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Pulsed electric field processing of white grapes (cv. Garganega): Effects on wine composition and volatile compounds

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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<sup>-1</sup> 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.

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

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16

17 **Abbreviated running title:**

18 Pulsed Electric Fields on white grapes and effects on wine composition

19

20 **Abstract**

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

32

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

34

## 35 **1 Introduction**

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

40 When a high-voltage current is applied to food products, this may induce structural  
41 modifications of certain cell membrane constituents, such as some carrier proteins and  
42 phospholipid bilayers (Tsong, 1991). The dielectric polarization of phospholipids and their re-  
43 orientation, promoted by the electric field applied, provoke the formation of hydrophilic pores  
44 in the membrane itself (Tsong, 1991). This phenomenon is described as dielectric breakdown  
45 (Zimmermann, Pilwat & Riemann, 1974), electroporation (Tsong, 1991) or  
46 electropermeabilization (Teissie, Golzio & Rols, 2005), and may be reversible or irreversible,  
47 depending on the intensity of the electric field applied (Maged & Amer Eissa, 2012; Vega-  
48 Mercado, Góngora-Nieto, Barbosa-Cánovas & Swanson, 2007). This results in an increased  
49 permeability of the membrane itself to small molecules (Ortega-Rivas & Salmerón-Ochoa,  
50 2014), swelling and cell breakdown (Vega-Mercado et al., 2007).

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

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

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

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

## 83 **2 Materials and Methods**

### 84 *2.1 Reagents and materials*

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

## 97 *2.2 PEF treatments*

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

101 PEF equipment consisted in a 8 kV, 30 A PEF generator (Model H.V.18kV\_30A\_Alintel  
102 Generator) and a 100 x 30 mm i.d. poly(methyl methacrylate) cylindrical cell provided with  
103 two toroidal stainless steel electrodes. Both the cell and the generator were supplied by Alintel  
104 S.r.l. (Pieve di Cento, BO, Italy). The mash was continuously pumped into the cell, by a  
105 single-screw volumetric pump (Model MXF30INCA, Liverani – Lugo, RA, Italy), at a flow  
106 rate of 200 l h<sup>-1</sup>. PEF treatments were carried out, in three repetitions each, at an electric field  
107 strength of 1.5 kV cm<sup>-1</sup>, with a duration of the single pulse of 0 μs (no pulse, Untreated), 8 μs  
108 (corresponding to a total specific energy of 11 kJ kg<sup>-1</sup>) and 16 μs (corresponding to a total  
109 specific energy of 22 kJ kg<sup>-1</sup>). For both the PEF treatments, PEF generator provided squared  
110 wave pulses, with a frequency of 600 Hz. Experiments were carried out at room temperature



111 (20 °C). The temperature increase of the mash, measured after the treatments, was lower than  
112 5 °C for all the samples.

### 113 *2.3 Winemaking protocols*

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

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

124 At the end of alcoholic fermentation, samples were racked in 0.75 l glass bottles,  
125 supplemented with 60 mg l<sup>-1</sup> of potassium metabisulfite and sealed with crown cap closures.

126 All the wines were stored at 20 °C until analysis.

### 127 *2.4 Analytical determinations*

#### 128 *2.4.1 Pressing yield*

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

#### 131 *2.4.2 Microbiological analysis*

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

134 Falcon tubes. Samples were aseptically transferred in a stomacher bag and treated for 1 min in  
135 a Stomacher 400 homogenizer (Seward Ltd, Worthing, SXW, United Kingdom).  
136 After homogenization, 1 ml of each sample was transferred in a 15 ml sterile tube and mixed  
137 with 9 ml of saline-peptone water (9 g l<sup>-1</sup> sodium chloride and 1 g l<sup>-1</sup> bacteriological peptone).  
138 After vortexing for 1.5 min in a VWR vortex mixer (International PBI, Milan, Italy),  
139 additional decimal dilutions were made in the same solution. The diluted samples were plated  
140 on Malt Extract Agar and incubated at 25 °C for 48-72 h, under aerobic conditions. Total  
141 yeast colonies were counted.

#### 142 2.4.3 *Alcoholic fermentation kinetics*

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

#### 149 2.4.4 *FTIR analysis*

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

159 2.4.5 *Color and total phenolics*

160 Wine color and Total Phenolic Index (TPI) were determined on the wines, fifty days after the  
161 end of alcoholic fermentation. Concerning color, analyses consisted in measuring the  
162 absorbance of the samples at 420 nm, in 10 mm optical path length quartz cuvettes (Hellma  
163 Analytics, Mülheim, Germany); readings were performed against distilled water. For TPI, the  
164 samples were previously diluted ten times with distilled water and absorbance was read at 280  
165 nm in the same conditions. TPI was calculated multiplying by 10 the absorbance measured at  
166 280 nm.

167 2.4.6 *Browning assay*

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

174 2.4.7 *Aroma compounds*

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

#### 182 2.4.8 *Free and bound terpenes and norisoprenoids*

183 The musts collected after pressing and prior to the addition of pectolytic enzymes, were analyzed  
184 to assess the effects of PEF processing on the release of free and bound terpenic molecules from  
185 the grapes. The procedure used was a modification of the method published by Dziadas & Jeleń  
186 (2010). An aliquot of juice was sampled after pressing and centrifuged at 3000 rpm for 10 min.  
187 Hundred ml of the lipid phase was added with 100 µl of internal standard (1-heptanol, 500 µg  
188 ml<sup>-1</sup> in 96 % v/v ethanol) and loaded onto an Isolute<sup>®</sup> 500 mg, 6 ml, C18 SPE cartridge (Biotage,  
189 Uppsala, Sweden), previously conditioned with 25 ml of methanol and 25 ml of Milli Q grade  
190 water. Sample loading was followed by a washing step with 150 ml of Milli Q water. Free  
191 terpenes were then eluted with 25 ml of pentane: dichloromethane (2:1 v/v). The eluate was  
192 dehydrated with anhydrous sodium sulfate and stored at -20 °C until GC-MS analysis.

193 Bound terpenes were eluted from the same cartridge with 25 ml of HPLC grade methanol.  
194 The eluate was collected in conical tubes and evaporated in a vacuum centrifuge (Univapo  
195 100 H - Uniequip, Planegg, Germany). The residue was resuspended in 5 ml of 0.2 M citrate  
196 buffer (pH 5.00) and added with 200 µl of glycosidase preparation (25 g l<sup>-1</sup> in Milli Q grade  
197 water). The samples were stored at 40 °C for 20 hours, for allowing enzymatic hydrolysis,  
198 transferred in a 10 ml volumetric flask and supplemented with 100 µl of internal standard (1-  
199 heptanol). Bound terpenes and norisoprenoids were extracted five times with pentane:  
200 dichloromethane (2:1 v/v), by using the same procedure described in the Section 2.4.7. GC-  
201 MS analyses were carried out as follows.

#### 202 2.4.9 *GC-MS analyses*

203 The system used for GC-MS analyses was a GC-17A gas chromatograph coupled with a QP-  
204 5000 mass spectrometer (both by Shimadzu, Kyoto, Japan). Volatile compounds were  
205 separated on a J&W DB-Wax capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness)  
206 provided by Agilent Technologies Inc. (Santa Clara, CA, USA), under the following  
207 operating conditions: 40 °C for 1 min, then 4 °C min<sup>-1</sup> up to 240 °C, held for 15 min. The

208 injection (1  $\mu$ l) was made in splitless mode, with a splitless time of 60 s. Injector and detector  
209 temperatures were both set at 240 °C. Carrier gas was helium at a linear flow rate of 35 cm s<sup>-1</sup>.  
210 <sup>1</sup>. Electron impact mass spectra were recorded at 70 eV and volatile compounds were  
211 tentatively identified by comparison of their mass spectra and retention times with those of  
212 standard compounds, or by comparison of mass spectrum with those reported in the mass  
213 spectrum libraries Wiley 6 and NIST 107. Moreover, linear retention indices were calculated  
214 according to the retention times of *n*-alkanes, and compared with those reported in literature.  
215 Semi-quantitative analysis was based on the internal standard method, considering a response  
216 factor equal to 1.00.

### 217 *2.5 Statistical analyses*

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

## 224 **3 Results and Discussion**

### 225 *3.1 Effects of PEF processing on pressing yield and must composition*

226 PEF treatment determined an appreciable increase of the pressing yield. The percent yield in  
227 juice for the Control sample (Untreated) was 78.0 % w/w (average value of the three  
228 repetitions analyzed). This value increased to 84.9 % w/w for the sample treated at 11 kJ kg<sup>-1</sup>  
229 and to 81.4 % w/w for the one processed at 22 kJ kg<sup>-1</sup>, with an average percent increase with  
230 respect to the yield of the Control of + 8.9 % and + 4.3 % respectively. These percentages are

231 in agreement with those reviewed by Vorobiev & Lebovka (2012), who reported a 4 %  
232 increase of pressing yield (belt-press), after PEF processing of cider apple mash.

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

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

253 Concerning the effects of the treatments on must composition (Table 1), PEF processing did  
254 not affect neither the level of sugars in the juice, nor the YAN concentration. However, PEF  
255 provoked a slight variation of the acidic fraction. In particular, pH was significantly higher in  
256 the juice processed at  $11 \text{ kJ kg}^{-1}$ , with an average increase of + 0.08 units, with respect to

257 the Untreated sample. This slight increase of the pH might be explained with a higher degree  
258 of salification of organic acids, due to an enhanced extraction of cations from the skins. The  
259 significant variations measured for malic acid and for titratable acidity are actually negligible  
260 from the practical point of view.

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

### 267 *3.2 Effects of PEF processing on fermentation behavior and wine composition*

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

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

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

284 In effects, PEF was able to determine a more intense color and a higher level of total  
285 polyphenols in the wines analyzed (Table 3). Surprisingly, the lower was the specific energy,  
286 the more intense was the color development and the higher the TPI. This behavior is in  
287 opposition with the results published by Praporscic et al. (2007), who found that the PEF pre-  
288 treatment of the mash of three white grape varieties, led to an increased juice extraction yield,  
289 but to a lower color extraction. As mentioned in Section 3.1, the conditions of such  
290 experiment ( $0.75 \text{ kV cm}^{-1}$ , for a total PEF time of 0.3 s) were different with respect to the  
291 present operating conditions. Moreover, according to Teissie and colleagues (Teissie et al.,  
292 2005), the mechanical stress induced by the electric field applied on biological membranes  
293 also depends on the composition of the medium, particularly for what concerns its ionic  
294 strength; for this reason, compositional aspects connected to varietal differences, might have  
295 played a significant role in determining the behaviors observed in the two experiments. In  
296 addition, the differences found might be ascribed also to the pressing method used. In fact, it  
297 is well known that different pressing machines can determine a different extraction of color  
298 and phenolic compounds, depending on the pressure applied.

299 What it is relevant in the current experiment is that the samples processed with the lowest  
300 specific energy are those for which the highest extraction yield was achieved (see Section  
301 3.1), and those with the most intense color evolution (sample PEF 11, in Table 3). The size of  
302 the pores originated during the application of a PEF treatment depends on several factors,  
303 such as the intensity of the electric field applied (Zimmermann et al., 1974; Zimmermann,  
304 1986) and the pulse duration (Saulis & Salulè, 2012). Probably, the lower specific energy  
305 transferred to the samples treated at  $11 \text{ kJ kg}^{-1}$  and the lower duration of the pulse, were able  
306 to promote mainly the release of water (higher pressing yield) and small phenolic molecules  
307 in the juice after pressing. Such small polyphenols might have been easily oxidized,



308 provoking the intense browning measured in the wines after storage. Contrary, the  
309 presumably larger pore size originated by processing the mash at  $22 \text{ kJ kg}^{-1}$ , might have  
310 promoted the release of more complex and polymerized phenolic molecules, which might  
311 have contributed to achieve a greater stability of the phenolic fraction, potentially reducing  
312 the intensity of browning reactions. In fact, the reactivity of flavanols towards oxidation in  
313 aqueous phase (i.e. their antioxidant capacity) is reported to decrease with their complexity,  
314 e.g. from trimer to tetramer and with the glycosylation of the 3-hydroxyl group of the  
315 heterocycle (Plumb, De Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998).  
316 Despite PEF has been described as a technology able to inactivate polyphenol oxidase  
317 enzymes (e.g. tyrosinase), the conditions reported for such inactivation are greatly more  
318 intense in terms of electric field applied, with respect to those used in the current experiment  
319 (Yang, Li, & Zhang, 2004; Noci, Riener, Walkling-Ribeiro, Cronin, Morgan, & Lyng, 2008).  
320 For this reason, it seems unlikely that tyrosinase inactivation might be responsible for the  
321 lower color development in the samples processed at  $22 \text{ kJ kg}^{-1}$ .  
322 Anyway, despite the reasons of such behaviors shall be further investigated in future  
323 experiments, the wines obtained by PEF processing with a total energy transfer of  $22 \text{ kJ kg}^{-1}$ ,  
324 seemed to represent the best compromise between wine stability and the effects of PEF on the  
325 extraction of phenolic molecules. Such treatment led to a limited color development and a  
326 relatively small increment of total polyphenols in the wines, allowing the achievement of a  
327 potentially higher level of stability towards oxidations, as confirmed by the lower POM-test  
328 value detected for PEF 22 sample, with respect to the Untreated wine (Table 3).

### 329 *3.3 Effect of PEF processing on wine aroma composition*

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

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

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

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

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

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

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

380 Besides fatty acids, also the concentration of some volatile phenols (4-vinylphenol and 4-  
381 vinylguaiacol) was significantly reduced by PEF processing. It is well known that the  
382 presence of such compounds in white wines comes from the enzymatic decarboxylation of  
383 cinnamic acids, operated by yeasts. Vinyl phenols are generally recognized as defects in wine,  
384 because of their carnation and pharmaceutical olfactory notes (Ribéreau-Gayon et al.,

385 2006). What it is interesting in Table 4, concerning vinylphenols, is that the olfactory  
386 threshold of 4-vinylphenol and 4-vinylguaiacol is  $180 \mu\text{g l}^{-1}$  (López, Aznar, Cacho, &  
387 Ferreira, 2002) and  $40 \mu\text{g l}^{-1}$  (Guth, 1997; López et al., 2002), respectively. Vinyl-4-phenol is  
388 reported to the most unpleasant, with pharmaceutical and paint-like odor (Ribéreau-Gayon et  
389 al., 2006). PEF processing was able to decrease the concentration of such compound at a level  
390 which is below to the odor threshold reported, with a potential positive impact on the overall  
391 perception of the wines. The ability of PEF processing to potentially reduce the presence of  
392 vinylphenols in wine is probably connected with the reduction of the concentration of  
393 hydroxycinnamic acid precursors in the juice, by oxidation. In the case of the treatment at  $11$   
394  $\text{kJ kg}^{-1}$ , this hypothesis is supported by the significant color evolution observed for this set of  
395 samples (Table 3). Nevertheless, these findings need to be further investigated in the future.  
396 No significant impact of PEF processing was found on diols and the other compounds  
397 reported in Table 4.

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

#### 410 **4 Conclusions**

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

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

428

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533

## 535 **Figure Captions**

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

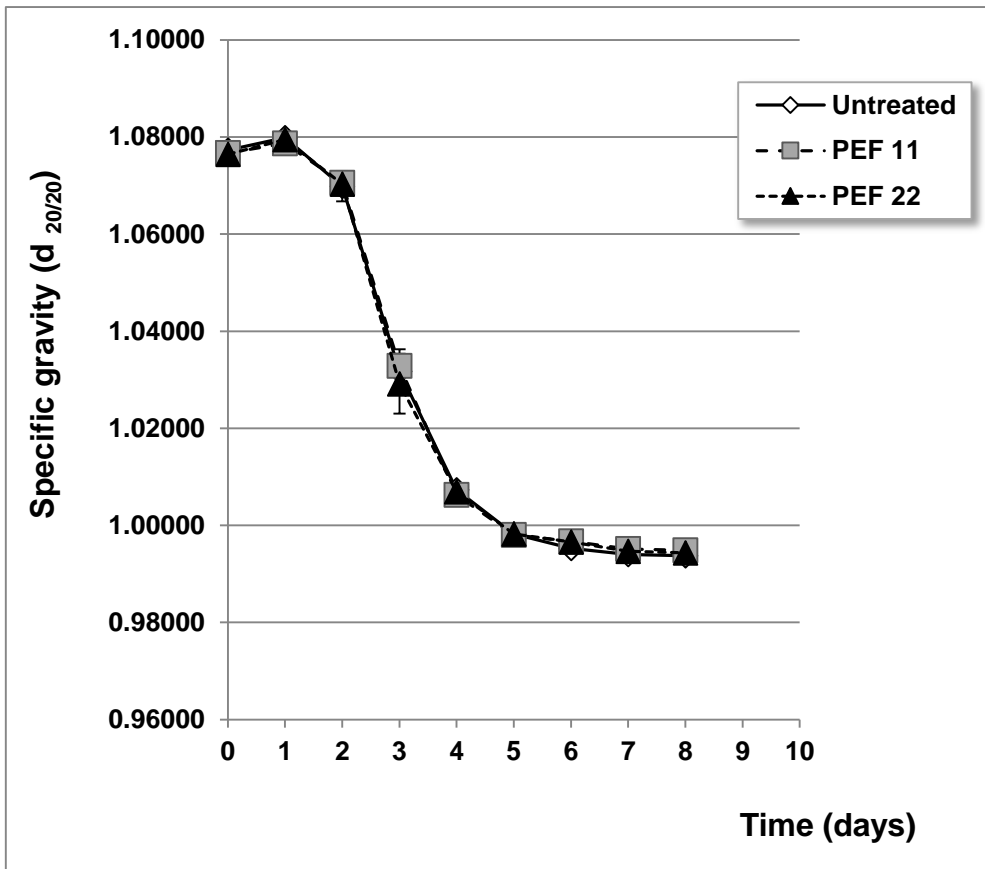
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Fig. 1

548 Table 1.

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

552

Sample	Reducing sugars (g l <sup>-1</sup> )		pH		Total acidity (g l <sup>-1</sup> )				
	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 <sup>-1</sup> )		YAN <sup>a</sup> (mg l <sup>-1</sup> )		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

<sup>a</sup> Yeast Assimilable Nitrogen

553 Table 2.

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

557

Sample	Total acidity (g l <sup>-1</sup> )			Volatile acidity (g l <sup>-1</sup> )			pH			Alcoholic strength (% v/v)						
	Mean	±	SD	Mean	±	SD	Mean	±	SD	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 <sup>-1</sup> )			Lactic acid (g l <sup>-1</sup> )			Tartaric acid (g l <sup>-1</sup> )			Citric acid (g l <sup>-1</sup> )						
	Mean	±	SD	Mean	±	SD	Mean	±	SD	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 <sup>-1</sup> )			Glycerol (g l <sup>-1</sup> )			Potassium (g l <sup>-1</sup> )			Ash (g l <sup>-1</sup> )						
	Mean	±	SD	Mean	±	SD	Mean	±	SD	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

558

559

560 Table 3.

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

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

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569 Concentrations (in  $\mu\text{g l}^{-1}$ ) of volatile compounds, detected in Control (Untreated) and PEF-  
 570 processed (PEF 11 and PEF 22) wines, fifty days after the end of alcoholic fermentation. Means and  
 571 standard deviations (SD) of three repeated samples are reported. Different letters mark significant  
 572 differences according to ANOVA and Tukey HSD Test, at  $p < 0.05$   
 573

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



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575 Table 5.

576 Concentrations (in  $\mu\text{g l}^{-1}$ ) of bound and free terpenes and norisoprenoids, detected in Control (Untreated) and PEF-processed (PEF 22) musts  
 577 (sampling after pressing and before pectolytic enzyme treatment). Means and standard deviations (SD) of three repeated samples are reported.

578 Different letters mark significant differences according to ANOVA and Tukey HSD Test, at  $p < 0.05$

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<u>Bound</u>															
Sample	<i>cis</i> -linalool oxide (furan)		linalool		$\alpha$ -terpineol		geraniol		nerol						
	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD					
Untreated	1,0	$\pm$ 0,2	<b>a</b>	3,6	$\pm$ 0,1	<b>a</b>	0,8	$\pm$ 0,1	<b>a</b>	21,0	$\pm$ 0,5	<b>a</b>	5,3	$\pm$ 0,5	<b>a</b>
PEF 22	1,1	$\pm$ 0,1	<b>a</b>	6,5	$\pm$ 0,1	<b>b</b>	1,1	$\pm$ 0,1	<b>b</b>	27,9	$\pm$ 0,7	<b>b</b>	7,5	$\pm$ 0,2	<b>b</b>
Sample	2,6-dimethyl-3,7-octadiene-2,6-diol		8-hydroxylinalool		geranic acid		3-hydroxy- $\beta$ -damascone		tetrahydroionone						
	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD					
Untreated	9,8	$\pm$ 0,1	<b>a</b>	29,5	$\pm$ 0,1	<b>a</b>	6,1	$\pm$ 1,2	<b>a</b>	9,5	$\pm$ 0,3	<b>a</b>	10,1	$\pm$ 0,6	<b>a</b>
PEF 22	9,5	$\pm$ 0,5	<b>a</b>	36,6	$\pm$ 0,1	<b>b</b>	8,7	$\pm$ 0,7	<b>b</b>	14,2	$\pm$ 0,7	<b>b</b>	12,7	$\pm$ 0,6	<b>b</b>
Sample	3-oxo- $\alpha$ -ionol		dihydro- $\beta$ -ionone		3-oxo-7,8-dihydro- $\alpha$ -ionol (Blumenol C)		3-hydroxy-7,8-dihydro- $\beta$ -ionol		3-oxo-retro- $\alpha$ -ionol						
	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD	Mean	$\pm$ SD					
Untreated	34,4	$\pm$ 0,3	<b>a</b>	5,4	$\pm$ 0,8	<b>a</b>	39,0	$\pm$ 0,5	<b>a</b>	7,6	$\pm$ 0,8	<b>a</b>	6,1	$\pm$ 0,0	<b>a</b>
PEF 22	43,8	$\pm$ 0,2	<b>b</b>	8,7	$\pm$ 1,6	<b>b</b>	52,0	$\pm$ 5,0	<b>b</b>	8,8	$\pm$ 0,9	<b>a</b>	8,8	$\pm$ 0,3	<b>b</b>

*Continue*

Table 5.

<i>Free</i>					
Sample	<i>cis</i> -linalool oxide (furan)	linalool	$\alpha$ -terpineol	geraniol	nerol
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Untreated	<i>n.d.</i> <sup>a</sup>	2,4 $\pm$ 0,2 <b>a</b>	<i>n.d.</i>	8,6 $\pm$ 0,2 <b>a</b>	<i>n.d.</i>
PEF 22	<i>n.d.</i>	2,4 $\pm$ 0,0 <b>a</b>	<i>n.d.</i>	12,0 $\pm$ 1,2 <b>b</b>	<i>n.d.</i>

Sample	2,6-dimethyl-3,7-octadiene-2,6-diol	8-hydroxylinalool	geranic acid	3-hydroxy- $\beta$ -damascone	tetrahydroionone
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Untreated	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
PEF 22	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>

Sample	3-oxo- $\alpha$ -ionol	dihydro- $\beta$ -ionone	3-oxo-7,8-dihydro- $\alpha$ -ionol (Blumenol C)	3-hydroxy-7,8-dihydro- $\beta$ -ionol	3-oxo-retro- $\alpha$ -ionol
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Untreated	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
PEF 22	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>

<sup>a</sup> *n.d.*: not detected

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**Supplementary Material**

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## 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<sup>-1</sup> specific energy (SE), PEF gave a limited evolution of wine color

At 22 kJ kg<sup>-1</sup> SE, PEF apparently increased the stability of wine towards oxidations