IVES Technical Reviews
vine & wine

https://doi.org/10.20870/IVES-TR.2023.7763

Bioprotection as an alternative to SO_2 in the pre-fermentation phase

Sara Windholtz[1,2], Claudia Nioi[1,2], Cécile Thibon[1,2], Georgia Lytra[1,2], Stéphane Bécquet[3], Emmanuel Vinsonneau[4], Joana Coulon[5], Isabelle Masneuf-Pomarède[1,2]

[1] Univ. Bordeaux, Bordeaux INP, Bordeaux Sciences Agro, INRAE, OENO, UMR 1366, ISVV, F-33140 Villenave d'Ornon, France
[2] Bordeaux Sciences Agro, Bordeaux INP, INRAE, OENO, UMR 1366, ISVV, F-33170 Gradignan, France
[3] Vigneron Bio Nouvelle Aquitaine, Montagne, France
[4] Institut Français de la Vigne et du Vin, Pôle Bordeaux-Nouvelle Aquitaine, France
[5] BioLaffort, 11 Rue Aristide Bergès, 33270 Floirac, France

Additives have been used in the food industry for many years, to prevent food spoilage and extend shelf life. These chemical additives are a source of controversy and their use must be reduced in the face of societal demand. In enology, this applies in particular to sulfur dioxide (SO_2). There has been recent research on bioprotection as an alternative to sulfite addition in the pre-fermentation phase. This technical article discusses the many advantages of using bioprotection agents.

Background

Many alternatives to sulfur dioxide (SO_2), both physical and chemical, are available on the market or are currently under trial[1]. Among them, one solution is bioprotection by the addition of living microorganisms. This practice, already used in the food sector, involves adding microorganisms capable of colonizing the medium. Their presence limits or even inhibits the growth of other undesirable microorganisms, without impairing the product's sensory properties. In enology, recent research has focused on the detailed impact of using bioprotection as an alternative to SO_2 during the pre-fermentation stages of winemaking.

Competition for space in grape must

In 2017, three protocols were studied using Merlot: bioprotection (BP) applied at 5 g/hL (without SO_2 addition), Ø: without SO_2; SO_2: 5 g/hL[2].

The bioprotection used (in the form of ADY) was a mixture (50/50) of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*.

The manufacturer's recommendations for rehydration of the bioprotection agent were followed. It was applied by spraying it directly on the grapes. During the pre-fermentation phase at 10 °C, three samples were taken: on filling the tank and then after 24 hours and 48 hours of cold soaking. Analysis using metabarcoding and high-throughput sequencing was used to characterize the microbial biodiversity of the grape must and determine the relative abundance of different genera and species within the fungal population (Figure 1). The species used for bioprotection represented an average of 50% of the microflora in the grape must studied. The relative abundance of *T. delbrueckii* (light blue) increased during cold soaking, while the reverse was true for *M. pulcherrima*. The strong presence of these two strains limits the space available for undesirable microorganisms such as *Hanseniaspora*, *Aspergillus* and *Aureobasidium* in the must. The same observation was made with other red Bordeaux musts[3]. In addition, the use of bioprotection limits the early establishment of native strains of *Saccharomyces cerevisiae*, in contrast to the other two protocols. Similar results were also observed in white must using different bioprotection products.

O_2 consumption by bioprotection

Yeasts consume oxygen as part of their metabolism. The use of bioprotection at a rate of 5 g/hL, corresponding to a concentration in the order of 2×10^6 cells/mL, leads to consumption of dissolved O_2 in the must, as shown by initial trials with whites[4]. O_2 was consumed more rapidly in the presence of bioprotection (BP), in contrast to the SO_2-free control (Ø), where O_2 consumption is likely due to the activity of polyphenol oxidases. The use of bioprotection maintained significantly higher glutathione (GSH) concentrations at the end of alcoholic fermentation compared with the control (Figure 2.B). This antioxidant compound is naturally present in must and is also synthesized by yeast during the alcoholic fermentation. In addition, the presence of bioprotection microorganisms seems to limit must browning (visual assessment) (Figure 2.A). Further investigations[5] have demonstrated that O_2 consumption could be linked to not only the species, but also the yeast strain used for bioprotection. Thus, *Metschnikowia pulcherrima* has an Oxygen Consumption Rate (OCR) significantly greater than that of the other species (Figure 2.C). This means that it consumes O_2 more quickly than other species. In addition, within the same species (e.g. *L. thermotolerans*), OCR values vary significantly from one strain to another. This ability to consume O_2 could explain the decrease in populations of acetic acid bacteria observed when using bioprotection[5].

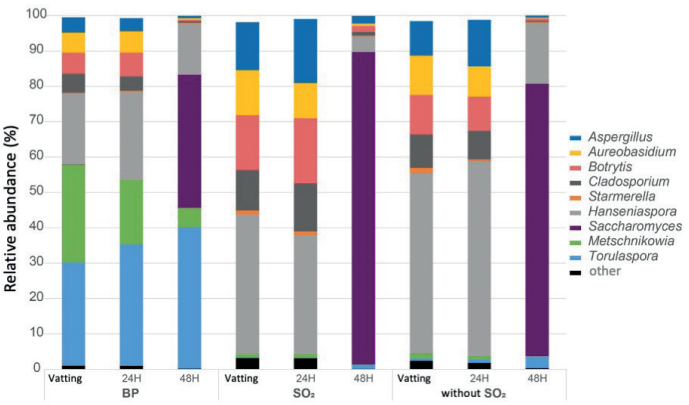

FIGURE 1. Relative abundance (%) of fungal populations in a 2017 Merlot must[2].

Aromatic profile and sensory impact of bioprotection

In addition to their use at low dose for bioprotection, non-*Saccharomyces* yeasts are marketed for their biotechnological properties: they can limit the production of volatile acidity, enhance the fruity aroma of

The translation of this article into English was offered to you by Moët Hennessy.

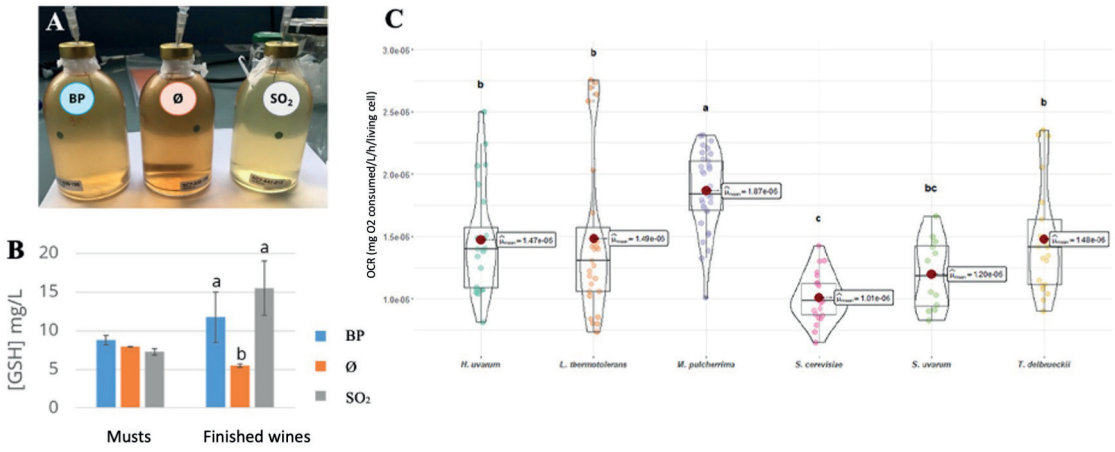

FIGURE 2. O2 consumption by bioprotection. (A) Sémillon must; (B) [GSH] in Sémillon musts and wines; (C) mean OCR by species (grape juice). BP: 5 g/hL bioprotection; Ø: without SO2; SO2: 5 g/hL. ANOVA (p-value <0.05).

wines or increase their acidity in a context of climate change. To this end, they are applied at high doses compatible with their contribution to the fermentation process (15-30 g/hL), in co-inoculation or sequential inoculation with selected strains of *S. cerevisiae* (to ensure full completion of the alcoholic fermentation).

Here, the aim was to study the chemical and sensory impact of different non-*Saccharomyces* yeasts, used either as low-dose bioprotection agents, or applied at high dose in sequential inoculation with *S. cerevisiae*[6]. The aroma compounds in the wines were analyzed and then separated according to the protocol for application of non-*Saccharomyces* yeasts (Figure 3.A). The results show that wines from sequential inoculation are correlated with acetates of higher alcohols, while those used for bioprotection (with inoculation at a lower dose and without looking for fermentation activity) are correlated with fatty acid ethyl esters. From a sensory point of view, there is a marked impact on fruit perception in young Merlot wines, with wines from sequential inoculation being the most intense, followed by wines obtained using bioprotection, which in turn are more intense than the control wine.

In another experiment, sensory analyses were carried out on these wines after 18 months' bottle ageing[7]: the wines made using bioprotection were not sensorially different from wines made without SO_2, but did differ from wines made with sulfite additions. Nevertheless, the "fresh blackcurrant" descriptor in wines made with bioprotection was scored as being more intense than in wines made with sulfite additions.

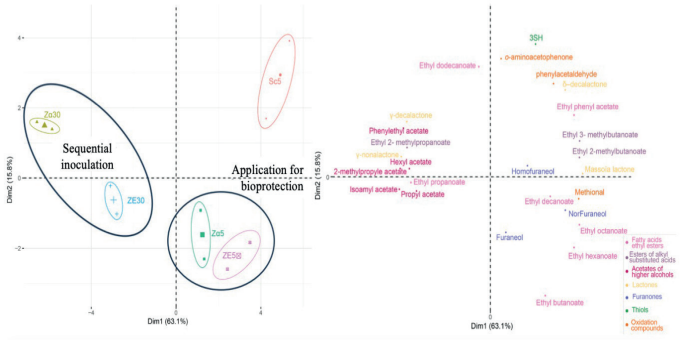

FIGURE 3. Principal Component Analysis of Merlot wines with different applications of non-*Saccharomyces* yeasts. Sc5: Control, addition of *Saccharomyces cerevisiae* at 5 g/hL to grapes; Zα5: *T. delbrueckii* applied for bioprotection at 5 g/hL to grapes; ZE5: mixture of *M. pulcherrima* and *T. delbrueckii* applied for bioprotection at 5 g/hL; Zα30: *T. delbrueckii* applied on filling the tank at 20 g/hL and addition of *S. cerevisiae* after a loss of 10 density points (sequential inoculation); ZE30: *M. pulcherrima* and *T. delbrueckii* applied on filling the tank at 20 g/hL an addition of *S. cerevisiae* after a loss of 10 density points (sequential inoculation).

In conclusion, the use of non-*Saccharomyces* yeasts for bioprotection is a promising alternative to sulfur dioxide in the early stages of winemaking, provided the grapes are healthy. All the results indicate that bioprotection offers:

1/ Partial protection against oxidation phenomena, by limiting early browning of musts through consumption of dissolved O_2, thus preserving GSH concentrations in white wines;

2/ Antimicrobial properties, limiting the relative abundance of certain fungal populations in grape must by competing for space and limiting populations of acetic acid bacteria;

3/ Chemical and sensory properties, characterized by the production of fatty acid ethyl esters, enhancing fruit perception in young wines;

4/ Sensory properties after bottle ageing, enhancing the "fresh blackcurrant" score. ■

1 Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative Methods to SO2 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

2 Windholtz, S., Vinsonneau, E., Farris, L., Thibon, C., & Masneuf-Pomarède, I. (2021). Yeast and Filamentous Fungi Microbial Communities in Organic Red Grape Juice : Effect of Vintage, Maturity Stage, SO_2, and Bioprotection. *Frontiers in Microbiology, 12*, 748416. https://doi.org/10.3389/fmicb.2021.748416

3 Windholtz, S., Dutilh, L., Lucas, M., Maupeu, J., Vallet-Courbin, A., Farris, L., Coulon, J., & Masneuf-Pomarède, I. (2021). Population Dynamics and Yeast Diversity in Early Winemaking Stages without Sulfites Revealed by Three Complementary Approaches. *Applied Sciences, 11*(6), Article 6. https://doi.org/10.3390/app11062494

4 Windholtz, S., Nioi, C., Redon, P., Masneuf-Pomarede, I., & Thibon, C. (2021). *Does bioprotection by adding yeasts present antioxidant properties ?* [Poster]. 8th edition of Macrowine virtual congress. https://www.infowine.com/intranet/libretti/0/19546-38%20Sara%20WINDHOLTZ%20Poster%20Macrowine%202021.pdf

5 Windholtz, S., Nioi, C., Coulon, J., & Masneuf-Pomarède, I. (2023). Bioprotection by non-*Saccharomyces* yeasts in oenology : Evaluation of O_2 consumption and impact on acetic acid bacteria. *International Journal of Food Microbiology*, 110338. https://doi.org/10.1016/j.ijfoodmicro.2023.110338

6 Windholtz, S. *et al.* Non-*Saccharomyces* yeasts as bioprotection in the composition of red wine and in the reduction of sulfur dioxide. LWT 149, 111781 (2021).

7 Pelonnier-Magimel, E., Windholtz, S., Pomarède, I. M., & Barbe, J.-C. (2020). Sensory characterisation of wines without added sulfites via specific and adapted sensory profile. *OENO One, 54*(4), Article 4. https://doi.org/10.20870/oeno-one.2020.54.4.3566