Applications of pulsed electric fields in winemaking

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Abstract. Wineries can take advantage of the ability of pulsed electric fields (PEF) to electroporate the cell membranes of grape skins and microbial cells to improve several kind of processes. The PEF permeabilization of red grape skin cells permits winemakers to reduce the duration of maceration and/or to improve a wine's color and concentration of polyphenolic compounds without impairing its sensorial attributes. The capability of PEF to inactivate spoilage microorganisms while preserving the physicochemical and sensorial properties of must and wines may help enhance wine quality by guaranteeing reproducible fermentations and reducing or replacing the use of SO2 for wine stabilization. It has been also demonstrated that PEF triggers yeast autolysis thereby accelerating the release of mannoproteins from cell walls and decreasing the duration of aging on lees.

1 Introduction

Pulsed electric fields (PEF) is a non-thermal processing technology that has been investigated in recent years to improve the wine-making process. PEF treatment consists of the intermittent application, in the form of pulses, of a potential difference lasting on the order of a millionth of a second (μs) to a product placed between two electrodes. As a consequence, an electric field (E) is generated whose intensity depends on both the potential difference (V) and the distance between the electrodes (d): E=V/d.

When the applied electric field exceeds a certain threshold value, a phenomenon called electroporation occurs, resulting in the uncontrolled transport of substances through the cytoplasmic membrane of cells due to the formation of pores. The external electric field required to electroporate cells depends, among other factors, on the cell size. While fields lower than 5 kV/cm are required to electroporate plant tissue cells, the formation of pores in membranes of microbial cells requires the application of higher electric fields (above 10 kV/cm).

Wineries can utilize the ability of pulsed electric fields (PEF) to electroporate the cell membranes of grape skins and microbial cells to improve various processes. The PEF permeabilization of red grape skin cells allows decreasing maceration time and/or enhancing a wine's colour and concentration of polyphenolic compounds without compromising its sensory characteristics. The capacity of PEF to deactivate spoilage microorganisms while preserving the physicochemical and sensory properties of must and wines can help to enhance wine quality by ensuring reproducible fermentations and reducing or replacing the use of SO_2 for wine stabilization. It has also been demonstrated that PEF triggers yeast autolysis, thereby accelerating the release of mannoproteins from cell walls and reducing the duration of aging on lees.

2 Improvement in the extraction of phenolic compounds during red winemaking by PEF

Maceration-fermentation is the most critical stage in the red winemaking process. This stage is essential for obtaining high-quality red wines but also represents a stage with high requirements in energy and manpower. During maceration-fermentation, the solid parts of the grape berries remain in contact with the fermenting must for several days (7-10 days) for the extraction of polyphenols, mainly located in the cells of the grape skins. These compounds are key factors in red wine since they are involved in their sensory properties (color, flavor, astringency, and bitterness), aging behavior, and the beneficial effects for health attributed to moderate wine consumption. The maceration process poses significant challenges for the wine industry, such as the reduction of the effective volume of the maceration-fermentation tanks. Twenty percent of the tanks are taken by the solid parts of the grapes consequently reducing production capacity. Furthermore, this process requires very high energy consumption, for controlling fermentation temperature and periodically pumping the wine over the skin mass that rises to the top of the maceration-fermentation tanks.

Recent studies have shown that using PEF to electroporate grape skin cells can increase the extraction

rate of phenolic compounds during the maceration-fermentation step in red winemaking (Fig. 1).

The treatment is usually applied to crushed and destemmed grapes with the aim of causing irreversible electroporation of the cytoplasmic membrane of grape skin cells, which contain most of the necessary compounds for producing high-quality red wine.

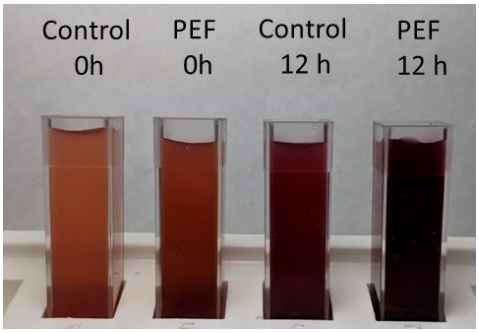

Figure 1. PEF effect on the extraction of components responsible for wine colour.

The benefits of electroporation have been observed in various grape varieties, including Tempranillo, Grenache, Graciano, Mazuelo, Merlot, Aglianico, Cabernet Sauvignon, and Cabernet Franc, harvested in Spain, France, Lebanon, Italy, and New Zealand [1].

Semi-industrial trials conducted in wineries have confirmed the initial results obtained at the laboratory and pilot plant scales. For example, in one trial, twelve tons of Grenache grapes were processed at a flow rate of 2,500 $kg.h^{-1}$, and the wines obtained from untreated and PEF-treated (4.0 $kV.cm^{-1}$) grapes after 3 and 6 days of maceration were compared [2]. PEF pre-treatment of grapes reduced maceration time from 6 to 3 days without causing a significant decrease in red wine color or the concentration of polyphenolic compounds. The higher tannin concentration observed in PEF-treated wines was due to a higher degree of extraction of tannins located in the grape skins rather than in the seeds. The results of a triangle and preference test showed significant sensory differences between the wines obtained with untreated or PEF-treated grapes after 3 and 6 days of maceration when aged either in bottles or in oak barrels. Panelists were able to distinguish between the wines and the majority (86%) preferred wines made from grapes treated with PEF.

3 Inactivation of wine-spoiling microorganisms by PEF

Microbial processes play a crucial role in the winemaking process, but the presence of spoilage microorganisms can pose a significant risk to the quality of the final product, resulting in substantial economic losses.

Wineries commonly use SO_2 and sterilizing filtration before bottling to control microbial growth. However, the widespread use of SO_2 is being questioned due to the sensitivity of certain individuals with allergies and the increasing rejection of chemical preservatives by consumers [3]. With regard to sterilizing filtration, filters with a nominal pore size of 0.45 µm are generally used, but they are not effective against smaller-sized bacteria. This operation is always a controversial issue in wineries due to its potential impact on wine's chemical and sensory characteristics, especially in red wine. Consequently, the wine industry is actively seeking alternative methods for microbial control without altering the properties of the wine

PEF presents a promising alternative for microbial control in wineries as they can inactivate microorganisms at lower temperatures than those used in thermal processing. Several studies have investigated the potential of PEF for inactivating microorganisms that are involved in wine fermentation processes, such as Saccharomyces bayanus and Oenococcus oeni, or spoil wine-related, such as Brettanomyces bruxellensis, Lactobacillus plantarum, and Lactobacillus hilgardii [4]. The viability of all these microorganisms was shown to be affected by moderate electric field strengths (15 kV/cm), and combining SO_2 with PEF treatments has been found to have a synergistic effect (Fig. 2).

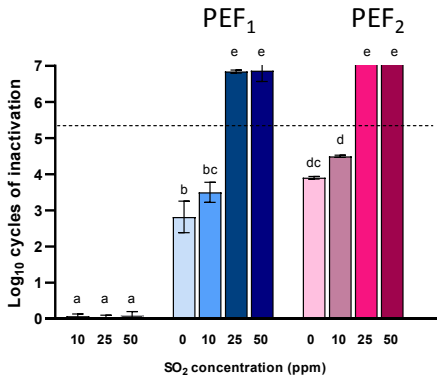

Figure 2. Inactivation of *Brettanomyices bruxellensis* combining PEF_1 (15 kV/cm, 39 kJ/kg) or PEF_2 (15 kV/cm, 78 kJ/kg) with 0, 10, 25 or 50 ppm of SO_2 immediately after PEF (A, C) and incubated for 24 h (C, D). Dotted lines represent the quantification limit (<30 CFU/mL).

Moderate PEF treatments, either alone or in combination with sublethal doses of SO2, have been successful in stabilizing wines microbiologically for at least four months [6]. Moreover, these treatments had negligible impacts on the physicochemical and sensory parameters of the wine, indicating that PEF presents a viable physical process that could potentially replace or reduce SO_2 doses or serve as an alternative to sterilizing filtration in the wine industry.

In summary, the results of these studies reveal the great potential of PEF for microbial control in winemaking, providing an effective solution that could minimize the use of chemical preservatives and their potential negative impact on wine quality

4 Accelerating mannoprotein release during aging on lees

Mannoproteins present in wine have positive effects such as reducing haze formation, preventing tartaric salt

precipitation, decreasing astringency, and improving mouthfeel, aroma intensity, and color stability [7]. The traditional method of enriching wine with mannoproteins involves "aging on lees," which means keeping the wine in contact with the yeast sediment after fermentation. This process requires labor costs, periodic stirring (bâtonnage), and sensory analyses. It also immobilizes winery stocks and may increase the risk of wine oxidation and microbial contamination with bacteria and Brettanomyces.

Recent studies have shown that pulsed electric fields (PEF) can trigger the autolysis of S. cerevisiae and accelerate the release of mannoproteins [8]. The electroporation of the cytoplasmic membrane by PEF could facilitate the contact of endogenous lytic enzymes with the yeast cell wall where the mannoproteins are located. The figure indicates that the concentration of mannoproteins in Chardonnay wine increased significantly in samples containing PEF-treated yeasts (5 and 10 kV/cm, 75 µs) (Fig. 3).

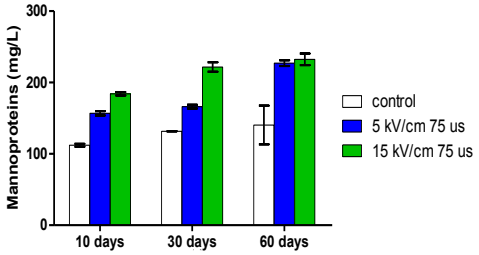

Figure 3. Effect of PEF treatments of different intensities on the mannoproteins release from S. cerevisiae during aging on the lees.

After 30 days of incubation, the wine containing yeast treated under the most intense PEF conditions reached the maximum mannoprotein concentration value. However, it took six months to reach the same level in the wine containing untreated yeast. Aging on the lees with PEF-treated yeast did not affect the chromatic characteristics, total polyphenol index, total volatile acidity, pH, ethanol, or CIELAB parameters of the wine. In contrast, mannoproteins released from yeast treated with PEF reduced wine turbidity and showed foaming properties similar to those released during the traditional "aging on lees."

5 Conclusion

PEF technology has multiple applications in wineries, as it can improve various operations and meet the food industry's current demands, such as energy efficiency, sustainability, and the reduction of chemical usage. The availability of commercial PEF units that can respond to wineries' processing capacity demands and the authorization of PEF technology as a new oenological practice for white and red winemaking by the International Organization of Vine and Wine (Resolution OIV-OENO 634-2020) provide a definite impetus for rapid PEF technology implementation in wineries.

References

1. M.A. Maza, I. Álvarez, J. Raso, *Applications of Pulsed Electric Fields in Winemaking*. In: J. Raso, V. Heinz, I. Alvarez, S. Toepfl, (eds) *Pulsed Electric Fields Technology for the Food Industry.* Food Eng. Rev. Springer (2022)
2. M.A. Maza, J.M. Martínez, P. Hernández-Orte, G. Cebrián, A.C. Sánchez-Gimeno, I. Álvarez, J. Raso, *Influence of pulsed electric fields on aroma and polyphenolic compounds of Garnacha wine,* Food Bioprod. Process. **116**, 249-257 (2019)
3. M.T. Lisanti, G. Blaiotta, C. Nioi, L. Moio, *Alternative Methods to SO_2 for Microbiological Stabilization of Wine*. CRFSFS **18**(2), 455-479 (2019)
4. E. Puértolas, N. López, S. Condón, J. Raso, I. Álvarez. *Pulsed electric fields inactivation of wine spoilage yeast and bacteria.* Int. J. Food Microbiol **130**(1), 49-55 (2009)
5. C. Delso, A. Berzosa, J. Sanz, I. Álvarez, J. Raso, *Pulsed electric field processing as an alternative to sulfites (SO2) for controlling saccharomyces cerevisiae involved in the fermentation of Chardonnay white wine*, Food Res. Int. **165**, 112-525 (2023)
6. C. Delso, A. Berzosa, J. Sanz, I. Álvarez, J. Raso, *Microbial Decontamination of Red Wine by Pulsed Electric Fields (PEF) after Alcoholic and Malolactic Fermentation: Effect on Saccharomyces cerevisiae, Oenococcus oeni, and Oenological Parameters during Storage*. Foods **12**, 278 (2023)
7. J.A. Pérez-Serradilla, M.D. Luque de Castro, *Role of lees in wine production: A review,* Food Chem, **111**(2), 447-456 (2008)
8. J.M. Martínez, C. Delso, M.A. Maza, I. Álvarez, J. Raso, *Pulsed electric fields accelerate release of mannoproteins from Saccharomyces cerevisiae during aging on the lees of Chardonnay wine*, Food Res. Int. **116**, 795-801 (2019)