



# The synergism between SO<sub>2</sub> and ethanol is the “villain” of yeasts at the beginning of the second fermentation of sparkling wines

Bruno Cisilotto<sup>1,2</sup>, Fernando Joel Scariot<sup>1</sup>, Luisa Vivian Schwarz<sup>1</sup>, Ronaldo Kauê Mattos Rocha<sup>1</sup>, Ana Paula Longaray Delamare<sup>1</sup>, Sergio Echeverrigaray<sup>1</sup>

<sup>1</sup> Laboratory of Enology and Applied Microbiology, Institute of Biotechnology, University of Caxias do Sul, Brazil.

<sup>2</sup> Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS), Campus Bento Gonçalves, Brazil.

In our paper, we show that at a pH commonly found in sparkling base wines (i.e., 2.8-3.3), a synergism occurs between the SO<sub>2</sub> added as a preservative and the ethanol present in the base wine. This synergism causes high stress on yeasts that lead to an important loss of viability, which in turn causes an increase in the lag phase time, slow fermentation and difficulties in finishing the *prise de mousse*.

## Hypothesis creation and experimental design

The hypothesis that synergism between SO<sub>2</sub> and ethanol is the major stress factor at the beginning of the second fermentation of sparkling wines emerged from the observation that yeast population viability decreased and lag phases differed in length in some fermentations within the first week after *pied-de-cuve* inoculation.

As mentioned in the introduction and the discussion of our paper<sup>1</sup>, the stress caused by ethanol and SO<sub>2</sub> is described in the literature. There are calculations and formulas in the oenological chemistry literature which theorise the fraction of molecular SO<sub>2</sub> taking into account the concentration of ethanol<sup>2</sup>, as well as studies showing the synergism caused by SO<sub>2</sub> and ethanol in “finished” wines with wine spoilage yeasts. In addition, it is recommended that free SO<sub>2</sub> concentrations in sparkling base wines be less than 10 mg/L before starting the second fermentation<sup>3</sup>. However, we did not find any research in the scientific literature describing the synergism between SO<sub>2</sub> and ethanol and showing the stress caused in yeast strains commonly used for the second fermentation of sparkling wines.

With this in mind, we created an experimental fermentative model to test the behaviour of the yeast *Saccharomyces cerevisiae* EC-1118® (strain specifically marketed to produce sparkling wines) in different environments. The treatments comprised a synthetic medium with i) added SO<sub>2</sub>, ii) added ethanol, iii) added SO<sub>2</sub> and ethanol, and iv) neither SO<sub>2</sub> nor ethanol added (control). In this way, it was possible to evaluate and compare the behaviour of the yeasts in each fermentative system via analyses with stress markers, cell vitality and viability markers and the expression of genes related to SO<sub>2</sub> stress.

## Interpreting the results

The results obtained in our experiments showed that synergism between SO<sub>2</sub> and ethanol intensifies stress and affects the yeast population in a significant way, with a decrease in vitality and viability, increased production of reactive oxygen species (ROS), decreased intracellular pH, increased production of acetaldehyde, and increased expression of genes related to stress caused by SO<sub>2</sub><sup>1</sup>. The same effect of decreasing yeast vitality and viability was observed in an industrially conducted second fermentation with real wine<sup>1</sup>. Figure 2 illustrates the effect of each treatment in terms of viability and fermentation kinetics. In this case, the experiment was conducted at a temperature of 20 °C. Cell viability was assessed with

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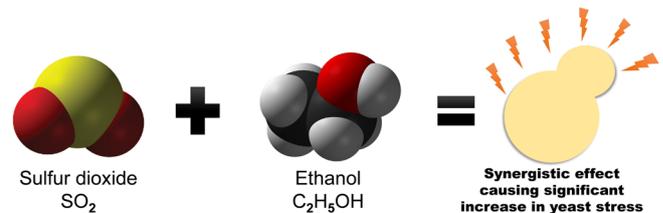


FIGURE 1. Informative graphic summary of the synergistic effect of SO<sub>2</sub> and ethanol.

DNA intercalating dye propidium iodide (PI) using a flow cytometer (Figure 2A) and the evaluation of reducing sugars using the method with 3,5-dinitrosalicylic acid (DNS) as reducing agent and microplate reader with absorbance at 595 nm (Figure 2B) (for more information see article). The results in Figure 2 show the behaviour of an inoculum adapted with ethanol in the different treatments, simulating the beginning of a second fermentation. In the treatments with the addition of SO<sub>2</sub> only, the yeasts can be seen to have very similar behaviour to the control, despite undergoing a small reduction in viability during the first hours of adaptation. In the treatment with ethanol alone, there is no loss of viability, but there is a decrease in fermentation speed and consumption of sugars due to the inhibition of growth of the population<sup>1</sup>. In the treatment with 5 % ethanol and 20 mg/L SO<sub>2</sub> it is interesting to note that, although the yeasts underwent a slightly

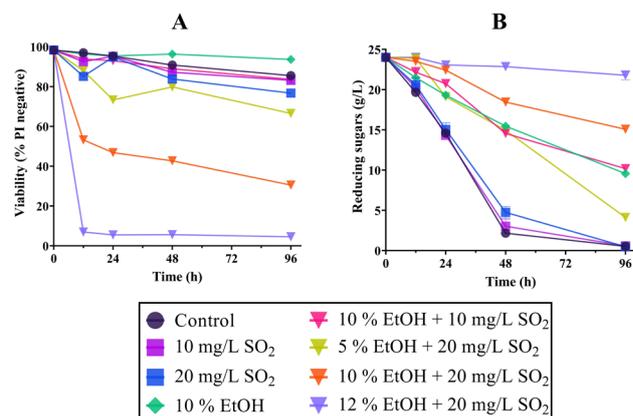


FIGURE 2. Influence of each treatment on the viability and kinetics of fermentation. Adapted from Cisilotto *et al.* (2021)<sup>1</sup>.

more pronounced loss of viability at the beginning and suffered more stress<sup>1</sup>, the fermentative kinetics increased speed after 48 h with an increase in the percentage of yeast viability, showing that the amount of ethanol plays an important role in the synergistic effect. In treatments with quantities equal to or above 10 % ethanol and with an initial amount of SO<sub>2</sub> above 10 mg/L, there was a marked decrease in the viability of the population and consequently a reduction in the speed of sugar consumption. This slows down fermentation and can eventually lead to the cessation of fermentation.

### Final remarks

Despite several studies looking for a substitute for SO<sub>2</sub><sup>4</sup> for use in winemaking, one that has such a comprehensive effect (antioxidant and antimicrobial activity), as well as being low cost and safe for human consumption, does not yet seem to exist. Thus, we will probably continue to use SO<sub>2</sub> as a wine preservative for a long time. However, in the case of base wines used for sparkling wines, which is an intermediate product, the doses of free SO<sub>2</sub> must be very precise and personalised. For this precise dosage, besides the concentration of free SO<sub>2</sub>, and the pH index, ethanol concentration, storage temperature, wine turbidity and ionic strength of each wine must be considered. The amount of yeast cells inoculated for the second fermentation of sparkling wines plays an important role in the quality of the product; an excess of cells at the time of inoculation can cause sensory defects. The lower the concentration of ethanol and the amount of free SO<sub>2</sub> in the base wine, the better the yeast adaptation and consequently the better the fermentation kinetics; the excess of cells at inoculation and excessive reduction in percentage of viable cells at the beginning of the process can thus be avoided. Carrying out

a linear and standardised fermentation can avoid a series of logistical complications and unnecessary expenses, especially in sparkling wines made in the traditional method in which the re-inoculation of the *pie-de-cuve* implies the opening of the bottles. Therefore, balanced concentrations of SO<sub>2</sub> and ethanol will minimise not only yeast stress at the beginning of the second fermentation of sparkling wines but also the “stress” of the oenologist responsible for conducting this process. ■

**Sources:** Sourced from the research article: “Yeast stress and death caused by the synergistic effect of ethanol and SO<sub>2</sub> during the second fermentation of sparkling wines” (OENO One, 2021).

- 1 Cisilotto, B., Scariot, F. J., Vivian Schwarz, L., Mattos Rocha, R. K., Longaray Delamare, A. P., & Echeverrigaray, S. (2021). Yeast stress and death caused by the synergistic effect of ethanol and SO<sub>2</sub> during the second fermentation of sparkling wines. *OENO One*, 55(4), 49–69. <https://doi.org/10.20870/oeno-one.2021.55.4.4809>
- 2 Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding Wine Chemistry* (first edit). Wiley.
- 3 Alexandre, H. (2019). Yeasts and Sparkling Wine Production. In P. Romano, M. Ciani, & G. H. Fleet (Eds.), *Yeasts in the Production of Wine* (pp. 395–432). Springer New York. [https://doi.org/10.1007/978-1-4939-9782-4\\_13](https://doi.org/10.1007/978-1-4939-9782-4_13)
- 4 Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative Methods to SO<sub>2</sub> for Microbiological Stabilization of Wine. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 455–479. <https://doi.org/10.1111/1541-4337.12422>