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.

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 sul- phites” 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 an 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.
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.

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 environmentally 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 winemaking, based on the condition of the must, the level of SO$_2$ added, then this factor can be taken into consideration based on the winemaking itine-rary chosen and the wine style desired.

Figure 2: Wine yeast sulfate assimilation pathway

Figure 3: SO$_2$ production by different wine yeast strain at different temperature
THE RESULTS

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$_2$S 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 4: Total SO$_2$ production by wine yeast in synthetic media MS 300

Figure 5: Total SO$_2$ production during alcoholic fermentation in three wines comparing Lalvin ICV oKay® with the reference yeast
A QUICK SUMMARY

The best strategy for SO₂ management is the 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 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 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.