ± 0.01	a	11.13 ± 0.03	a
PEF 22	5.11 ± 0.07	a	0.21 ± 0.01	a	3.50 ± 0.00	a	11.10 ± 0.03	a

Sample	Malic acid (g l^{-1}) Mean ± SD		Lactic acid (g l^{-1}) Mean ± SD		Tartaric acid (g l^{-1}) Mean ± SD		Citric acid (g l^{-1}) Mean ± SD	
Untreated	2.10 ± 0.01	a	0.17 ± 0.08	b	1.70 ± 0.14	a	0.34 ± 0.02	a
PEF 11	2.19 ± 0.02	a	0.00 ± 0.00	a	1.59 ± 0.01	a	0.35 ± 0.00	a
PEF 22	2.12 ± 0.00	a	0.18 ± 0.04	b	1.65 ± 0.02	a	0.36 ± 0.02	a

Sample	Total dry extract (g l^{-1}) Mean ± SD		Glycerol (g l^{-1}) Mean ± SD		Potassium (g l^{-1}) Mean ± SD		Ash (g l^{-1}) Mean ± SD	
Untreated	18.5 ± 0.0	a	5.91 ± 0.13	a	0.9 ± 0.0	a	2.2 ± 0.1	a
PEF 11	20.3 ± 0.1	c	6.27 ± 0.11	b	1.0 ± 0.0	b	2.4 ± 0.0	c
PEF 22	19.4 ± 0.1	b	6.20 ± 0.00	b	1.0 ± 0.0	b	2.3 ± 0.0	b

Table 3.

Wine color (Abs 420 nm), Total Phenolic Index (TPI) and POM-test value, determined on Control (Untreated) and PEF-processed (PEF 11 and PEF 22) wines, fifty days after the end of alcoholic fermentation. Means and standard deviations (SD) of three repeated samples are reported. Different letters mark significant differences according to ANOVA and Tukey HSD Test, at p <0.05

Sample	Abs 420 nm	TPI	POM-test
	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	0.146 ± 0.007 a	8.7 ± 0.2 a	24.6 ± 2.1 b
PEF 11	0.377 ± 0.003 c	20.3 ± 0.1 c	4.6 ± 0.2 a
PEF 22	0.261 ± 0.010 b	12.0 ± 0.1 b	8.3 ± 3.8 a

Table 4.

Concentrations (in µg l^{-1}) of volatile compounds, detected in Control (Untreated) and PEF-processed (PEF 11 and PEF 22) wines, fifty days after the end of alcoholic fermentation. Means and standard deviations (SD) of three repeated samples are reported. Different letters mark significant differences according to ANOVA and Tukey HSD Test, at $p < 0.05$

Compound	Untreated		PEF 11		PEF 22	
	Mean ± SD		Mean ± SD		Mean ± SD	
<u>*ethyl esters*</u>						
ethyl hexanoate	1025 ± 62	a	992 ± 29	a	1017 ± 67	a
ethyl octanoate	1927 ± 133	a	1671 ± 98	a	1706 ± 74	a
ethyl 3-hydroxybutanoate	283 ± 15	a	322 ± 23	a	298 ± 31	a
ethyl decanoate	1441 ± 390	a	1451 ± 547	a	1118 ± 148	a
ethyl 4-hydroxybutanoate	3271 ± 256	b	2701 ± 311	ab	2441 ± 154	a
ethyl hexadecanoate	283 ± 59	a	596 ± 66	b	349 ± 62	a
<u>*acetic esters*</u>						
3-methyl-1-butanol acetate	6041 ± 285	b	3926 ± 275	a	3730 ± 287	a
hexyl acetate	322 ± 14	b	204 ± 12	a	212 ± 27	a
2-phenethyl acetate	679 ± 64	b	366 ± 54	a	443 ± 64	a
<u>*other esters*</u>						
ethyl lactate	1521 ± 48	b	1284 ± 22	a	1334 ± 17	a
diethyl succinate	312 ± 17	a	279 ± 12	a	316 ± 72	a
<u>*alcohols*</u>						
2-methyl-1-propanol	19957 ± 1912	a	24856 ± 1671	b	22698 ± 1599	ab
2- and 3-methyl-1-butanol	206748 ± 2864	a	240285 ± 29686	a	200394 ± 3430	a
1-hexanol	1222 ± 106	a	1670 ± 39	c	1460 ± 15	b
trans-3-hexen-1-ol	135 ± 31	a	142 ± 12	a	131 ± 37	a
cis-3-hexen-1-ol	112 ± 18	a	120 ± 19	a	125 ± 7	a
2-ethyl-1-hexanol	140 ± 17	a	75 ± 48	a	109 ± 46	a
2-phenylethanol	47393 ± 3120	b	43394 ± 3581	ab	40004 ± 933	a
<u>*diols*</u>						
2,3-butanediol	7643 ± 2903	a	5977 ± 1250	a	5042 ± 763	a
1,3-butanediol	2247 ± 1059	a	1886 ± 687	a	1421 ± 299	a
1,2-propanediol	334 ± 210	a	318 ± 189	a	139 ± 15	a
<u>*organic acids*</u>						
acetic acid	4306 ± 941	a	2868 ± 1169	a	2851 ± 365	a
2-methylpropanoic acid	898 ± 30	a	868 ± 77	a	845 ± 29	a
butanoic acid	470 ± 15	b	400 ± 8	a	445 ± 28	ab
3-methylbutanoic acid	745 ± 14	b	656 ± 65	ab	605 ± 9	a
hexanoic acid	5621 ± 1146	a	5099 ± 1195	a	4666 ± 506	a
octanoic acid	11131 ± 1343	b	8368 ± 1041	a	9502 ± 697	ab
decanoic acid	3971 ± 586	b	2811 ± 423	a	3181 ± 317	ab
<u>*volatile phenols*</u>						
4-vinylguaiacol	202 ± 22	c	86 ± 6	a	137 ± 27	b
4-vinylphenol	210 ± 14	b	104 ± 33	a	112 ± 36	a
<u>*other compounds*</u>						
diidro-2(3*H*)-furanone (γ-butyrolactone)	634 ± 35	a	632 ± 25	a	657 ± 66	a
3-(methylthio)-1-propanol (methionol)	747 ± 73	a	918 ± 111	a	708 ± 83	a

Table 5.

Concentrations (in µg l^{-1}) of bound and free terpenes and norisoprenoids, detected in Control (Untreated) and PEF-processed (PEF 22) musts (sampling after pressing and before pectolytic enzyme treatment). Means and standard deviations (SD) of three repeated samples are reported. Different letters mark significant differences according to ANOVA and Tukey HSD Test, at $p < 0.05$

Bound

Sample	*cis*-linalool oxide (furan)	linalool	α-terpineol	geraniol	nerol
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	1,0 ± 0,2 **a**	3,6 ± 0,1 **a**	0,8 ± 0,1 **a**	21,0 ± 0,5 **a**	5,3 ± 0,5 **a**
PEF 22	1,1 ± 0,1 **a**	6,5 ± 0,1 **b**	1,1 ± 0,1 **b**	27,9 ± 0,7 **b**	7,5 ± 0,2 **b**

Sample	2,6-dimethyl-3,7-octadiene-2,6-diol	8-hydroxylinalool	geranic acid	3-hydroxy-β-damascone	tetrahydroionone
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	9,8 ± 0,1 **a**	29,5 ± 0,1 **a**	6,1 ± 1,2 **a**	9,5 ± 0,3 **a**	10,1 ± 0,6 **a**
PEF 22	9,5 ± 0,5 **a**	36,6 ± 0,1 **b**	8,7 ± 0,7 **b**	14,2 ± 0,7 **b**	12,7 ± 0,6 **b**

Sample	3-oxo-α-ionol	dihydro-β-ionone	3-oxo-7,8-dihydro-α-ionol (Blumenol C)	3-hydroxy-7,8-dihydro-β-ionol	3-oxo-retro-α-ionol
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	34,4 ± 0,3 **a**	5,4 ± 0,8 **a**	39,0 ± 0,5 **a**	7,6 ± 0,8 **a**	6,1 ± 0,0 **a**
PEF 22	43,8 ± 0,2 **b**	8,7 ± 1,6 **b**	52,0 ± 5,0 **b**	8,8 ± 0,9 **a**	8,8 ± 0,3 **b**

Continue

Table 5.

Free

Sample	*cis*-linalool oxide (furan)	linalool	α-terpineol	geraniol	nerol
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	*n.d.* [a]	2,4 ± 0,2 **a**	*n.d.*	8,6 ± 0,2 **a**	*n.d.*
PEF 22	*n.d.*	2,4 ± 0,0 **a**	*n.d.*	12,0 ± 1,2 **b**	*n.d.*

Sample	2,6-dimethyl-3,7-octadiene-2,6-diol	8-hydroxylinalool	geranic acid	3-hydroxy-β-damascone	tetrahydroionone
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
PEF 22	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*

Sample	3-oxo-α-ionol	dihydro-β-ionone	3-oxo-7,8-dihydro-α-ionol (Blumenol C)	3-hydroxy-7,8-dihydro-β-ionol	3-oxo-retro-α-ionol
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Untreated	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
PEF 22	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*

[a] *n.d.*: not detected

Supplementary Material

Click here to download Supplementary Material: Supplementary Material_Comuzzo et al.doc

Highlights

PEF pre-treatment of white grapes did not change basic composition of musts and wines

PEF pre-treatment of grapes did not modify the behavior of alcoholic fermentation

PEF increased the extraction of varietal aroma precursors from grapes

At 22 kJ kg^{-1} specific energy (SE), PEF gave a limited evolution of wine color

At 22 kJ kg^{-1} SE, PEF apparently increased the stability of wine towards oxidations