The use of Sulphur Dioxide in Must and Wine

Sulphur dioxide (SO2) is the most widely used and controversial additive in organic winemaking.
The use of sulphur dioxide (SO_2) in organic winemaking is one of the most critical point and the differences concerning the SO_2 use among the different countries and wine producing.

Sulphites are naturally produced by the yeasts during the wine processing (ORWINE: Code of good practice, 2009). The addition of SO_2 is traditionally considered as an efficient method to protect and preserve the wine at different stages of its elaboration.

Sulphitation is allowed by all the standards for organic wine processing, but with restrictions compared to the wine regulation.

The European Wine Regulation fixes total SO_2 maximum doses in the end product; they vary according to wine types, and notably in relation to the presence of residual sugars, going from 150mg/l for red wines to 400mg/l for sweet wines from Botrytised grapes, such as Sauternes, Beerenauslese, Ausbruch, Tokaj. Additional quantities, up to 40 mg/l of SO_2, are allowed "*when approved for all wines except those with final rates upper than 300 mg/l of* SO_2 *end*".

Nature of SO2 permitted additives

- **pure SO_2** under gas or liquid formula (E220)
- **Potassium- metabisulphite** (E224)
- **Potassium- bisulphite** (E228)

Relevance of SO_2 in wine-making
Sulphur dioxide (SO_2) is an antimicrobial compound that has been used in winemaking for centuries.
It is widely used as preservative, generated originally by burning sulphur but later obtained by adding sulphite or bisulphite. It greatly improved fermentation processes by inhibiting the growth of undesirable bacteria and yeasts; furthermore it inactivates certain enzymes during the wine making process (Romano and Suzzi 1993).
Its many properties make it an indispensable aid in winemaking.
Yeasts, themselves, produce SO_2 during fermentation of grape juice, so it is very unlikely that SO_2-free wine could ever be produced (**results from ORWINE-WP3 – see below**). The formation of SO_2 is a strain characteristic and it can be produced by "natural – spontaneous" yeast as well as commercialised selected yeasts. There are significant strain specific differences. Most of the yeast strains (*Saccharomyces cerevisiae*) produce 10-20 mg/L SO_2 during fermentation. A smaller amount of strains is able to produce more than 30-40 mg/L SO_2 or even more (Eschenbruch 1974, Suzzi and Romano 1982, Suzzi et al. 1985). This can increase the total amount of bound SO_2 that can cause too high levels with regard to the legal limits. On the other hand an increased amount of bound SO_2 can also inhibit the development of lactic acid bacteria which were necessary for malolactic fermentation which is used to degrade acid levels in wines with a biological treatment.
Therefore the selection of yeast strains with a low SO_2-formation is recommended for winemaking.
Furthermore a high formation of acetaldehyde (ethanal) by yeasts during fermentation has an impact on bound SO_2-levels. Acetaldehyde is one of the most important binding compounds of SO_2.
Formation of acetaldehyde by yeasts is affected by the yeast strain, fermentation conditions and nutrient supply in the grape must.
Therefore a yeast strain with a low ability to form SO_2 and with a low requirement for nutrients is supposed for wine production as well as a sufficient nutrient composition in the grape must.
Sometimes a high SO_2-formation of yeasts is linked with a high production of hydrogen sulphide and other undesirable volatile sulphur compounds that can cause off-flavours in wine (Romano and Suzzi 1993).

Consequently, the total absence of sulphur dioxide in wine is rare, even in the absence of SO_2 use (sulphiting).

The principal properties of SO_2 are as follow:

1) **Antiseptic:** The Molecular SO_2 inhibits the development of micro-organisms. It has a greater activity on bacteria than on yeasts. The effectiveness of a given concentration is increased by lowering the initial population. During storage, SO_2 hinders the development of all micro-organisms (haze formation by yeasts, re-fermentation of sweet wines, unintentional malo-lactic fermentation, various bacterial spoilages).
The antiseptic action of SO_2 looks like specific for wine-making: Most of the undesirable bacteria and yeasts are more sensitive to SO_2 than the wine yeast *Saccharomyces cerevisiae* except *Kloeckera apiculatus,* which develop before the others, produce lower quality wines with lower alcohol (Romano and Suzzi, 1992).
The antiseptic action of SO_2 with respect to yeasts can appear in different ways. In one hand it can be stop the fermentation of sweet wines (mutage -traditionally used in Germany). On the other hand, it protects these same wines from possible re-fermentations (Troost 1980, Fischer 2003). The subsequent disappearance of free SO_2 permits the revival of yeast activity. Bound sulphur dioxide does not have antiseptic action on yeasts.
One of the principal roles of sulphiting in wine-making is to obtain must much less susceptible to bacterial development, while undergoing a normal alcoholic fermentation. This protection is most necessary in the case of must that are rich in sugar, low in acidity, high in pH and high in temperature. The risk of bacterial infection (undesirable malo-lactic bacteria, acetic acid bacteria) and of stuck fermentation are highest at these cases.

Sulphur dioxide is fungistatic at high pHs and at low concentrations, and it is a fungicide at low pHs and high concentrations. At normal pH levels (3,2 – 3,5), HSO_3^- is the main form present in musts and wines, and it is exclusively fungistatic.

2) **Antioxidant:** SO_2 protects wine from chemical and enzymatic oxidation.
The chemical consumption of oxygen by SO_2 is slow. Such oxidation requires the presence of catalysers, notably iron and copper ions (Ribéreau-Gayon et al 2000).
The inactivation by SO_2 of certain oxidation enzymes is one of the famous action of SO_2 in wine-making. SO_2 inhibits the functioning of oxidation enzymes (tyrosinase, laccase) which excessively intense the oxidation of the phenolic compounds of must and wine. It prevents madeirization, browning of phenolic compounds.
The anti-oxygen effect of sulphur dioxide is involved in wine storage, its role is insignificant during winemaking. In this case, SO_2, protects against oxidations by destroying oxidase or, at least, blocking their activity, if destruction is not total.
In must, enzymatic oxidations are more significant than chemical oxidations because they are more rapid. In wine, however, chemical oxidations play an unquestionable role, since oxidative enzymes no longer exist.

3) **Binding of Acetaldehyde** (Ethanal) and other similar products (e.g. pyruvic acid). In this case SO_2 protect wine aromas and makes the flat character disappear. Acetaldehyde is one of the most important binding compounds of SO_2. Other substances likely to fix small amounts of SO_2 are gluconic -, galacturonic- or pyruvic acid etc.. SO_2 can also bind with phenolic compounds of red wines and the reaction is directly visible by the decoloration produced. The reaction is reversible: the colour reappears when the free SO_2 disappears.

3.3. SO_2 – Management (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

Relevance of the use of sulphur dioxide in musts and wines

Sulphites are nowadays considered as fundamental additives in different stages of wine production for their antimicrobial, antioxidant and anti-oxidising activity.

In musts and wines sulphur dioxide inhibits the growth of bacteria and wild yeasts whilst selected yeasts (*Saccharomyces* ssp.) show a certain tolerance toward the additive. This is very important from the technological point of view as it determines the predominance by the selected strains in the fermenting medium (selected just on the basis of their resistance to SO_2).

In addition to their activity in the selection of fermenting micro-organisms, sulphites have other important microbiological effects. Bacteria are very sensitive to sulphur dioxide and for this reason sulphiting is a good technique for avoiding malolactic fermentation (when not desired) as well as for reducing the risk of microbial pollutions (e.g. development of acetic bacteria or uncontrolled lactic fermentations).

The antioxidant action of SO_2 in musts consists mainly in the inhibition of enzymatic oxidations. The addition of sulphites stops the oxygen consumption in the must itself by the inhibition of the enzymes which catalyse the oxidation of phenolic compounds (polyphenol-oxydases). One of these enzymes, normally present in the grape (tyrosinase), is totally inactivated by a relatively low addition of sulphur dioxide (approx. 50 mg/L), whilst another enzyme, produced by *Botrytis cinerea* and derived from rotten grapes (laccase) is less sensitive to sulphur dioxide. Hence the risk of browning and oxidations is higher in the musts produced from botrytized grapes.

Another advantage related to the use of sulphites in the early steps of wine-making process is their ability to bring about a greater extraction of anthocyanins and phenolics during the maceration of red grapes. Sulphur dioxide can denature some proteins, located in the membranes of the grape skin cells, producing micro-leaks and improving the extraction of colouring matter. Moreover, sulphur dioxide can bind anthocyanins making them more soluble and extractable, especially in a water-alcoholic medium. The problem of this kind of interaction is the slight loss of wine colour that resulting fact. The resulting must formed by the interaction of sulphur dioxide with colour compounds (anthocyanins), is not coloured.

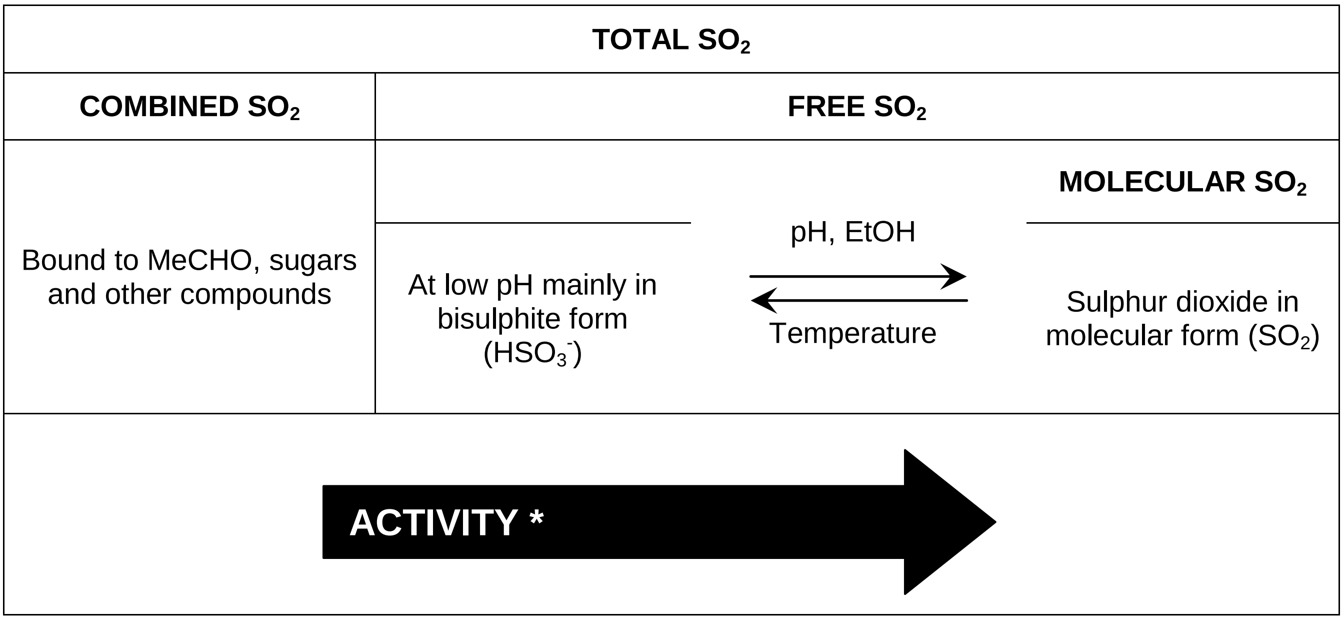
If antioxidising activity is mainly affecting the must and the inhibition of enzymes its use in the completed wine is based on its ability to directly react with oxygen in the presence of metallic catalysts (such as iron or copper). This kind of reaction reduces the oxygen availability in the medium and its ability to react with other substances (e.g. polyphenols).Thus sulphur dioxide is particularly important in the conservation of wine.

State of sulphur dioxide in musts and wines

In must and wine sulphur dioxide are in equilibrium between different forms viz. total SO_2, free SO_2 and molecular SO_2.

Different compounds (sugars, carbon compounds) are able to act as SO_2-binding molecules. Acetaldehyde (MeCHO) is the most reactive. The product formed by its interaction with the bisulphite ion is stable and its formation reduces the activity of the additive with regards to both its antimicrobial action and its antioxidant properties. The fraction of SO_2 bound by acetaldehyde and other compounds represents the combined fraction of the additive itself.

The following Figure 79, describes explain the equilibrium of sulphur dioxide in musts and wines.



*** Against micro-organisms, polyphenoloxydasic enzymes and molecular oxygen**

Fig. 79: Schematisation of the equilibrium of sulphur dioxide in wine

In wine at low pH, free sulphur dioxide is mainly present as the bisulphite ion (HSO_3^-); even though this form shows a good activity both against the micro-organisms and against oxidation, the most active form of the additive is the molecular one (SO_2).
The percentage of free sulphur dioxide in molecular form depends on the pH, being higher when the pH is lower. Thus the effects of sulphites are more intense when the pH is low. Alcoholic degree and temperature also affect the equilibrium between bisulphite ions and molecular SO_2 the molecular fraction increases at higher alcoholic concentrations and temperatures.
As already mentioned acetaldehyde is the most important SO_2-binding compound in must and wine. Some yeasts strains can produce MeCHO as a reaction to the presence of high levels of sulphites in their growing medium; this means that when sulphur dioxide is added in high amounts to the must it can cause an increase of acetaldehyde production by the yeasts and, as a consequence, a lower ratio between free and total SO_2 at the end of alcoholic fermentation[1].
For this reason, wine-makers are inclined to limit the use of sulphites before alcoholic fermentation, with the advantage of reducing acetaldehyde production. This results in a more favourable ratio between free and total SO_2, and, consequently, to a wider margin of action as regards any subsequent addition of the additive.

Toxicity of sulphites

Despite the fundamental reactions outlined above sulphur dioxide is well known as a poisonous and allergenic substance (LD_{50}: 0,7-2,5 mg/kg b.w. depending on animal species; maximum daily intake: 0,7 mg/kg b.w.[2]), and for this reason it could have a strong impact on the perception of the consumers as regards human health.
Based on EC Regulation 1991/2004, sulphites must be declared on the label if their overall content in wine is higher than 10 mg/L. This represents a serious problem for wine producers (when speaking about the opportunity to reduce the levels of SO_2) and it is an important issue particularly for the “organic sector”. Even conventional wine-makers are oriented

[1] Increase of sulphur dioxide in combined form; for example, 100 mg/L of total SO_2 added before alcoholic fermentation can become, at the end of sugar depletion, 60-70 mg/L, with less than 10 mg/L in the free form.
[2] Ribéreau-Gayon et al., 1998. Traité d'Œnologie. Microbiologie du vin, Vinifications. Vol. I. Dunod, Paris.

towards a lowering of the amount of SO_2 in their products and perhaps the consumers expect to find only minor levels of the additive in wines from organic viticulture. Moreover, some questions related to the use of sulphites in oenology are still undecided. For example: "How much is it possible to reduce sulphur dioxide levels without risking taste and quality degradation or increasing microbial contamination or oxidation during the vinification or the storage in barrels or bottles?"

5.4. Natural production of SULPHITE (SO_2) by yeast during alcoholic fermentation

(Werner, M.; Rauhut, D.)

During alcoholic fermentation yeasts naturally produce sulphur dioxide (SO_2) as a metabolic intermediate of the sulphate reduction pathway (Romano and Suzzi (1993), Ribéreau-Gayon et al., (2006)). Yeast strains can be categorized into ***low SO_2 producers*** i.e. *Saccharomyces cerevisiae* var. *ellipsoideus* and ***high SO_2 producers*** i.e *Saccharomyces bayanus* Sacardo. Certain yeast strains can produce up to 300 mg/L of sulphite during fermentation. Dott and Trüper (1976) described that the sulphite reductase of the sulphite-producing yeast strains might be altered. As a consequence sulphite (SO_2) will be accumulated in the cell and finally be released into the must. Former assumptions about mutations being the cause of the sulphite production could not be confirmed. Today producers of commercial dried yeast consider this important property of the yeast during the selection process. It is only when wine-makers wish to induce a spontaneous fermentation that the properties of the fermenting yeast strains cannot be guaranteed. The majority of today's commercial yeast strains are considered to be low SO_2 producers, showing a production up to 20 mg/L of total SO_2. Only few yeast strains appear to have a higher production (up to 80 mg/L SO_2).

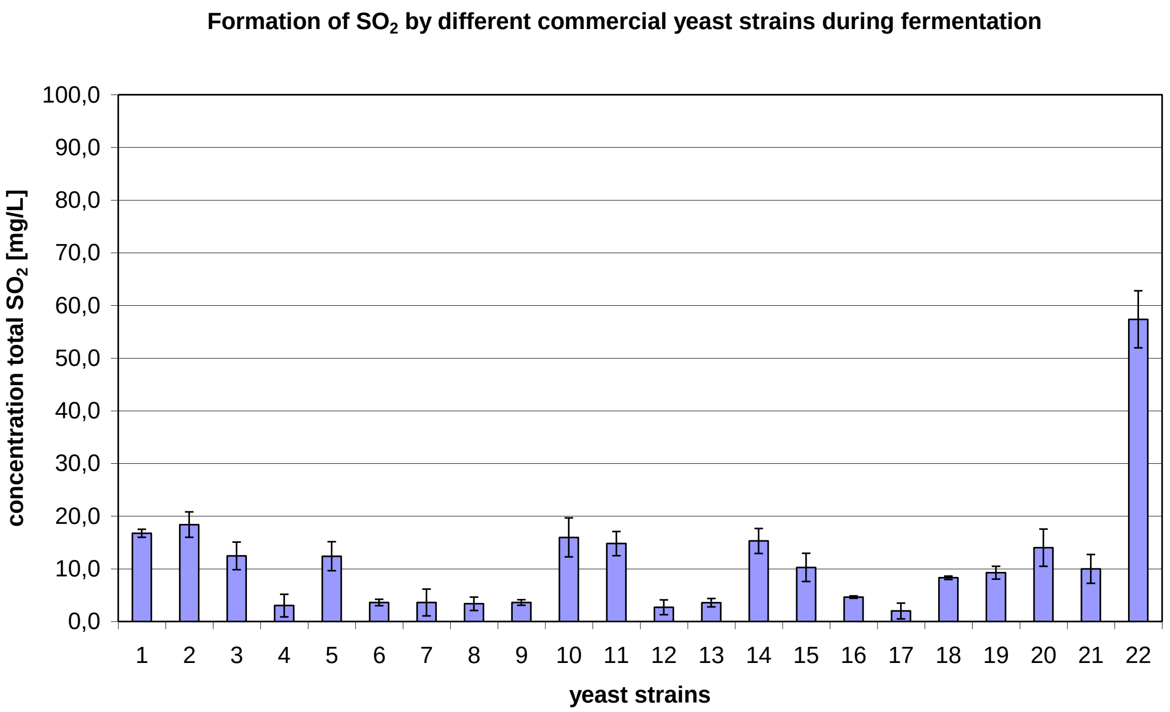


Fig. 87: Production of SO_2 by 22 commercial yeast strains during fermentation. Mean value of the triplicate. Bars show the standard deviation.

Figure 87 shows the SO_2 production of 22 commercial yeast strains used in Europe. No. 1 to 21 were recommended by the yeast producers as low SO^2 producers. No. 22 is a reference strain with a high SO_2 production. The fermentations were performed with 2007 Riesling must, which was pasteurised in order to eliminate any undesired micro-organisms. The fermentation temperature was 18°C, the inoculation dosage was 30 g/hl pure dried yeast. Rehydration was done by water (35°C) for 25 minutes. The results show predominantly two groups of yeast strains. One group produces under 10 mg/L total SO_2, the other group produces between 10 and 20 mg/L total SO_2. Only one yeast strain reaches a concentration of 57 mg/L of total SO_2.

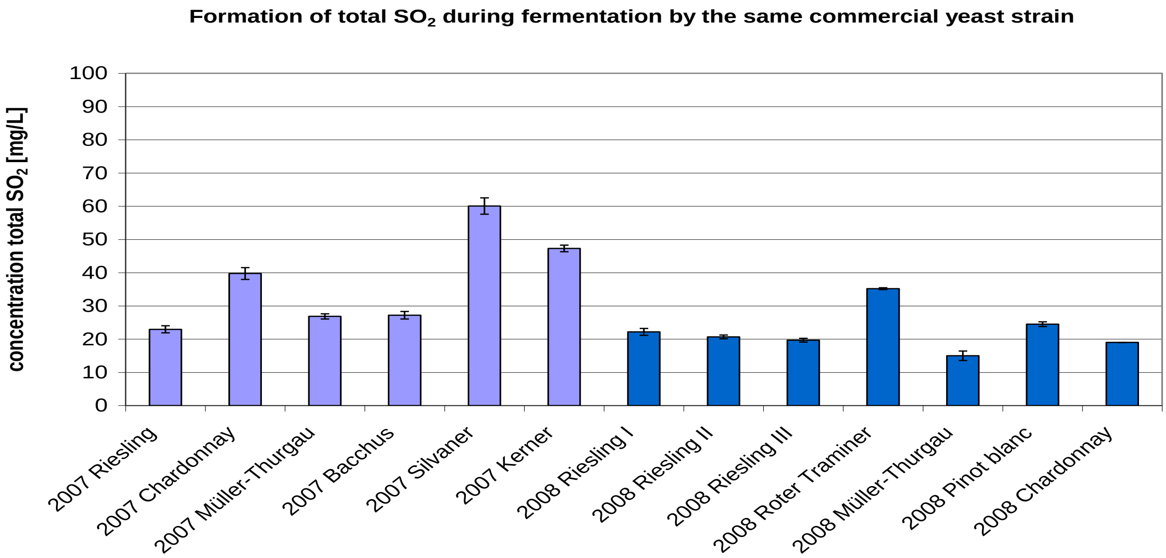


Fig. 88: Production of SO_2 by one commercial yeast strain during fermentation in must from different grape varieties. Mean value of the triplicate. Bars show the standard deviation.

Figure 88 shows the concentration of SO_2 after the alcoholic fermentation by the same commercial yeast strain in must from different grape varieties (vintage 2007 and 2008). Fermentation conditions were the same as for the comparison of yeast strains. All the different grape juices were pasteurised, in order to eliminate any undesired micro-organisms. The results show that the formation of SO_2 during fermentation depends also on the yeast variety and the composition of the grape juice. The grape juices in figure 88 were all fermented with the same commercial yeast strain, but the concentration of total SO_2 varies from 15 to 60 mg/L after the alcoholic fermentation. This indicates that even a yeast strain that is considered as a low SO_2 producer can produce higher concentrations in certain grape juices in certain years.

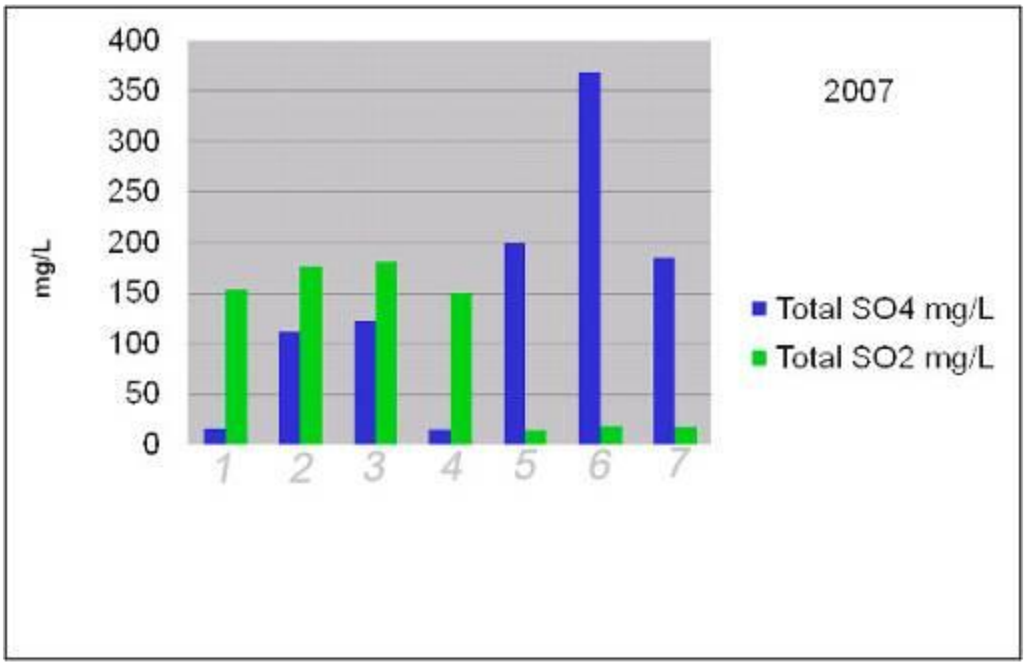


Fig. 89: Production of SO_2 by two different commercial yeast strains during alcoholic fermentation in Chardonnay must with the addition of ammonium sulphate and ammonium phosphate.

Variant 1-4: yeast strain 1; variant 5-7: yeast strain 2; variant 1 and 5: control; variant 2, 3 and 6: addition of ammonium sulphate, variant 4 and 7: addition of ammonium phosphate. Source: partner IFV.

Figure 89, shows that the concentration of sulphate plays an important role in SO_2 production during the alcoholic fermentation. Sulphate is present in the natural must or it can be introduced by the addition of ammonium sulphate, a nutrient. Alternatively ammonium can be added as ammonium phosphate. As the results in figure 89 show, not every yeast strain has the same ability to produce SO_2 on the basis of SO_4. Yeast strain 2 does not use sulphate, neither the natural nor the added sulphate in a relevant amount. This explains why this yeast strain can be considered as a low SO_2 producer. The yeast strain 1 shows a high ability to produce SO_2 on the basis of SO_4, even if it is only naturally present in the must. This yeast strain can be considered as a high producer of SO_2.These results were only obtained in white and rosé wines.

The sulphur dioxide produced by the yeast will be bound to SO_2 binding compounds. Thus it will be included in the estimate of the amount of total SO_2 in the wine, which is limited by regulations, but it will not be available as active free SO_2. The final requirement for SO_2 by the specific wine is determined by many wine compounds, such as acetaldehyde, 2-keto-glutarate and pyruvate, but also the amount of sugar. Only by adding an adequate amount of sulphur dioxide will the wine be finally protected by a certain amount of active free SO_2.

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