# Inactivation of *Saccharomyces bayanus* after the alcoholic fermentation in a red wine by Pulsed Electric Fields (PEF) and $SO_2$

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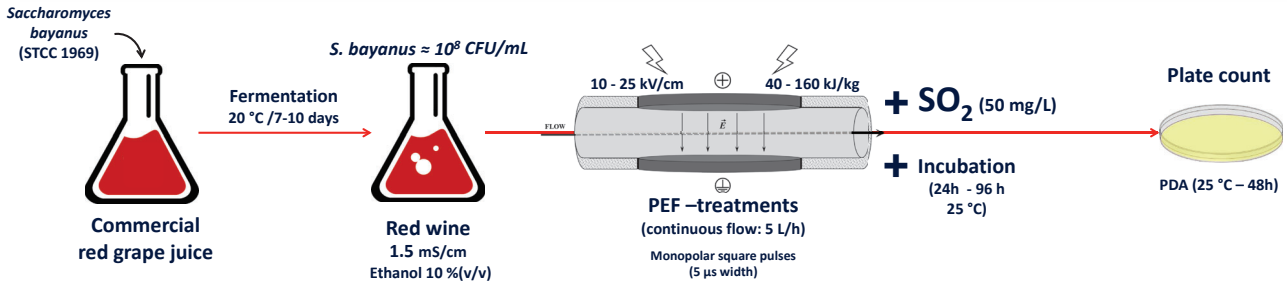
## Introduction

**Sulfur dioxide ($SO_2$)** is an additive widely used in the wine industry due to its antimicrobial and antioxidant properties. However, as the presence of $SO_2$ in the wine may have adverse effects on sensitive individuals, the winemaking industry is interested in reducing or eliminating the use of this potentially dangerous preservative. **Pulsed Electric Fields (PEF)** is well-known as a gentle technology for microbial decontamination. Several studies have already reported the potential of PEF for inactivating **spoiling microorganisms in wine**. However, further investigation is required for defining PEF process conditions that may be applied at **industrial scale** with the PEF unit currently available. Microbial decontamination by PEF could be conducted at different stages of winemaking. After alcoholic fermentation, controlling *Saccharomyces* spp. involved in the transformation of sugars in ethanol is required to promote the growth of malolactic bacteria and to prevent the wine spoiling.

## Objective

**The aim of this study was to characterize the resistance of *Saccharomyces bayanus* after alcoholic fermentation of must to PEF treatments in combination with $SO_2$**

## Material and methods




## Results and discussion

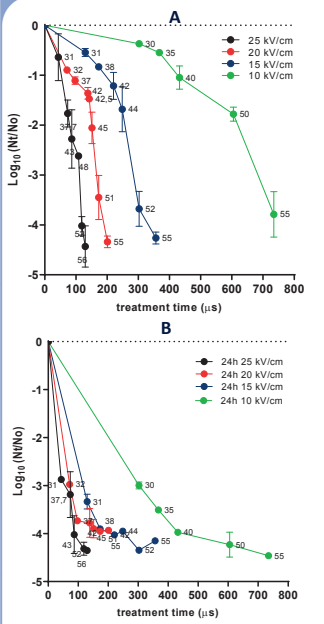



**Figure 1.** Inactivation of ***S. bayanus*** in red wine by PEF treatments at 10, 15, 20 and 25 kV/cm. **(A)** Survival curves obtained by plating the wine samples after the PEF treatment; (**B**) Survival curves obtained by plating the wine samples after 24 h of incubation at 25 °C. Numbers correspond to **exit temperatures** after the PEF treatment.

**Figure 1A** shows the influence of the electric field on the inactivation of *S. bayanus* at different treatment times after wine fermentation. The increment of the electric field reduces the number of pulses required to obtain a given inactivation level. While more than 700 µs were required to inactivate around 4 $Log_{10}$ cycles the population of *S. bayanus* at 10 kV/cm, at 25 kV/cm similar levels of inactivation were obtained with around 120 µs. However, similar levels of inactivation were obtained by treatments of different electric field strength when the exit temperature was the same.

**Figure 1B** shows that the numbers of survivors to the different PEF treatments applied drastically decreased when the wine samples were stored for 24 h at 25 °C. This effect was much more significant for those treatments in which the exit temperatures were below 50 °C. For example, the inactivation of a treatment at 15 kV/cm for 220 µs that corresponded to an exit temperature of 45 °C increased from 2 $Log_{10}$ cycles up to 4 $Log_{10}$ cycles after 24 h of incubation. This increment in the lethality after incubation permitted to reach 4 $Log_{10}$ cycles of inactivation with treatments of lower total specific energy (77.8 kJ/kg) that corresponded to an exit temperature of around 40 °C. These results suggest the presence of a proportion of the population sublethally damaged by PEF-treatment that was not able to repair the injuries caused by the PEF after 24 h of incubation in the wine.

The inactivation effect of PEF on *S. bayanus* suspended in wine added with $SO_2$ (50 mg/L) just after the PEF treatment and after 24 and 96 hours of storing at 25 °C is shown in **Figure 2**. $SO_2$ did not affect the yeast population in the first 24 hours of incubation but inactivation of around 1 $Log_{10}$ cycle was achieved after 96 h of incubation.

The lethality of the two assayed PEF treatments (15kV/cm for 130µs and 15kV/cm for 220µs) was higher than 5 Log10 cycles and higher than 6 $Log_{10}$ cycles after 24 and 96 hours of incubation respectively in the wine containing $SO_2$. In both cases, a synergistic effect was observed, the inactivation achieved by combining PEF with $SO_2$ was higher than the addition of the individual effects. This synergistic effect seems to indicate the existence of a subpopulation of PEF injured *S. bayanus* cells that can recover their damage in wine but not in wine added with $SO_2$.

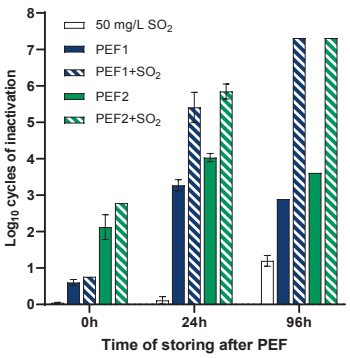



**Figure 2.** Inactivation of ***S. bayanus*** in red wine by **PEF1** (15kV/cm, 130µs, 30°C), **PEF2** (15kV/cm, 220µs, 45°C), **$SO_2$** (50 mg/L) and a combination of them after the treatment and **24 and 96 hours of incubation.**

## Conclusions

Results obtained in this investigation reveal the **potential of application of moderate PEF treatments** applicable with the current PEF unit commercial available for **reducing or even eliminating the use of $SO_2$** for controlling the yeast involved in wine fermentation. Further investigation is still required with other target microorganisms at different steps of winemaking as well as the effect of PEF in the physico-chemicals and sensory properties of final wines stabilized by PEF.

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