



The Wine EXPERT

Practical Winemaking Information

SO₂ PRODUCTION BY WINE YEAST DURING ALCOHOLIC FERMENTATION

What is sulphur dioxide?

Sulphur dioxide is a molecule commonly known as SO₂. It is used in many dried fruits (figs, raisins, apricots, etc) as an antimicrobial agent and has been used in winemaking by the Romans, when they discovered that burning sulphur candles inside empty wine vessels keeps them fresh and free from vinegar smell.

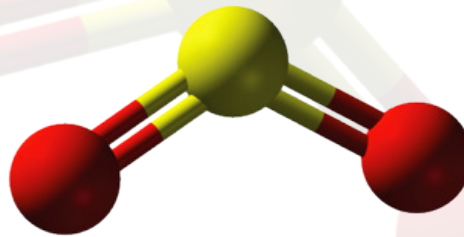


Figure 1: Molecular structure of sulphur dioxide.(SO₂)

Why it is important in wine?

Sulphur dioxide is used during several steps of the winemaking process. It is added to prevent the unwanted developments of microorganisms, as an anti-oxidant, as an antioxidant to inhibit polyphenol oxidases (laccase and tyrosinase) and as a solvent.

However, sulphites can have a negative impact on wine sensory properties, can delay the onset of malolactic fermentation, and can cause some health concerns in case of high concentrations in the final wine. That's why SO₂ levels in wine are regulated. On wine bottles, "contains sulphites" must be displayed on the label when found above 10 mg/L. Consequently, it is important in the winemaking process to control and manage the SO₂ content of wine in order to maintain the lowest possible concentration while preserving its interesting properties.

SO₂ can be added in wines in several forms such as liquid gas, SO₂ solution, potassium metabisulphite powder or effervescent tablets .

SO₂ is not only an exogenous compound, as it can also be produced by yeast as it will be discussed in this document.

The many forms of SO₂

Sulphur dioxide can be found in many forms in wines, and it will have an impact on the final concentration found in the product. It is important to understand the nature of the form that it takes in the wine and the impact it has.

- Free SO₂: the active and most efficient form of the sulphites found in wines. This is the form that will be active as an antimicrobial agent, as well as an antioxidant. It is called free because it is not bound or attached to any other compounds.
- Bound SO₂: When the SO₂ is added to wine or must, a portion will be bound by sugars and by aldehydes (such as acetaldehyde) and by ketones. This form of SO₂ is not active.
- Total SO₂: Free + Bound SO₂.
- Molecular or Active SO₂: the molecular SO₂ is the most active and efficient form of the free SO₂. This form of SO₂ is more precise than the free SO₂ in the degree of protection that it offers to the wine. It's calculated with a formula taking in account pH, temperature, the % of alcohol and the free SO₂. The pH of the wine is one of the main factors intervening in the balance molecular, free and total SO₂. Generally, a concentration between 0,35 mg/L and 0,60 mg/L of molecular SO₂ will allow for a proper protection of the wine.

SO₂ formation during fermentation

Saccharomyces cerevisiae wine yeast, whether selected or spontaneous, will produce SO₂. Wine yeasts are able to produce from a few mg/L of sulphites to more than 90 mg/L, depending on the fermentation conditions and the yeast strain. It was reported by Delteil (1992) that 30% of indigenous wine yeast from Côte Rôtie (France) were strong SO₂ producers. Sulphur dioxide is an intermediate metabolite in the sulfate assimilation pathway (figure 2) leading to sulphur amino acid synthesis. Under certain conditions, it may be synthesized in excess then excreted into the medium. Furthermore, sulphites are precursors for the synthesis of sulphide, a highly undesirable by-product. Although the sulfate assimilation pathway has been widely studied, little is known about the parameters that influence sulphite production, and the molecular basis responsible for the differences between yeast strains has not yet been completely identified.

The best strategies to avoid such situation is 1) to select a wine yeast that will produce very little SO₂, 2) to know if your selected yeast has a high demand for nitrogen during fermentation and 3) to properly manage alcoholic fermentation.

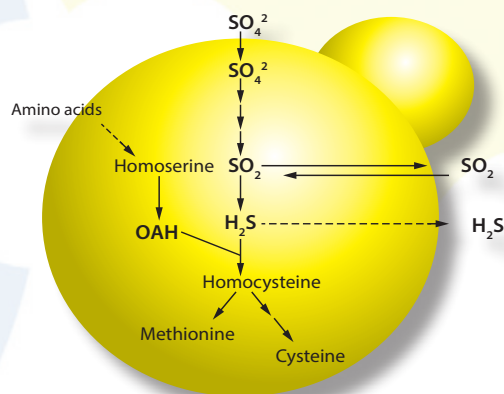


Figure 2 : Wine yeast sulfate assimilation pathway

THE RESULTS

The parameters influencing the production of sulphur compounds by yeast are:

1. Temperature: it has been shown that at low temperature (16 °C), sulphur production is greater than at 28 °C (Figure 3).
2. The wine yeast used: we know that the production of SO₂ by wine yeast is genetically and environmentally determined. All wine yeast, selected or spontaneous, will produce various concentration of SO₂. (Figure 4).

Many wine yeast were characterized based on their SO₂ production in a synthetic media. Figure 4 (on next page) illustrate the range of concentration produced by the different wine yeast from the lowest at 5 mg/L to the highest at 90 mg/L. The concentration of SO₂ produced are those that are intrinsically produced by the wine yeast since there were no sulphur addition to the synthetic must. When selecting a yeast for winemaking, based on the condition of the must, the level of SO₂ added, then this factor can be taken into consideration based on the winemaking itinerary chosen and the wine style desired.

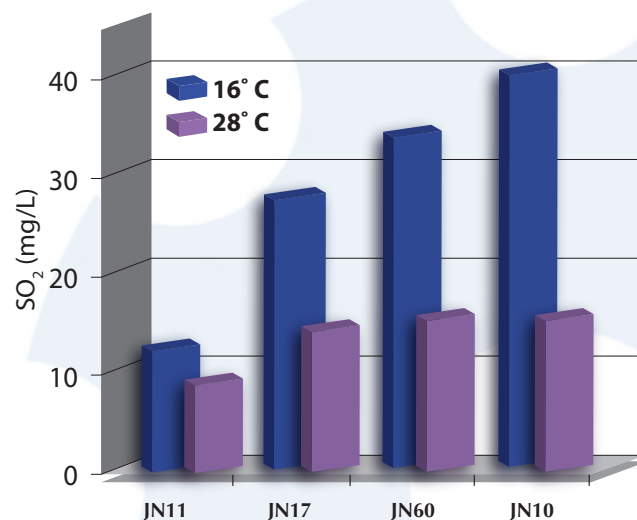


Figure 3: SO₂ production by different wine yeast strain at different temperature

THE RESULTS

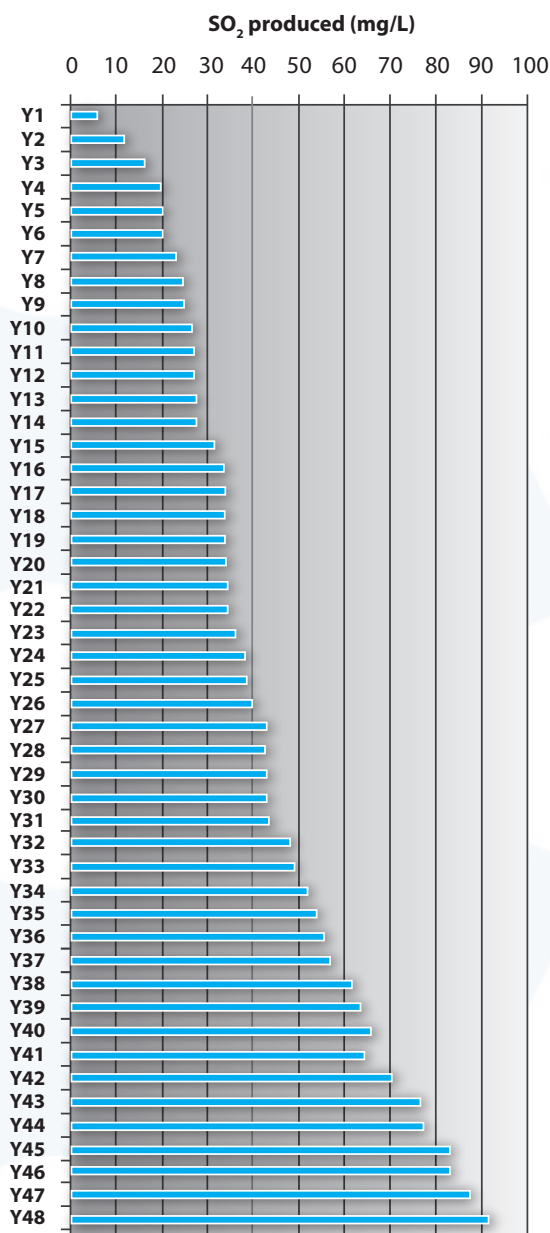
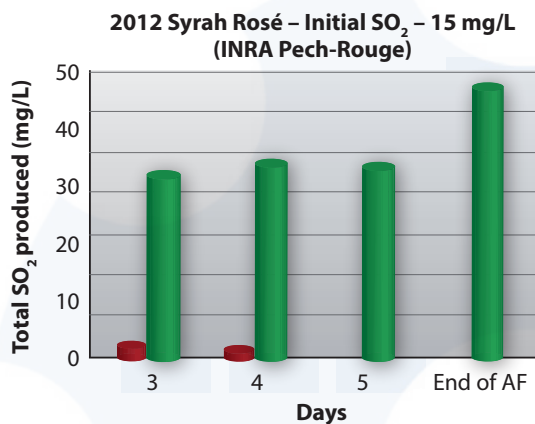
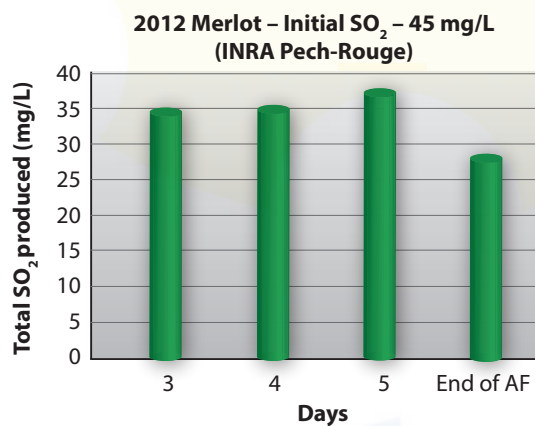
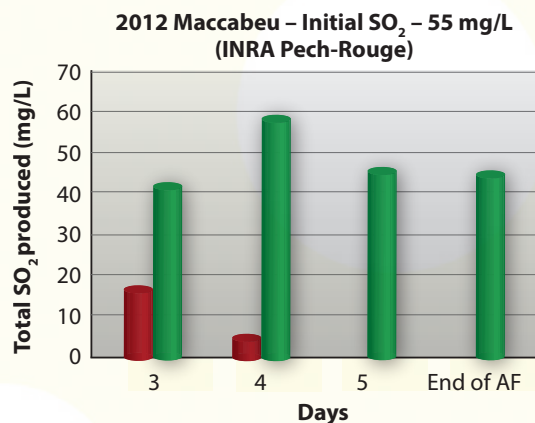


Figure 4: Total SO₂ production by wine yeast in synthetic media MS 300

For example, if malolactic fermentation is desired, and knowing the sensitivity of wine bacteria to SO₂, then a wine yeast producing lower concentration of sulphite can be selected. Recent wine yeast selection has also been focused on finding a micro-organism able to produce less or no SO₂. During a collaborative work between Lallemand, Institut Coopératif du Vin (France) and the SupAgro INRA (France), a natural wine yeast, Lalvin ICV oKay[®] was obtained with a directed breeding strategy approach that produces very low levels of SO₂, H₂S and acetaldehyde (SO₂ binding compound). This wine yeast has shown in all situations, a lower production of SO₂, as seen in Figure 5. In the different wines, Lalvin ICV oKay[®] was in some instances, not producing any SO₂, as shown when there is no red column for

this specific yeast. Oenological and sensory properties of the Lalvin ICV oKay[®] have been shown to be very positive to produce quality wines. Moreover, since this wine yeast produces little or no SO₂ during alcoholic fermentation, malolactic fermentation is compatible when needed.



■ Lalvin ICV oKay[®] yeast
■ Reference yeast

Figure 5: Total SO₂ production during alcoholic fermentation in three wines comparing Lalvin ICV oKay[®] with the reference yeast

Bruno Blondin



Bruno Blondin has a Ph.D. in Food Science from Université de Montpellier (France) and is a professor and Scientific Director at the Supagro (INRA Montpellier). Bruno is the expert on yeast metabolism. He is the author of 42 publications, and 2 patents. He is also an expert at the OIV (Organisation Internationale de la Vigne et du Vin) and he is a member of the scientific council of the IFV (Institut Français de la Vigne et du Vin). His research activities with the microbiology groupe of the UMR Oenological Science INRA-Montpellier Supagro-UM1 are focused on functional genetics of wine yeast, namely the genetics of sulphur compounds produced during fermentation. His work has resulted in understanding the origin and production of sulphur compounds, and help better control their formation. He is responsible for microbiology and biotechnology courses for the Viticulture/Enology diploma at Montpellier Supagro.

A WORD FROM OUR EXPERT

The exogenous supply of SO₂ is not alone in determining a wine's final SO₂ content; in fact, wine yeasts also produce a significant amount of SO₂. In the yeast's metabolism, SO₂ is an intermediate component in the synthesis of sulfur-containing amino acids (methionine and cysteine). This pathway is specifically active in the growth phase—once the very small quantities of sulfur containing amino acids present in the grape must have been exhausted—to meet the anabolic demand for protein synthesis. Nonetheless, certain yeasts can produce amounts of SO₂ that exceed their needs or absorption capacities and are therefore excreted into the medium. Depending on the strain, the production varies between a few milligrams and more than 100 mg/L.

Until now, little was known about the molecular bases responsible for the differences in production among yeast strains. However, a new study recently identified the *MET2* and *SKP2* genes as responsible for controlling the production of SO₂, and also of H₂S and acetaldehyde (SO₂ binding compounds). QTL mapping—a genetic study that identifies regions of the genome involved in this phenotype—has identified new alleles of these genes in a very low SO₂-producing strain.

These two alleles have especially powerful control over SO₂ production as they intervene at two key steps of sulphur metabolism. First, by limiting its synthesis, the *SKP2* gene allele controls, in post translation, the efficacy of an enzyme involved in the synthesis of SO₂. Second, the *MET2* gene allele increases its incorporation by enhancing the synthesis of carbon precursors.

A QUICK SUMMARY

The best strategy for SO₂ management is to keep the lowest efficient level of SO₂ while respecting legal, health and chimerical requirements. Knowing the production of SO₂ by wine yeast is part of the strategy of proper management of SO₂ in wine.

The production of SO₂ by wine yeast is not only regulated by must or fermentation conditions, or by stress factors, but is rather mainly an intrinsic characteristic, genetically determined, that varies from one wine yeast to another. With extensive research to understand and characterize selected wine yeast, we can show the different levels of SO₂ that a wine yeast can produce. When this factor is important in the wine to be fermented, whether for malolactic compatibility, wine style or market need, it can become a criteria for the wine yeast to use in a particular vinification. The new wine Lalvin ICV oKay® is a good choice for alcoholic fermentation when SO₂ production is a concern as it produces little or no SO₂, H₂S or acetaldehyde. Lalvin ICV oKay® is an innovative yeast selection (*Patent pending PTC/IB220131050623*) particularly interesting on white and rosé wines, ensuring low levels of volatile acidity and promoting aromatic esters. It brings freshness and balance in the mouth.

