The Wine EXPERT

Practical Winemaking Information

SO_2 PRODUCTION BY WINE YEAST DURING ALCOHOLIC FERMENTATION

What is sulphur dioxide?

Sulphur dioxide is a molecule commonly known as SO_2. 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.

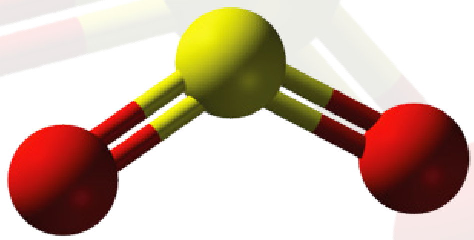

Figure 1: Molecular structure of sulphur dioxide.(SO_2)

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 antioxidasic to inhibit polyphenol oxidases (laccase and tyrosinase) and as a dissolvent.

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 SO_2 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 SO_2 content of wine in order to maintain the lowest possible concentration while preserving its interesting properties.

SO_2 can be added in wines in several forms such as liquid gas, SO_2 solution, potassium metabisulphite powder or effervescent tablets .

SO_2 is not only and exogenous compounds, as it can also be produced by yeast as it will be discussed in this document.

The many forms of SO_2

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 SO_2: 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 SO_2: When the SO_2 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 SO_2 is not active.
- Total SO_2: Free + Bound SO_2.
- Molecular or Active SO_2: the molecular SO_2 is the most active and efficient form of the free SO_2. This form of SO_2 is more precise than the free SO_2 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 SO_2. The pH of the wine is one of the main factor intervening in the balance molecular, free and total SO_2. Generally, a concentration between 0,35 mg/L and 0,60 mg/L of molecular SO_2 will allow for a proper protection of the wine.

SO_2 formation during fermentation

Saccharomyces cerevisiae wine yeast, whether selected or spontaneous, will produce SO_2. 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 SO_2 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 SO_2, 2) to know if your selected yeast has a high demand for nitrogen during fermentation and 3) to properly manage alcoholic fermentation.

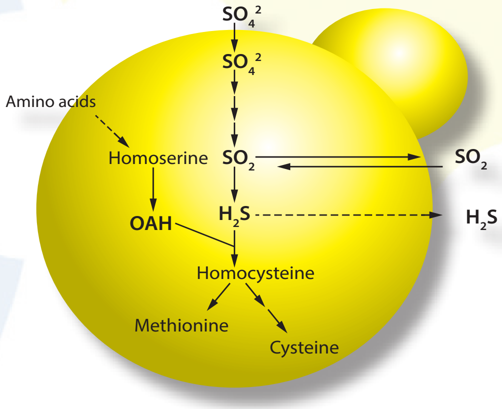

Figure 2 : Wine yeast sulfate assimilation pathway

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 SO_2 by wine yeast is genetically and environmentaly determined. All wine yeast, selected or spontaneous, will produce various concentration of SO_2. (Figure 4).

Many wine yeast were characterized based on their SO_2 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 SO_2 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 winema-king, based on the condition of the must, the level of SO_2 added, then this factor can be taken into consideration based on the wine-making itine-rary chosen and the wine style desired.

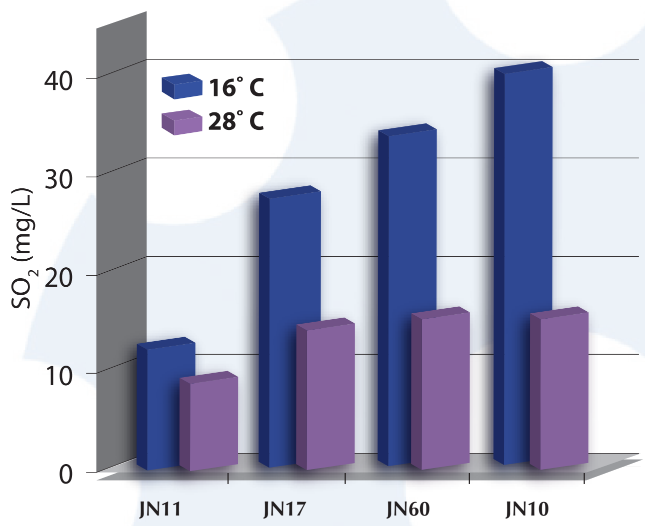

Figure 3: SO_2 production by different wine yeast strain at different temperature

THE RESULTS

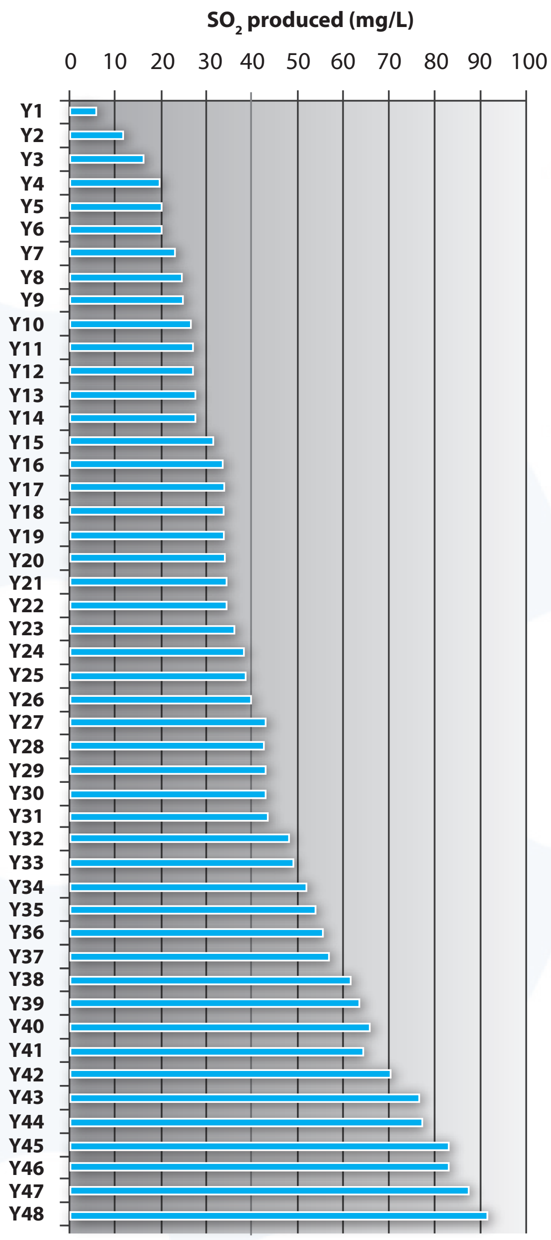

Figure 4: Total SO_2 production by wine yeast in synthetic media MS 300

For example, if malolactic fermentation is desired, and knowing the sensitivity of wine bacteria to SO_2, then a wine yeast producing lower concentration of sulphite can be selected. Recent wine yeast selection has also been focused on finding a microorganism able to produce less or no SO_2. 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 SO_2, H_2S and acetaldehyde (SO_2 binding compound). This wine yeast has shown in all situations, a lower production of SO_2, as seen in Figure 5. In the different wines, Lalvin ICV oKay® was in some instances, not producing any SO_2, 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 SO_2 during alcoholic fermentation, malolactic fermentation is compatible when needed.

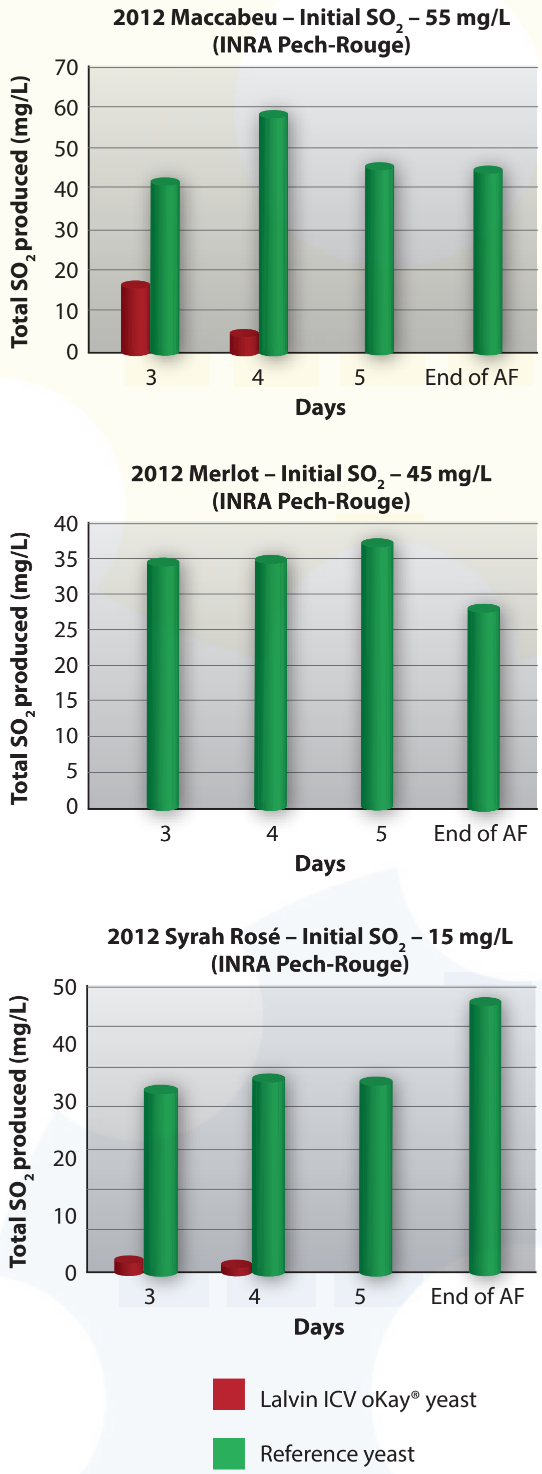

Figure 5: Total SO_2 production during alcoholic fermentation in three wines comparing Lalvin ICV oKay® with the reference yeast

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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). Burno 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 SO_2 is not alone in determining a wine's final SO_2 content; in fact, wine yeasts also produce a significant amount of SO_2. In the yeast's metabolism, SO_2 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 SO_2 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 reBillycently identified the *MET2* and *SKP2* genes as responsible for controlling the production of SO_2, and also of H_2S and acetaldehyde (SO_2 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 SO_2-producing strain.

These two alleles have especially powerful control over SO_2 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 SO_2. Second, the *MET2* gene allele increases its incorporation by enhancing the synthesis of carbon precursors.

A QUICK SUMMARY

The best strategy for SO_2 management is the keep the lowest efficient level of SO_2 while respecting legal, health and chimerical requirements. Knowing the production of SO_2 by wine yeast is part of the strategy of proper management of SO_2 in wine.

The production of SO_2 by wine yeast is not only regulated by must or fermentation conditions, or by stress factors, but is rather mainly an intrinsic characteristics, 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 SO_2 that a wine yeast can produced. 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 SO_2 production is a concern as it produces little or no SO_2, H_2S 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.

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