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Pulsed Electric Fields – assisted vinification

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Abstract

Pulsed Electric Fields (PEF) treatments can be profitably applied as pretreatment to the extraction process from vegetable tissues, in order to increase the release of valuable compounds. In the present work we propose to apply PEF treatments during the vinification process for the permeabilization of the grape skins, in order to increase the polyphenolic content after maceration of red wines made from four Italian grape varieties, Aglianico, Piedirosso, Nebbiolo and Casavecchia. Prior to the fermentation/maceration step, the grape skins were treated at different PEF intensities (field strengths of 1.5 and 3.0 kV/cm and energy inputs of 10 and 20 kJ/kg), with their permeabilization being characterized by electrical impedance measurements. The fresh wine, obtained after pressing, was characterized for alcohol content, total acidity, pH, color intensity, total polyphenols, anthocyanins content and antioxidant activity.PEF treatment on Aglianico grapes induced a significantly higher release of polyphenols (+100%) and anthocyanins (+30%), thus improving the color intensity (+20%) and the antioxidant activity of the wine (+40%), while preserving the other organoleptic characteristics. Similar results were obtained over two different harvest years (2008 and 2009), confirming the repeatability of the process. In contrast, there was only a minor impact on the polyphenolic release kinetics of Piedirosso, Nebbiolo and Casavecchia grapes, despite the significant degree of cell membrane permeabilization, with it probably being due to the predominance of mass transfer limitations through the vacuole membrane.

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1. Introduction

Red wine is made from the must of red grapes that undergoes fermentation together with the grape skin. Therefore, during the red winemaking process the grape skin stays in contact with the juice

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throughout the fermentation process. In this step, yeasts convert most of the sugars of the grape juice into ethanol but also phenolic compounds are extracted from the grape skin.

Traditional winemaking process can extract only a fraction of the large amounts of different phenolic compounds, located in the grape skin, due to the resistance to the mass transfer of cell walls and cytoplasmatic membranes. Phenolic compounds in red wine, such as anthocyanins, tannins and their polymers, are responsible for both the color as well as the body of the wine [1]. In addition, the presence of phenolic compounds is also responsible for the health-beneficial properties of the wine [2]. The phenolic content and composition of wines depends on the initial content in grapes, which is a function of variety and cultivation factors [1,3], but also on the winemaking techniques [4]. It has been found that several techniques such as high fermentation temperature, extending maceration time, must or grape freezing, as well as use of maceration enzymes can enhance the extraction of phenolic compounds through the degradation or permeabilization of the grape skin cells [4]. Nevertheless, these techniques suffer from some drawbacks such as higher energy costs, worsening of the wine quality or long production times

Recently, it has been demonstrated that the application of a PEF treatment of moderated intensity (field strength from 0.1-10 kV/cm, energy input from 1-50 kJ/kg) may represent a viable option for enhancing the extraction of phenolic compounds from skin cells during the maceration steps, without altering wine quality and with moderate energy consumption [5-10].

During the PEF process, an electric field of moderate intensity (0.1-10 kV/cm) is applied in form of repetitive very short voltage pulses (from 1 μs up to 1 ms) to food tissue placed between two electrodes with the aim of inducing electroporation of cell membranes enhancing the diffusion of solutes.

Measurements of the changes in the electrophysical properties such as complex impedance of untreated and treated biological system have also been suggested as a simple and reliable method for obtaining a measurement of the extent of membrane permeabilization of plant cells [11].

The present study is addressed to investigate the feasibility of the PEF treatment in the red wine vinification, by proving the repeatability of the results over two different grape harvests and addressing the issue of extending its applicability to different grape varieties.

2. Materials and Methods

Four different Italian grape varieties were tested: Aglianico, Piedirosso, Nebbiolo and Casavecchia, which were manually harvested during the 2008 and 2009 vintages from vineyards in the province of Avellino (Italy). After harvest, the grapes of each variety were crushed and de-stemmed, and then potassium metabisulfite was added (5 g/100 kg). Batches of the crushed grapes with a total weight of 650 g were assigned either as control or subjected to PEF treatment prior to the fermentation/maceration step. Furthermore, other batches of the product were treated with a commercial enzymatic preparation (Everzym Color, Everintec, Italy) added to the crushed grapes prior to the maceration phase (Table 1).

PEF treatments with monopolar square wave pulses of different intensities (Table 1) were carried out in a laboratory scale batch system already described in a previous work [10].

In order to quantify the cellular degree of permeabilization attained by each treatment, the cell permeabilization index Z_p [12], was evaluated on the basis of the measurement of the absolute values of the complex impedance of the intact and PEF treated tissues in the low (1 kHz) and high (10 MHz) frequency ranges. The value of this index varies between 0, for the intact tissue, and 1, for the fully permeabilized tissue.

Alcoholic fermentation was carried out at 25±3°C with a selected yeast (Zymaflore F15, Laffort Oenologie, France). After the fermentation period, the samples were pressed in a manually operated basket press to obtain fresh wine, which was then stored in glass demijohns. The vinification was carried out in triplicate for all of the experimental conditions.

The fresh wine obtained was analyzed for total acidity (titrimetric method), pH (Crimson pH meter), color intensity (spectrometric analysis), total polyphenols and anthocyanins content (spectrophotometer kits), antioxidant activity (DPPH method).

All the tests were carried out in triplicate on samples prepared and treated independently. All the results reported, are the average of the measurements taken, plus or minus the standard deviation.

Table 1. Enzyme and PEF-treatment conditions

	Treatment conditions
Control	-
Enzyme	2 g/100 kg of grapes
PEF1	1.5 kV/cm, 10 kJ/kg
PEF2	3.0 kV/cm, 10 kJ/kg
PEF3	3.0 kV/cm, 20 kJ/kg

3. Results and Discussion

The fresh wine obtained after the must pressing at the end of fermentation/maceration stage was analyzed in order to identify the different characteristics induced by the PEF treatment in comparison to both the control and the enzyme addition samples (Table 2).

The analyses were carried out on wines produced from Aglianico grapes in two different harvesting years, 2008 and 2009. The grape skins were treated by PEF and compared with control wine from untreated grape skins. In particular, the grapes harvested in 2008 were treated according to the PEF1 conditions (described in Table 1) and the grapes harvested in 2009 were treated according to the PEF1, PEF2 and PEF3 conditions (also reported in Table 1).

Table 2. Physicochemical characterization of the fresh wine obtained from Aglianico grapes untreated and PEF-treated in the harvest years 2008 and 2009.

	Control 2008	PEF1 2008	Control 2009	PEF1 2009	PEF2 2009	PEF3 2009
Alcohol content (v/v %)	11.8±0.1	12.0±0.1	9.1±0.1	10.1±0.1	11.3±0.1	10.5±0.1
pH	3.2±0.02	3.2±0.03	3.4±0.02	3.4±0.02	3.4±0.02	3.4±0.02
Total acidity (g/l tartaric acid)	11.2±0.2	11.1±0.2	7.1±0.2	7.8±0.3	7.6±0.2	7.9±0.1
Colour intensity (a.u)	9.8±0.05	11.0±0.06	6.8±0.06	8.3±0.04	7.5±0.04	7.4±0.07
Total polyphenols (g/l)	1.6±0.02	2.1±0.03	1.1±0.01	2.1±0.02	2.2±0.02	2.4±0.03
Free anthocyanins (mg/l)	477±7	734±12	300.7±10	352.9±15	387.2±11	395.4±15
Antioxidant activity (mg/ml ascorbic acid)	0.16±0.01	0.18±0.01	0.12±0.01	0.17±0.01	0.17±0.01	0.17±0.01

In particular, the main physicochemical parameters were monitored, such as alcohol content, pH, total acidity and colour intensity, as well as health-promoting properties, such as the content of total polyphenols, free anthocyanins and the antioxidant activity.

As shown in Table 2, the control wines were slightly different in alcohol content, pH and total acidity, as well as polyphenolic content, which can be reasonably ascribed to the variety from one year to the other. Nevertheless, it must be highlighted that no significant difference of the effect of the PEF treatment could be observed between the wine produced after 2008 harvest and the wine produced after 2009 harvest. In particular, under the same treatment conditions (PEF1) the increase in total polyphenols, free anthocyanins and antioxidant activity was comparable for the two wines.

Moreover, the results reported in Table 2 also shows that the increase in severity of the treatment, both in terms of higher electric field intensity (which increased from 1.5 to 3 kV/cm for the same specific energy, going from PEF1 to PEF2) or higher specific energy (which increased from 10 to 20 kJ/kg at 3 kV/cm, going from PEF2 to PEF3) led to a higher content of total polyphenols and free anthocyanins. On the other side, the antioxidant activity did not seem to be significantly affected.

The same outstanding results, which were obtained with the PEF-assisted vinification of Aglianico grapes, described in details elsewhere [11], were not obtained for different grape varieties, such as Piedirosso, Nebbiolo and Casavecchia.

In Fig. 1, the effect of different permeabilization techniques, based on enzyme addition and PEF pretreatment at different severity, are shown in terms of percentage variation with respect to respective control wine of four quality indicators, namely colour intensity, content of total polyphenols and free anthocyanins and antioxidant activity.

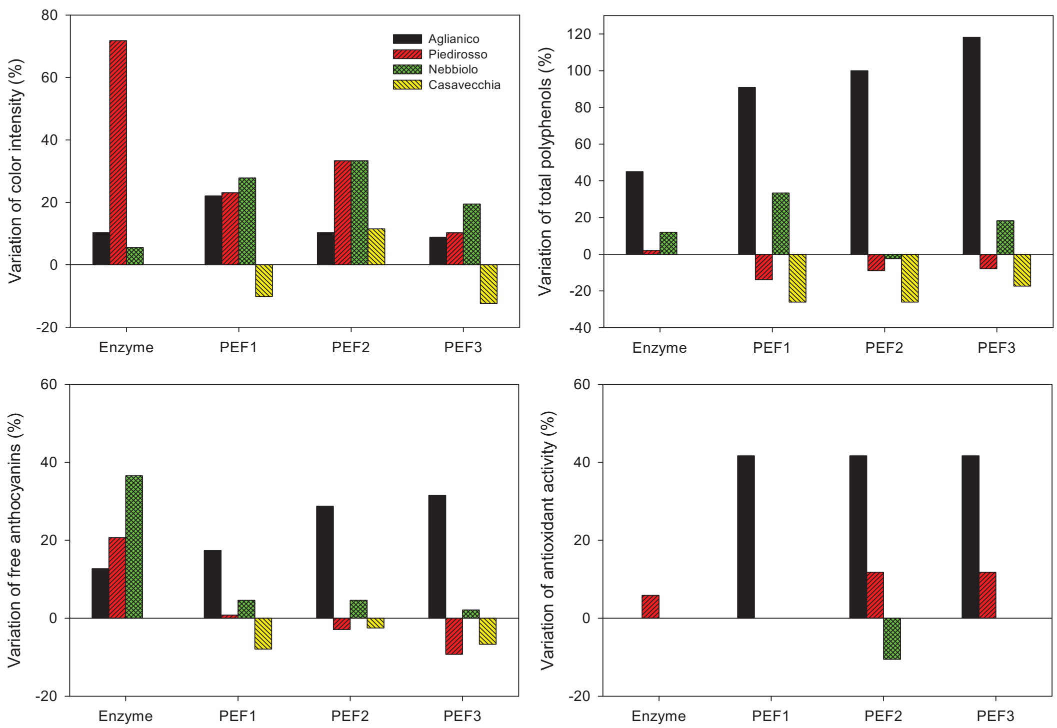

Fig. 1. Differential variation, with respect to control wine, of color intensity, total polyphenols, free anthocyanins and antioxidant activity of the wines obtained from enzyme-treated and PEF treated grapes of Aglianico, Piedirosso, Nebbiolo and Casavecchia cultivars

Interestingly, while enzyme addition always resulted in a positive impact on the examined quality parameters, the same did not happen for the PEF treatment. In particular, the impact of PEF treatment was always positive with respect to control and higher than enzyme addition only for Aglianico, while for Piedirosso, Nebbiolo and Casavecchia PEF treatment was always less efficient than enzyme addition and, in some cases, also negative in comparison to control.

The explanation of the observed behaviour can be probably ascribed to the fact that permeabilization of the cell membrane is the key determinant in governing the release of polyphenolic compounds from the grape skins into the wine. This is evident when considering the measured permeabilization of the cell membrane of the grape skin as a function of PEF treatment, reported in Table 3. The three PEF treatments of different intensity all caused a significant permeabilization of the cell membranes of the different grape varieties, and the degree of permeabilization increased with the severity of the treatment, both increasing the intensity of the electric field applied (from PEF1 to PEF2) and the specific energy delivered (from PEF2 to PEF3).

The measured cell permeabilization obviously does not correspond to the release of polyphenolic compounds, as shown in Fig. 1. In fact, while for Aglianico there is a clear correlation between permeabilization and total polyphenols concentration and free anthocyanins concentration, it is not the case for Piedirosso, Nebbiolo and Casavecchia, for which, in some cases, the PEF treatment resulted in the reduction of some parameters.

Polyphenols and anthocyanins are mainly contained within the vacuoles of the cells, and therefore their extraction encounters two main resistances to mass transfer, which are formed respectively by the vacuole membrane and the cell membrane. It appears that the PEF treatment is effective in permeabilizing the vacuole membranes of skin cells of Aglianico grapes significantly better than those of skin cells of the other grape varieties. Since PEF treatment causes permanent membrane permeabilization provided that a critical transmembrane potential is induced across the membrane by the externally applied electric field, it can be assumed that the tested grape varieties are characterized by different vacuole sizes, and only the size of Aglianico vacuoles are large enough to be permeabilized by the applied PEF treatment.

The reported results show that PEF treatment can be profitably implemented in the vinification process of Aglianico grapes. In comparison with the use of a pectolytic enzyme, the most effective PEF treatment resulted for Aglianico grapes not only in the polyphenols content, color intensity and antioxidant activity, but also in lower operational costs. In fact, the cost for the enzymatic treatment is of about 4 € per ton of grapes (the average cost of the enzyme is about 200 €/kg, and the amount used is 2 g per 100 kg of grapes), while the energy cost for the PEF treatments was estimated in about 0.8 € per ton of grapes (with the energy costs assumed to be 0.12 €/kWh) in the case of the most effective treatment. Obviously, the evaluation of the industrial feasibility of the integration of the PEF technology in the wine production should take into account also other parameters, such as the investment costs, the risks associated with a novel technology and the needs for qualified technicians to run and maintain it, the estimation of the market driving forces as well as the optimization of the PEF treatment for grapes characterized by difficult extractability of the polyphenols.

Table 3. Permeabilization index Z_p of the skin of different grape varieties, as a function of PEF treatment intensity

PEF treatment	Z_p Aglianico	Z_p Piedirosso	Z_p Nebbiolo	Z_p Casavecchia
PEF1	48.6±1.5%	68.6±4.6%	67.7±5.9%	82.2±6.3%
PEF2	61.1±0.6%	91.2%±1.2%	82.4±1.3%	86.3±1.4%
PEF3	75.9±3%	94±1.2%	86.8±0.3%	91.3±0.1%

4. Conclusion

This work shows that PEF treatments can be profitably used in the vinification process to increase the content of polyphenolics in the red wine, and consequently color intensity and antioxidant activity, provided that the vacuoles of the grape skin cells are permeabilized.

For PEF treatment ranging from 1.5 to 3 kV/cm of applied electric field and a delivered specific energy between 10 and 20 kJ/kg, Aglianico grapes were effectively permeabilized, leading to a wine with a higher color intensity (+20%), total polyphenols (+100%) and free anthocyanins (+30%) content as well as higher antioxidant activity (+40%), without the addition of pectolytic enzymes.

Similar results were obtained over two harvests, confirming the robustness and repeatability of the process.

On the other side, for different grape varieties, such as Piedirosso, Nebbiolo and Casavecchia, no significant effect of the PEF treatment was evident, probably because a higher applied electric field would be necessary to permeabilize their smaller grape skin cell vacuoles.

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