An assessment of potential applications with pulsed electric field in wines

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Abstract. Pulsed electric fields (PEF) is a non-thermal processing technology that uses instantaneous, pulses of high voltage for a short period in the range of milliseconds to microseconds; the application of high intensity electric field on toasted wood chips leads to a quick diffusion of extractable molecules. Currently most PEF studies, in the field of oenology, have been focusing on the application of PEF as a pretreatment of grape musts by examining the microbial inactivation and the enhancement of polyphenol extraction. In this study a post-treatment of wine is introduced as method to enhance the wood flavor in the wine with a green noninvasive technology. Major phenolic aldehydes that have been identified as the characteristic compounds of oak volatile compounds were selected as markers and were analyzed instrumentally to compare the influence of PEF processing to non-treated samples. PEF treated samples brought about higher concentrations of the examined oak compounds in the samples treated with PEF, which may explain the advantages of its application. The modulation of the intensity of the electric field and the period of pulses influenced the concentrations of the volatile phenols that were leached out. Differences found between the assayed treatments indicate that PEF application could be a potential practice for a rapid extraction of volatile compounds from oak.

1. Introduction

The aging process of wine, in which wine is in direct contact with wood for a specific time, is important for its organoleptic characteristics. During this process, small amounts of air insert into the barrel from its pores. Wine should have good structure and body, together with antioxidant capacity, in order to equilibrate the negative effect of the oxygen (Ortega-Heras et al. 2004).

Although aging conditions, such as the wine cellar temperature, humidity and the dwelling time in barrels affect the characteristic of wine (Arapitsas et al. 2004; Dubois, 1989) the most important factor is the raw material of the oak barrel and its treatment (Arapitsas et al., 2004; Miller & Howell, 1992), as these two factors determine compounds extracted on wine. The traditional way of aging wine in barrels acquires a lot of time, usually a few years, depending on the style of wine.

At present, for cost-effective reasons, alternatives to the oak barrel are being looked at to carry out the wine–ageing process. Therefore, alternative forms have been tested in the past few years to expedite the extraction of aroma compounds from the oak. One of these techniques consists of adding oak chips to wine in order to give a woody aroma (Garde-Cerdan et al., 2006).

Extraction of oak wood compounds into wine is of great importance since they can improve the wine's aroma and mouth feel. Major compounds that are degraded from wood are aromatic aldehydes, also known as volatile oak phenolics. Major degradation product of lignin is vanillin which gives wine a typical vanilla flavor. Other extracted aromatic aldehydes are: syringaldehyde, coniferyladehyde, sinapaldehyde, and furfural (Margalit 2010).

Furfural derives from the degradation of monosaccharides produced by partial hydrolysis of hemicelluloses and celluse, together with Maillard reactions which take place during the interaction with oak (Arapitsas et al. 2004; Ortega-Heras et al., 2006). Oak lactone originates from oak lipids and its odor attributes a woody and coconut character (Arapitsas et al. 2004). Guaiacol is a product of lignin thermal decomposition, and has a smoky flavor.

As the demand of wine market is increasing, new and economically effective methods are conducted to accelerate the extraction of oak aroma compounds (Zhang et al., 2012). Some of the new methods that have been introduced to accelerate wine aging process are ultrasonic waves, gamma irradiation, and electric fields, including pulsed electiric field (PEF) (Zhang et al., 2012).

Pulsed electric field relies on the use of high intensity pulsed electric fields for cell membrane disruption where induced electric fields perforate microbial membranes by electroporation. The high intensity pulsed electric filed processing system is a simple electrical system consisting of a high voltage source, a capacitor bank, a switch, and a treatment chamber. From the electrical point of view, the PEF treatment chamber represents the electrical load consisting of two or more electrodes filled with the liquid substance to be treated. The chamber has to be constructed in such a way that the electrical field acting on the liquid is more or less homogeneous across the entire active region.

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This goal can be reached in principle with planer, coaxial and axial electrode geometries (Loeffler, 2006).

The application of PEF is primarily used as a non-thermal processing for the inactivation of microorganisms, with the potential of being an alternative for pasteurization of liquid foods (Grahl et al., 1996; Álvarez et al. 2003). Several researchers have introduced the electric field treatment as an effective method to accelerate young wine aging (Zeng et al., 2008). Zhang et al. (2012) examined the influence of PEF on brandy aging in oak barrels and their results showed extraction enhancements of tannins, total phenols, and volatile phenols from the wood.

The study of Zeng et al, (2008) attempted the application of AC high voltage electric field to accelerate wine aging, by examining physiochemical attributes as well as sensory evaluation. Also, Corrales et al. (2008) drew the comparison between ultrasonics and pulsed electric field in the extractability of anthocyannins from grape by-products. Further researches have been conducted in wine processed with pulsed electric fields.

In the traditional way of aging, it has been observed that the bouquet of red and white wines develop after a short period in the barrel. During the aging of red wine various components of color, aroma and flavor develop. The color changes to deeper red and the flavor evolves becoming softer, with less astringency. The rate at which these changes occur is different for each wine, depending on both outside conditions and the wine's specific composition such as oxidative phenomena (O_2 and SO_2), temperature and time and its phenol composition (Ribereau-Gayon et al., 2006).

This study examines the extractability of oak volatiles in wine combined with PEF treatment, in order to expedite the aging process. Oak chips were added to wine samples and were treated with pulsed electric fields in different conditions of time (t_i = 200 ms and 5 sec) and voltage (E=7.7 and 1.2 kV/cm) to examine its effect in the extraction of volatile compounds compared to untreated wine samples. Moreover, organoleptic characteristics are examined of the treated and untreated wine samples in order to conclude whether PEF treatment leads to faster ageing than the traditional barrel ageing.

2. Material and methods

2.1. PEF equipment

The PEF equipment used was a static bench scale system. PEF equipments is consisted of a high voltage power generator (Eisco, India), a digital oscilloscope (model UTD 2062 C, ELV Elektronik AG, Germany) and a pulse generator (model UPG 100, ELV Elektronik AG, Germany).

The PEF generator used provides maximum voltage and current 5 kV and 0.8 kV respectively. This generator provides pulses of monopolar rectangular shape. Signals of voltage, current, frequency, and pulse waveform were monitored by the digital oscilloscope.

The treatment chamber (Val-Electronic, Greece) is consisted of three coaxial geometries, as shown in Fig. 1. A bronze cylinder 5 mm thick, 125 mm height and 28 mm diameter. A U shaped cylinder, where the liquid is filled in, 20 mm diameter and 130 mm height, which is inserted in the bronze cylinder. And a stainless steel electrode of 5 mm diameter and 120 mm height which screws to the U shaped cylinder. Electric field strength E is evaluated as $E = U/d$, where U is the applied voltage and d is the distance between the electrode and the bronze cylinder, where $d = 0,65$ cm.

a) b)

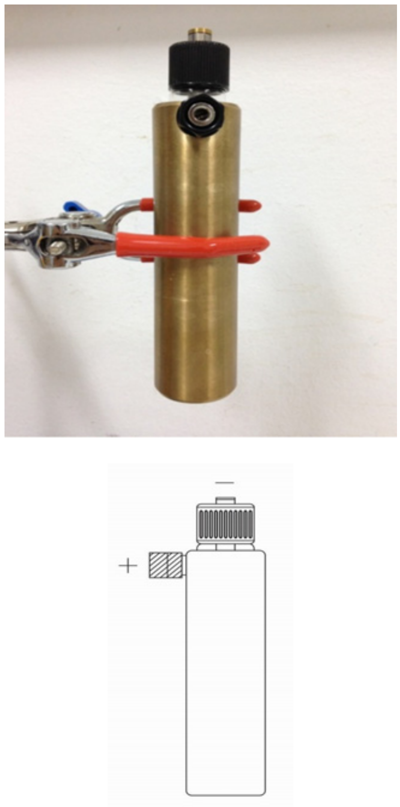

Figure 1. a) PEF treatment chamber, b) schematic aspect of PEF treatment chamber.

For each case the time of treatment is calculated as

$$t = (t_i + t_p) \times P \text{ in (s)}$$

t_i=the pulse duration (s)
t_p=pause time (s)
P=number of pulses.

The treatment conditions used in the experiments are:

- *E*=7.7 kV/cm, t=1500 s or 25 min (t_i=5 s, t_p=5 s, P=150)
- *E=1.2* Kv/cm, t=1800 s or 30 min (t_i=200 ms, t_p=200 ms, infinite pulses).

The detailed treatment procedure is as follows: For each sample 0.2 gr of toasted oak chips were added to 25 ml wine sample in the U shaped cylinder. For the PEF treated samples a voltage of 5 kV and 0.8 kV was applied between the electrodes for 25 minutes.

Under the same experimental procedure and condition, all wine samples treated with 0 kV/cm were used as control for comparison.

2.2. GC/MS analysis

2.2.1. Ultrasound-assisted extraction

All samples were immediately Ultrasound-assisted extracted after PEF treatment. 25 mL of wine sample, 10 mL of saturated NaCl solution and 25 mL of Dichloromethane were added in a laboratory flask. The flask was then placed in an ultrasonic bath (Transsonic T570/h, Elma, Germany) for 10 min at room temperature. The samples were centrifuged at 3500 rpm for 5 min to separate the phases. The supernatant was then extracted a second time using the same volume of solvent in the ultrasonic bath for 10 min. The organic layer was then washed with distilled water in a separation funnel. The organic phase was dried on anhydrous sodium sulphate, filtered and then condensed in a vacuum rotary evaporator. Prior to chromatographic analysis, the sample was recovered with 50 μL of chloroform.1 μL was used for GC–MS analysis. To demonstrate replication, all extractions were done in triplicate.

Each sample was subjected to GC coupled with MS analysis, using an Agilent 6890 series GC System, equipped with 5975C VL MSD and a fused silica capillary column, $30\,\mathrm{m}P \times 0, 32\,\mathrm{mmi.d.} \times 0, 25\,\mu\mathrm{m}$ film thickness (HP-%MS 5%, phenylmethylsiloxane, Agilent Technologies). Samples (1.0 μl) injected using split mode with a split ratio of 100:1. The injector temperature hold at 250 °C, the carrier gas was helium at a flow rate 1 ml/min, and the oven temperature programmed as follows: initial 50 °C hold 2.5 °C min to 180 °C, then rate 2 °C/min to 230 °C, then rate 6 °C/min to 250 °C for 5 min and finally rate 5 °C/min to 270°C for 2 min. The above GC conditions translated using the Agilent GC Method Translation to match the mass spectrum retention times. Helium used as carrier gas at 25 cm/s. The temperature of the transfer line fixed at 280°C, the mass spectrometer operated in the electron ionization mode (EI) at an ionization voltage of 70 eV in the mass range 40–550 amu and a manifold temperature of 270°C All data was recorded using the Turbomass 5.0 ChemStation software.

For the quantification of Vanillin, Syringaldehyde, Furfural and Oak Lactone external calibration curves were arranged from standards.

2.2.2. *Wines:*

The wine samples used for this study were from the greek variety Agiorgitiko from the experimental winery of Technological Institute of Athens at the department of Oenology and Beverages Technology (2015).

2.2.3. *Oak chips*

French *Quercus sp.*, untoasted, oak chips were used in this study, purchased by Arabois. These oak chips were extra small in size and heterogeneous in shape.The chips were toasted at 200 °C for 2 hours before being used. In each case 0.2 gr of oak chips were added to 25 ml of sample.

2.2.4. *Chemicals*

Dichloromethane, chloroform, sodium chloride and anhydrous sodium sulphate were purchased from Chem Lab (Zedelgem, Belgium). Vanillin, syringaldehyde, oak lactone and furfural were purchased from. All chemicals are of analytical grade.

2.2.5. *Statistical Analysis*

Every analysis was repeated three times.

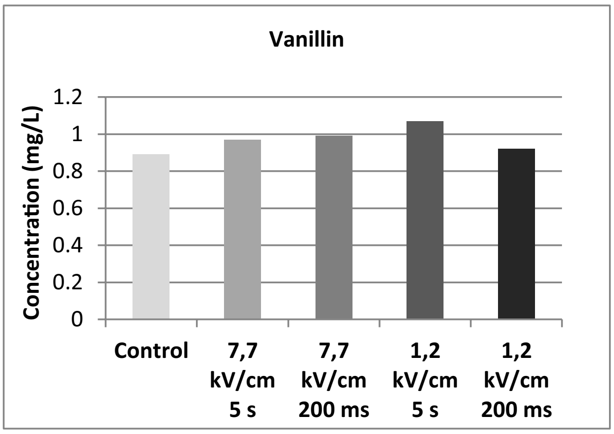

Figure 2a. Concentration of Vanillin for untreated (0 kV/cm) and PEF-treated (7.7 kV/cm for 5 sec and 200 ms and 1.2 kV/cm for 5 sec and 200 ms) red wines.

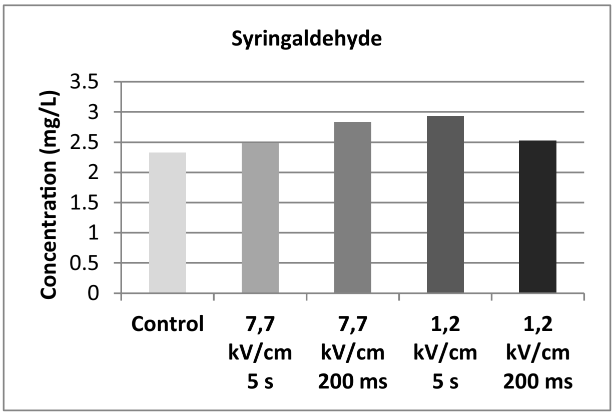

Figure 2b. Concentration of Syringaldehyde for untreated (0 kV/cm) and PEF-treated (7.7 kV/cm for 5 sec and 200 ms and 1.2 kV/cm for 5 sec and 200 ms) red wines.

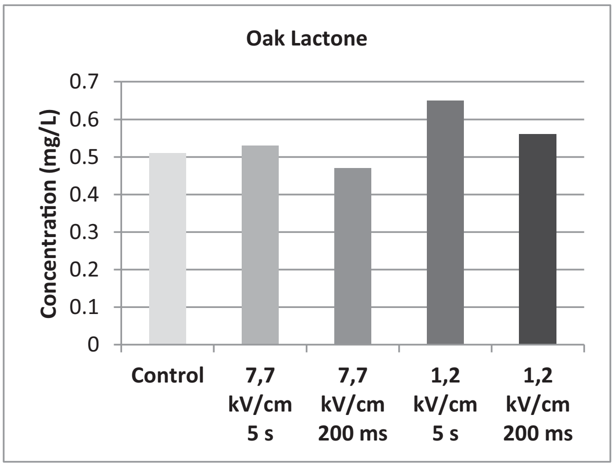

Figure 2c. Concentration of Oak Lactone for untreated (0 kV/cm) and PEF-treated (7.7 kV/cm for 5 sec and 200 ms and 1.2 kV/cm for 5 sec and 200 ms) red wines.

3. Results & discussion

Furanic aldehydes may be reduced during ageing to form their corresponding alcohols (Boidron et al., 1988; Garde-Cerdán & Ancin-Azpilicueta, 2006). Furfural can also be transformed through chemicals mechanisms (Tominaga, Blanchard, Darriet & Dubourdieau, 2000). Other studies (Garde-Cerdán et al., 2002; Gómez-Plaza et al., 2004;

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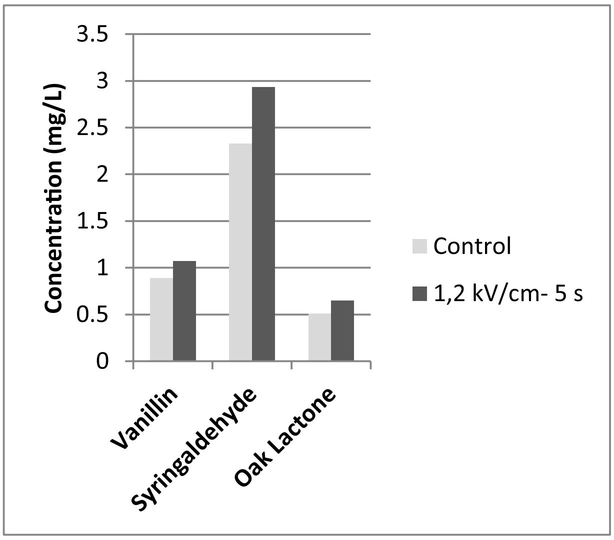

Figure 3. Concentration of Vanillin, Syringaldehyde and Oak Lactone for non-treated and PEF-treated wine samples.

Garde-Cerdán & Ancin-Azpilicueta, 2006) showed that the concentration of phenolic aldehydes is maximal after 10–12 months of ageing. Of this group of compounds most important is vanillin.

The present work studied the extraction rate of the most important volatile compounds of oak wood -vanillin, syringaldehyde, oak lactone and furfural by using oak chips and PEF treatment in order to minimize the time of ageing. In the non- treated wine samples, taking into consideration that the process time was the same for all samples, the compounds being extracted were sufficient enough to be quantified. To be particular, in the non-PEF treated sample, vanillin was extracted in 0.89 mg/L, Syringaldehyde in 2.33 mg/L, oak lactone in 0.51 mg/L and furfural was detected in traces. In Figs. 2a–c, the results of the extraction for the specific 3 volatile compounds in the control and in the samples after treated by PEF are shown. According to Perez-Prieto et al. (2003) after 3, 6 and 9 months of ageing red wine in once used American oak barrels vanillin was detected in the concentration of 140, 165 and 190 μg/L respectively, whereas in our study the extraction rate of the volatile compound of oak wood, vanillin, for PEF-treated sample for 30 min exceeds the 1 mg/lt.

In our study, in general terms, PEF showed the highest efficacy, for the molecular extraction of oak components. For pulse duration 5 sec, 180 pulses and 1,2 kV/cm, the extractability of vanillin and Syringaldehyde reached up to 1.07 mg/L and 2.93 mg/L respectively (Fig. 3). Moreover, oak lactone was increased to 0.65 mg/L, whereas furfural was "found" in traces and had a small extraction after the PEF treatment reaching to 0.03 mg/L.

Taking under advisement these results, different conditions of PEF treatment were examined in order to interpret the best combination of pulse duration and voltage in order to extract high concentration of the volatiles compounds. In order to achieve this, primarily the voltage was increased from 0.8 kV to 5 kV and the time was the same. Likewise, PEF results showed an enhancement in the concentration of the compounds compared with the control samples, but less comparing with the PEF treated samples of the first condition

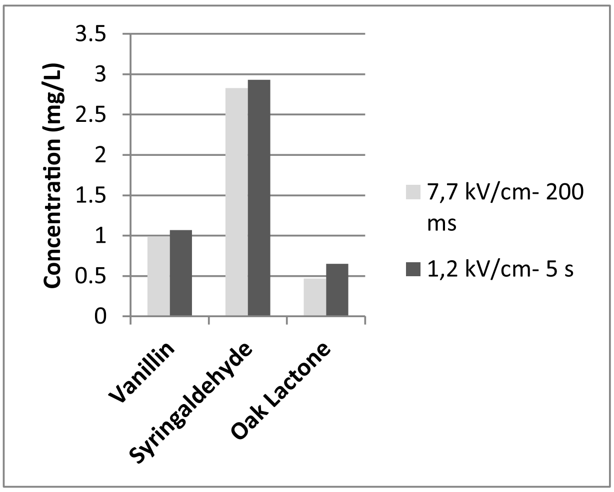

Figure 4. Concentration of Vanillin, Syringaldehyde and Oak Lactone for PEF-treated wine samples in different conditions.

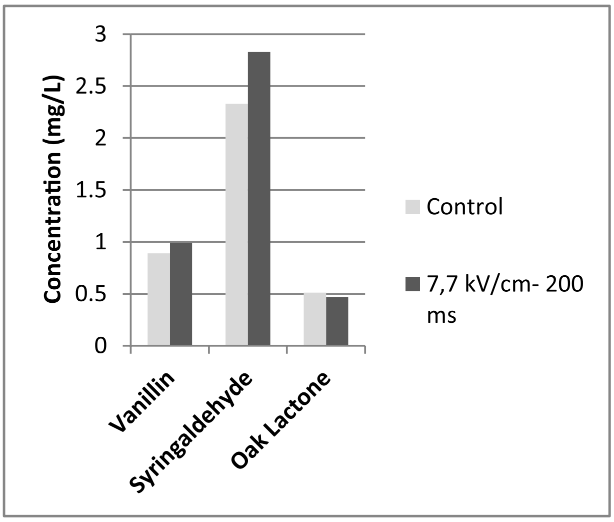

Figure 5. Concentration of Vanillin, Syringaldehyde and Oak Lactone for non-treated and PEF-treated (7.7 kV/cm and pulses 200 ms) red wines.

(1.2 kV/cm), as Vanillin and Syringaldehyde aspired to 0.99 mg/L and 2.83 mg/L respectively, while oak lactone had a smaller increase (Fig. 4). Thus, the best condition, for the fast extraction of volatile compounds is 1.2 kV/cm and pulse duration 5 sec.

Another outcome is that in PEF treatment of 1.2 kV/cm, which considers to be the condition with the greatest impact on the wine samples treated with PEF, as it was mentioned before, the quantity of the extracted compounds differs depending on the pulse duration. As shown in Fig. 5, in the treatment of short pulses (t_i =200 ms) the concentration of all examined compounds were higher than those of long pulses (t_i =5 s). More specific, vanillin's extraction was greater by 0.07% and syringaldehyde's by 1.93%; whereas in oak lactone a reduction was observed in the rate of 0.35%. Remarkably, in the case of long pulses (t_i =5 s) the extractability of furfural augments from traces to 0.03 mg/L.

All the samples, treated and untreated, were evaluated sensorially in order to detect if there was any difference in flavor and mouthfeel. According to Zeng et al. (2008), the results of the wine tasting showed that the

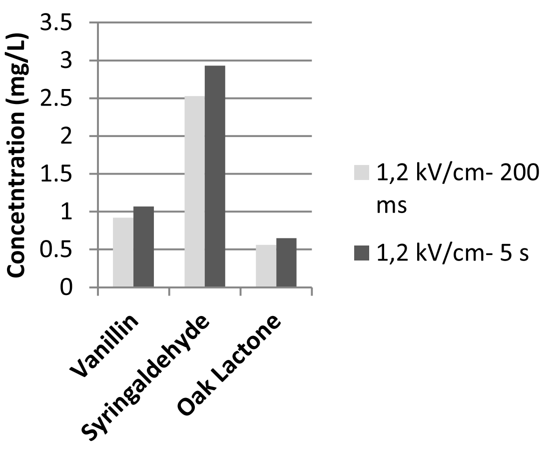

Figure 6. Concentration of the three extracted compounds, PEF-treated with 1.2 KV/cm in different pulse duration.

PEF treated samples had a better sensory quality than the untreated ones, and it was proved that the aging process was accelerated by the application of an AC electric field. In our study, we examined how the parameters (pulse duration, number of pulses and electric field strength) of PEF treatment can evolve the ageing process of wine. PEF treated wines were described by a softer mouthfeel and tannins. The pungent smell of fresh wine was covered by notes of vanilla and wood, as those of an aged wine. Therefore, the combined PEF treatment with oak chips can replace the long-during ageing in oak barrels; since it gives similar outcomes to the traditionally aged wine, but in shorter time.

4. Conclusion

The main aim of the present work was to study the extraction rate of the aromatic compounds –vanillin, Syringaldehyde, oak lactone and furfural from oak wood into wine treated with different conditions of PEF. In this study, it has been demonstrated that the application of PEF treatment to the wine enhanced the extraction of compounds from the wood. The concentration of all the compounds were higher after PEF treated with best condition 1.2 kV/cm and pulse duration 5 sec. Vanillin and Syringaldehyde reached up to 1.07 mg/L and 2.93 mg/L respectively, whereas oak lactone and furfural had a smaller influence reaching to 0.65 mg/L and 0.3 mg/L respectively. Therefore, it can be concluded that the volatile composition of wine was enhanced by PEF application and it can be further investigated in order to minimize the time of aging and the financial costs of this traditional process.

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