

SULPHUR DIOXIDE MANAGEMENT IN LOW INPUT WINEMAKING

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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-oxidasic activity.

In musts and wines sulphur dioxide inhibits the growth of bacteria and wild yeasts whilst selected yeasts (*Saccharomyces* spp.) 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 (selected just on the basis of their resistance to SO₂) in the fermenting medium.

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 SO₂ 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: in fact, the adduct formed by the interaction of SO₂ with color compounds (anthocyanins), is not colored.

If antioxidant activity is mainly related to the must, in wine, the activity of sulfites is not connected to the inhibition of enzymes, but to the ability of the additive to directly react with oxygen in 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.

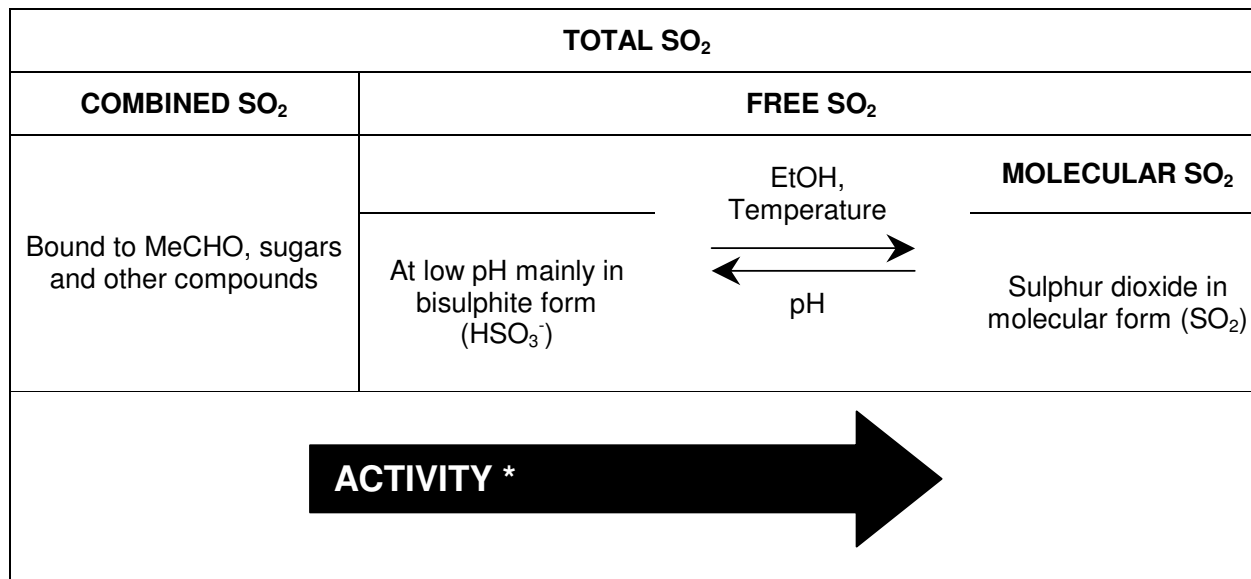
State of sulphur dioxide in musts and wines

In must and wine sulphur dioxide is in equilibrium between different forms viz. total SO₂, free SO₂ and molecular SO₂.

Different compounds (sugars, carbon compounds) are able to act as SO₂-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 SO₂ bound by acetaldehyde and other compounds represents the combined fraction of the additive itself.

The following Figure 1 describes the equilibrium of sulphur dioxide in musts and wines.

Figure 1: Schematisation of the equilibrium of sulphur dioxide in wine



* Against micro-organisms, polyphenoloxidase enzymes and molecular oxygen

At wine pH, free sulphur dioxide is mainly present as bisulphite ion (HSO₃⁻); 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 (SO₂).

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 SO₂; the molecular fraction increases at higher alcoholic concentrations and temperatures.

As already mentioned acetaldehyde is the most important SO₂-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 SO₂ at the end of alcoholic fermentation¹.

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 SO₂, 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 (LD₅₀: 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 SO₂) and it is an important issue particularly for the "organic sector". Even conventional wine-makers are oriented towards a lowering of the amount of SO₂ in their products and perhaps the consumers expect to find 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

¹ Increase of sulphur dioxide in combined form; for example, 100 mg/L of total SO₂ 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'Oenologie. Microbiologie du vin, Vinifications*. Vol. I. Dunod, Paris.

levels without risking taste and quality degradation or increasing microbial contamination or oxidation during the vinification or the storage in barrels or bottles?"

REDUCING SO₂ LEVELS

Nowadays different alternative practices and additives could be used in reducing the use of sulphites in wine-making, but the complete elimination of sulphites is, at the moment, still not possible.

The feasibility of sulfur dioxide reduction depends on the step of the vinification; in fact, not in all the phases of the production process suitable technologies are available to partially replace or reduce the additive.

In the following pages, a short review of the available alternatives to SO₂ (practices and additives) are presented in order to explain their usage

Correct management of selected yeasts inoculation

Performing alcoholic fermentation with no added sulphites means that the inoculation of selected yeasts takes place in a medium highly contaminated by wild micro-organisms. In these conditions, wild yeasts and lactic bacteria may grow and consume the assimilable nitrogen (YAN) which is a basic source of nutrition for *Saccharomyces* yeasts. This consumption occurs in the juice just in the first hours after pressing and generally leads unavoidably to a sluggish fermentation process.

In order to avoid this situation, when no SO₂ is used before alcoholic fermentation, a very early inoculation of the selected starter culture is strongly recommended. This practice allows *Saccharomyces* dominance during fermentation because the adaptation phase of the selected starter culture will be reduced. Obviously, the preparation of the starter should be done in strict accordance with the supplier instructions:

1. rehydration of the active dry yeast powder in warm water (35-40 °C) for 10-15 minutes;
2. eventual addition of nutrients during rehydration (e.g. yeast walls and thiamine, which are important growing factors for the yeasts);
3. careful addition of subsequent small aliquots of juice and agitation, to facilitate respectively the yeast acclimatization, and the production of fatty acids and sterols (fundamental factors for yeast metabolism);
4. Addition of the starter culture to the rest of the must.

When the fermentation is managed without sulphur dioxide, the control of yeast assimilable nitrogen is also recommended. Musts from organic grapes are generally not very rich in YAN and so need to be reintegrated, if possible, before yeast inoculation.

These actions (early inoculation of selected yeasts and control of YAN levels) reduce the risk of stuck or sluggish alcoholic fermentation and allow the complete transformation of the sugars even if no sulphites are added. Moreover, a lower addition of SO₂ before fermentation can reduce the production of acetaldehyde and so decrease the take up of the additive and so improve its potential activity in the later steps of the wine-making process.

Yeast – lactic bacteria co-inoculation

This recently introduced practice permits an effective and simultaneous management of both alcoholic and malolactic fermentation. For further details on this technology the reader can refer to a specific manuscript published on this journal ("Strategies to reduce SO₂ use in early phases of winemaking").

Lysozyme

Sulphur dioxide is able to affect bacteria metabolism, and for this reason, it represents one of the main tools in preventing microbial infection, as well as the behavior of malolactic fermentation when it is not desired.

From this point of view, according to different studies, a suitable alternative to sulphites is Lysozyme (500 mg/L of this egg derived protein have the same effect on lactic bacteria³ than 40 mg/L of SO₂; Gerbaux et al., 1997⁴).

In contrast to sulphites, this preservative is particularly active at high pH values and so it can be helpful in certain critical conditions which are propitious for microbial growth.

The use of Lysozyme should be carefully considered as its protein nature may cause an interaction with phenolic compounds with the consequent loss in colour of the red wines. Furthermore it may cause protein instability in white wines.

Lysozyme is extracted from eggs, and for this reason it can be an allergen. The risk connected with its use in wine-making is due to the persistence of its activity for different times after application. According to Bartowsky and co-workers⁵ (2004) 75-80 % of the initial activity is still detectable in white wines (Riesling) after six months, whilst no residual activity was detectable in red ones after only two days.

Hyper-oxygenation and hyper-reduction technologies

Hyper-oxygenation practice and hyper-reduction technologies can be also used to reduce levels of SO₂ in musts. The former consists in a massive addition of oxygen or air with the purpose of completely oxidising all the unstable substances. Hyper-reduction is based on the addition of ascorbic acid or other antioxidants to protect the must itself from oxidative reactions.

More detailed information on these techniques are available in two other specific manuscripts published on this journal ("Strategies to reduce SO₂ use in early phases of winemaking" and "Oxygen and Wine").

Conservation under inert gases

The direct reaction between sulphites and molecular oxygen is slow and requires the presence of catalysts such as iron or copper. In must this kind of reaction is not really important, because of the faster oxidations catalysed by polyphenoloxidases; on the contrary, in wine, despite its relative slowness, it can compromise the quality of the wine during aging.

Thus it is extremely important to maintain the containers (both steel tanks and wooden barrels) completely full during wine storage, to minimise the presence of atmospheric O₂ in the headspace of the tank itself. The use of inert gases such as nitrogen or argon can be useful in the management of the wine level inside steel tanks. These gases (as opposed to others such as carbon dioxide) show a low solubility in the wine itself and are able to significantly reduce the concentration of oxygen in the headspace, minimising the risk of oxidation.

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³ Lysozyme is not active on acetic bacteria or yeasts; it acts only against lactic bacteria

⁴ Gerbaux et al., 1997. Use of lysozyme to inhibit malolactic fermentation and to stabilize wine after malolactic fermentation. *Am. J. Enol. Vitic.*, 48: 49-54.

⁵ Bartowsky et al., 2004. The chemical and sensorial effects of lysozyme addition to red and white wines over six months cellar storage. *Australian Journal of Grape and Wine Research*, 10: 143-150.