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Pulsed electric field treatment to improve the phenolic compound extraction from Graciano, Tempranillo and Grenache grape varieties during two vintages

Noelia López-Giral [a,*], Lucía González-Arenzana [b], Carolina González-Ferrero [a], Rosa López [b], Pilar Santamaría [b], Isabel López-Alfaro [b], Teresa Garde-Cerdán [b,*]

[a] Centro Nacional de Tecnología y Seguridad Alimentaria (CNTA), Ctra. NA 134, Km. 53, 31570 San Adrián, Navarra, Spain
[b] Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja-CSIC-Universidad de La Rioja), Ctra. Mendavia-Logroño NA 134, Km. 90, 26071 Logroño, Spain

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ABSTRACT

The aim of this work was to study and compare the effect of pulsed electric field (PEF) treatment on the content of individual anthocyanins, phenolic acids, flavanols, and flavonols of three grape varieties from two vintages. For this purpose, the treatment showing to induce the highest release of phenolic compounds out of four different PEF applications assayed during the 2011 vintage was applied in 2012. The same continuous system was used over three grape varieties: Graciano, Tempranillo, and Grenache. Additionally, the influence of PEF on the physicochemical composition of the grapes was also studied. The effect of PEF treatment in the amount of individual phenolic compounds released and in the values of the physicochemical parameters analysed was qualitatively similar yet quantitatively different between the two years of study. Considering that the initial values of the physicochemical parameters were dependent on the vintage, it was observed that the lower the initial values the higher their increase after PEF treatment. Hence, the lower the initial values the higher the benefit obtained by PEF treatment. Furthermore, the influence of PEF treatment depended on the variety treated. For instance, Tempranillo was the variety showing the most significant differences in the content of individual anthocyanins upon PEF treatment, while Grenache variety showed major differences in the content of gallic acid, catechin, and epicatechin. Graciano variety showed almost no differences in the amount of individual phenolic compounds released after PEF application. For the three varieties, colour intensity, TPI, and total anthocyanins were increased by PEF application during the two assayed vintages, as well as antioxidant activity in 2012.

Industrial relevance: Pulsed electric field treatment is a suitable technology for the extraction of phenolic compounds. However, its suitability depends on the grape variety treated, and its quantitative effect depends on the initial phenolic composition of the grapes. Consequently, this technology could be used in the food industry to enhance the healthy properties of must and wine.

1. Introduction

Wine quality highly depends not only on the grape variety, but also on the regional and climate variations. In addition, wine quality also differs between vintages. One group of compounds that determine the product quality is the group of phenolic compounds (Lorrain, Chira, & Teissedre, 2011). Phenolic compounds are responsible for the organoleptic characteristics of wine, such as colour and astringency, being probably the quality parameters most affected by the factors mentioned above. The concentration and diversity of these compounds in both grapes and wine depend on many factors: climate and soil, harvest moment, grape processing method and maceration times (Díaz & Pérez, 2001).

In vitro and in vivo studies show that some phenolic compounds offer antioxidant and free radical-scavenging properties (Frankel, Kanner, German, Parks, & Kinsella, 1993), which may play a role in human health, including protection against cardiovascular diseases and cancer (Briviba, Abrahamse, Pad-Zobel, & Rechkemmer, 2002; Negro, Tommasi, & Miceli, 2003; Pérez-Magariño & González-San José, 2006). Antioxidant activity of red wines has been correlated with total anthocyanin and flavonol concentration. The content of total flavanols, among them catechin and epicatechin, is strongly related to this antioxidant capacity and free radical-scavenging activity (Arnous, Makris, & Kefalas, 2001). Minussi et al. (2003) found a positive correlation between wine total antioxidant potential and total polyphenol content, and especially with gallic acid, catechin, and epicatechin. Also, Frankel, Waterhouse, and Teissedre (1995) described a direct association between the total concentration of polyphenols and the inhibition of the oxidation of low-density lipoproteins (LDL). Moreover, they also

* Corresponding authors. Tel.: +34 948670159; fax: +34 948696127.
E-mail addresses: nlopez@cnta.es (N. López-Giral), Teresa.GardeCerdan@gmail.com (T. Garde-Cerdán).

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found that gallic acid and catechin were the phenolic compounds that showed a higher correlation coefficient with oxidation inhibition of LDL.

PEF treatment was able to improve the intracellular metabolite extraction (Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009). Hence, this technology induces cell permeabilisation, or electroporation, releasing anthocyanins from red grapes (Tedjo, Eshtiaghi, & Knorr, 2002). This is because anthocyanins and tannins are located in the vacuoles of the hypodermal cells of the grape skins. Moreover, flavonoids can also be expelled from the vacuole or can even appear compartmented within the cell cytoplasm (Markham, Gould, & Ryan, 2001). In addition, tannins are bound to cell-wall polysaccharides by hydrophobic interactions and hydrogen bonds (Glories, 1984; Pinelo, Arnous, & Meyer, 2006). Studies carried out on plants have confirmed that PEF permeabilises membranes, thus allowing the release of intracellular contents and leading to cell compaction without a significant impact on the cell walls (Bazhal, Lebovka, & Vorobiev, 2001; Fincan, 2003). Delsart et al. (2014) studied the effects of PEF on the organisation of the cell envelope and cellular components to see the characteristics of the passage of phenolic compounds through the tonoplast, plasma membrane and cell wall and they found that must of grapes treated by PEF of short duration had an impact mainly on vacuolar compounds. Tedjo et al. (2002) observed that after the application of 3 kV/cm and 50 pulses, 65% of the total membrane area was permeabilised. As a consequence, the total anthocyanin content was almost 3 times higher than untreated grapes. This improvement in the extraction of anthocyanins from the grape skins was also described by Corrales, Toepfl, Butz, Knorr, and Tauscher (2008), being the effect higher in anthocyanin monoglucosides than in acylated glucosides. Furthermore, these authors found that PEF increased the recovery of total phenolic compounds approximately two times higher than in untreated samples.

In recent years, it has been shown that batch PEF treatment applied to the grape skin before the maceration–fermentation stage increases the extraction of total phenolic compounds during different grape variety vinifications. This increase is dependent on the grape variety. In this regard, López et al. (2008a, 2008b) and Puértolas, López, Condón, Álvarez, and Raso (2010) studied the effect of PEF applied to several Spanish red grape skins, obtaining significantly higher concentrations of polyphenols and anthocyanins in red wine elaborated with skins treated by PEF. Donsì, Ferrari, Fruilo, and Pataro (2010, 2011) studied the effect of PEF in red grape skins from four Italian varieties, Aglianico, Piedirosso, Nebbiolo and Casavecchia, observing a significantly high release of polyphenols (up to 100%) and anthocyanins (up to 30%) in the case of the Aglianico variety. However, the effect on the other three grape varieties in the extraction of phenolic compounds was lower (improvement below 30%) or even non-existent. Likewise, Delsart et al. (2014) also observed an enhancement in the phenolic composition of wines obtained from Merlot and Cabernet Sauvignon grapes treated by PEF in batch. Regarding PEF application in continuous systems at pilot-plant scale, an improvement in the extraction of phenolic compounds from the grape skins has also been reported (Puértolas, Hernández-Orte, Saldaña, Álvarez, & Raso, 2010; Puértolas, López, Saldaña, Álvarez, & Raso, 2010; Puértolas, Saldaña, Álvarez, & Raso, 2010). In contrast, these authors did not carry out the study of phenolic compounds in detail in musts with and without PEF treatment.

Therefore, the aim of this investigation was to study the influence of a PEF treatment during two consecutive vintages for three grape varieties Graciano, Tempranillo, and Grenache, collected at La Rioja wine region — on individual phenolic compounds and general physicochemical parameters, such as colour intensity, total polyphenol index (TPI), total anthocyanins, and antioxidant capacity. In previous research, López-Alfaro et al. (2013) and Garde-Cerdán et al. (2013) studied the effect of four different PEF treatments (pulses of 10 and 20 μs with frequencies of 300 and 400 Hz: 10 μs–300 Hz (Treat1); 10 μs–400 Hz (Treat2); 20 μs–300 Hz (Treat3); and 20 μs–400 Hz (Treat4)) on stilbene and varietal aroma content for the three grape varieties as well as the oenological parameters in 2011. In the work by López-Alfaro et al. (2013) it was observed that, in all cases, the highest energy treatment (Treat4: 20 μs–400 Hz) was the one showing the highest increase in the extraction of total stilbenes. The variety most favoured by the application of PEF was Tempranillo, followed by Graciano and Grenache. The total stilbene concentration in the samples was increased with Treat4 (20 μs–400 Hz) by 200%, 60%, and 50%, respectively. In the work by Garde-Cerdán et al. (2013) an improvement in the volatile composition of the Grenache grapes was observed upon PEF treatment. However, this was not the case for Tempranillo and Graciano. Furthermore, colour intensity, total polyphenol index, and anthocyanins were other parameters improved by the PEF treatments. In general terms, Treat4 was the treatment yielding optimum results. These data were used to select the most suitable treatment to be applied in 2012 (i.e. 20 μs–400 Hz) and to subsequently compare both vintages.

To this point, the harvest was destemmed and crushed (must, skins and seeds) and was treated in a continuous PEF system under the treatment conditions described before as optimal for the release of phenolic compounds.

2. Materials and methods

2.1. Samples

The study was carried out in three red grape varieties: Graciano, Tempranillo and Grenache. 400 kg of grapes of each variety was harvested at their optimum maturity from the experimental vineyard of CIDA (Research Centre of the Spanish northern region of La Rioja) in 2011 and 2012. The grapes were destemmed and right after crushing (Zambelli, Padova, Italy) and sulphiting (70 mg/kg SO_2) operations, the grapes were separated into homogeneous batches divided into four (2011) and six (2012) 25 l stainless steel tanks (Superfustinox®, Milan, Italy). Two (2011) and three (2012) tanks were established as control and the other two (2011) and three (2012) were treated by PEF treatment right after harvesting the grapes. Similarly, control samples also run through pump and PEF equipment. The enzymatic activity of polyphenoloxidase and peroxidase was not evaluated as, in previous studies (not disclosed in this article), no residual enzyme activity was observed under the sulphiting conditions used. In addition, Ugliano (2009) reports that sulphitation of grapes and juice during crushing/pressing is very effective in controlling tyrosinase-driven juice oxidation, as tyrosinase is strongly inhibited by SO_2.

The oenological parameters, colour parameters and antioxidant capacity were determined in the obtained musts and then aliquots (50 ml) of each sample (control and PEF) were frozen at −40 °C in order to subsequently determine their phenolic composition by high performance liquid chromatography (HPLC).

2.2. Vinification

For each variety, the yeast Uvaferm VRB (Lallemand, St. Simon, France) was inoculated in the six tanks (three untreated and three treated by PEF) and alcoholic fermentation was carried out. The three deposits treated by PEF were devatted and its content pressed within 48 h, while the other three untreated tanks were devatted and the content was pressed upon completion of alcoholic fermentation. The alcoholic fermentation was monitored daily by measurement of the density. Fermentation was finished when sugar concentration was lower than 2.5 g/l.

2.3. PEF treatment

The homogeneously mixed grape paste (must, skins, and seeds) was pumped into the PEF treatment chamber using a membrane pump (PV8 Saniflo, Wilden, USA). The PEF equipment used in this research was an ELCRACK-HVP5 unit (DIL, Germany). The PEF treatment chamber used was a co-linear chamber ELCRACK DN25 of 2.50 cm of diameter and 2.38 cm distance between electrodes. The electrode area was of