AGROVIN

MICROSTAB PROTECT

Alternative to SO_2 usage

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Due to the increased demand of minimizing the use of sulfur dioxide wineries have been open to consider alternatives to minimize its usage.

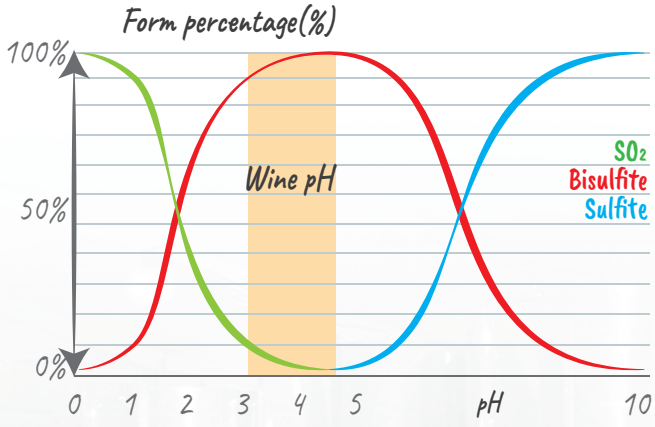
The efficiency of sulfur dioxide in winemaking

With only a few alternatives on the market, the antioxidant and microbial effects as well as oxidation protection make Microstab protect essential usage in the winery.

With minimal usage in the winery, sulfur dioxide is mainly focused in reducing the effects of oxidation. It can regenerate certain polyphenols in their reduced form and mixes well with acetaldehyde reducing its olfactory impact. Due to this effect, some wines would not be appropriately protected from oxidation. With high levels of acetaldehyde where sulfur dioxide mixed significantly, it is difficult to reach the desired levels of free sulfur dioxide. Furthermore, as demonstrated by Jackowetz et al. (2012), adding sulfur dioxide during fermentation within the wine yeasts.

On the other hand, this property is quite effective inhibiting oxidase enzymes' activity, especially tyrosinase, naturally present in grapes. At the same time, its effect on the laccase of grapes contaminated by botrytis is not so effective.

The antimicrobial effect of sulfur dioxide strongly influences the pH because it significantly impacts microorganisms.



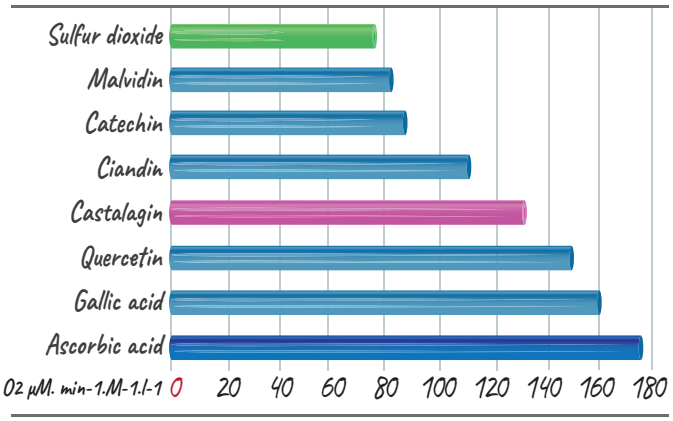
Picture 1: The balance between the different forms of SO_2 according to the pH of the wine.

Sulfur dioxide available alternatives:

Antioxidant effect

There are two antioxidants that have been in use in winemaking for quite some time. The first being tannins. Tannins have different antioxidant properties depending on their biological origin, but its is limited due to its organoleptic effect on the wine and cannot be added in large proportions.

The latter antioxidant would be ascorbic acid primarily used in early stages on winemaking due to its high reactivity against oxygen.



Picture 2: Tannin and ascorbic acid reactivity to oxygen.

The main disadvantage of ascorbic acid usage is that hydrogen peroxide is one of the resultants formed after its oxidation reaction. This peroxide is very oxidizing, and due avoid further oxidation; it needs an sulfur dioxide integration.

A new antioxidant, **glutathione**, has appeared in recent years. It is a tripeptide naturally present in the cells with strong antioxidant power. Its usage is permitted only in the form of inactive yeasts naturally enriched with glutathione.

Its antioxidant effect is carried out in three levels:

1. **Direct antioxidant.** Due to its low RedOx potential it can react with existing oxidants contained in the wine, protecting the desirable components from oxidation.

2. **Color protection**, GSH can join oxidized polyphenols giving rise to the GRP (Grape Reaction Product). This colorless compound, whish is not a substrate for polyphenol oxidase, prevents the browning of wines.

3. **Aroma protection**, Quinones are very reactive with thiols which causes a degradation in aromas. The GSH when joining with the Quinones prevents them from reacting with the thiols thus protecting aroma and oxidation.

Picture 3: Triple antioxidant effect of sulfur dioxide.

Oxidation protection

A series of tannins have been developed as an alternative to inhibit the oxidase effect of sulfur dioxide. These tannins react by precipitating proteins and are capable of inhibiting specific enzymatic processes. Gallic tannin, used during any production stage, inhibits tyrosinase's action and can also react with enzymes produced by botrytis, which do not react with sulfur dioxide.

Antimicrobial effect

There are also alternatives to SO_2 with and antimicrobial effect. DMDC (Dimethyl decarbonate) permits the effective yeast destruction. However, its protection is not prolonged due to rapid hydrolysis. Methanol is a product of its degradation and it is toxic to living beings apart from having stringent legal limits.

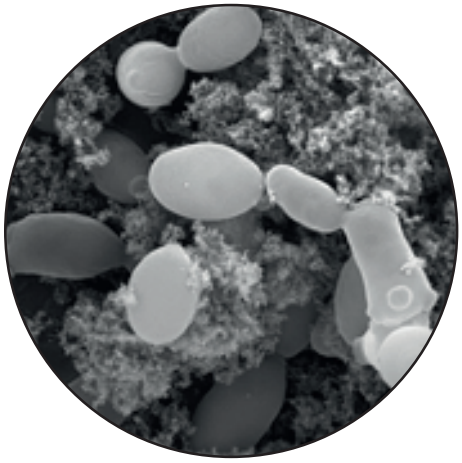
On the other hand, lysozyme is an enzyme widely distributed among living beings as an antibacterial protection mechanism. Its activity consists of breaking the Gram-positive bacteria cell walls, including lactic bacteria (*Oenococcoccus, Pediococcus* and *Lactobacillus*). Lysozyme is not active against Gram-negative bacteria such as acetic bacteria because its cell wall structure is different and resistant. It is not active on yeasts and therefore does not influence alcoholic fermentation. The effectiveness of lysozyme depends not only on the type of bacteria but also on the number of bacterial cells presented in the medium. Contrary to SO_2, lysozyme is more effective at high pH, when lactic bacteria's growth is more favored.

Sorbic acid of potassium sorbate is a preservative with a fungicide effect used mainly to avoid re-fermentation in sweet wines. It is not effective against Brett in the standard doses (10-25 g/hl). It is not suitable for red wines due to its instability in the presence of lactic bacteria's, which are capable of degrading sorbic acid to geraniol. Therefore, its usage is necessary to ensure a high level of free sulfur dioxide.

The approval of **chitosan** usage in winemaking opened the door to a new range of products with antimicrobial effects. Polysaccharides of fungal origin derived from chitin, gain a a substantial foothold on autochthonous yeast and lactic bacteria. Its usage makes it possible to reduce microorganisms' populations significantly.

Its effect on the microorganisms is carried out at levels. In the first phase, the polysaccharide joins the microorganisms, forming large floc that precipitate due to gravity. In the second phase, the chitosan provokes a destructuring of the membranes resulting in cell death.

The main advantage of this compound is that its effect is not determined by pH , which allows it to act on the wines with high pH where contaminations are more frequent.



Picture 4: Picture of *Brettanomyces* cells trapped in chains of chitosan molecules: Bijlana Petrova/ WSU.

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PROTECT

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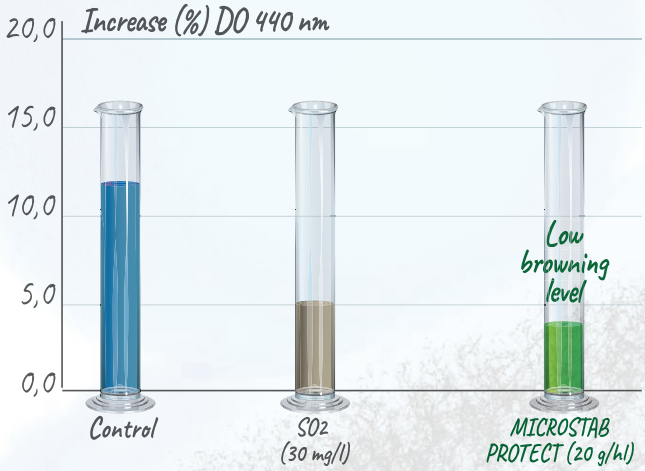
Specific preparation, which combines antimicrobial, antioxidant, and oxidation protection properties, as a result, it is proposed as an effective tool to reduce the levels of sulfur dioxide during winemaking.

- Substantially reduces or eliminates Brettanomyces populations, decreasing the risk of alterations due to the presence of spoilage yeasts.
- Effectively decreases populations of lactic bacteria. As with any other antimicrobial effect, the reduction of populations depends on the initial microbiological load.
- Antioxidant effect and oxidation protector. The natural antioxidant effect protects the aromatic fraction and limits the browning of the wines.
- IInactivates oxidation catalysts.. Reduces the activity of oxidase enzymes, which are responsible for the oxidation of phenols.
- Reduces metal content(Fe y Cu).

Antioxidant effect

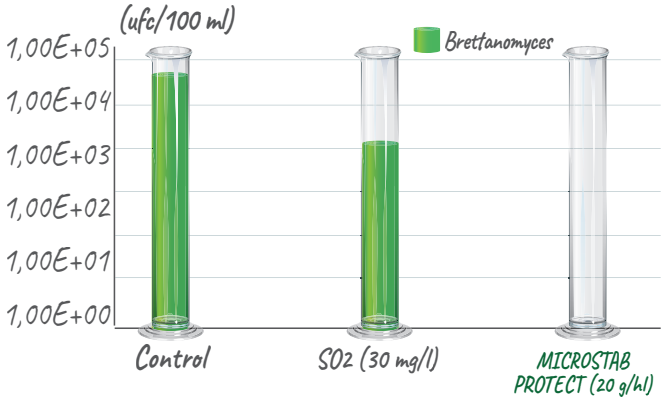
Due to its high content in glutathione, the results of usage combined with the antioxidant effect of the gallic tannin are higher, compared to the use of glutathione only.



Picture 5: Antioxidant protection reduces the level of browning of the sample.

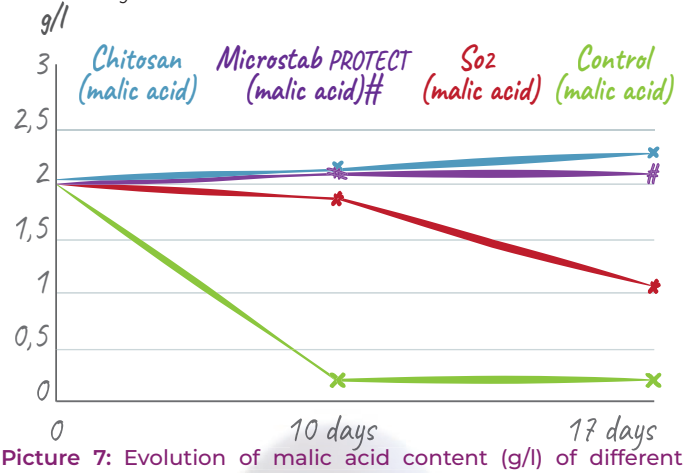
Antimicrobial effect

The antimicrobial effect is beneficial against Brett. Populations can be controlled without the addition of sulfur dioxide.



Picture 6: The effect of Microstab PROTECT on Brettanomyces.

The effect of **Microstab PROTECT** on lactic bacteria is also noticeable; being able to reduce the populations under the desirable limits to avoid the problems caused by contamination.



Picture 7: Evolution of malic acid content (g/l) of different treatments after 10 days of contact with red wine.

Microstab PROTECT can use at any time during the manufacturing process as a substitute or complement.

In red wines it can be used:

- **Wines with residual sugar.** Reducing the risk of lactic bacterias contamination.
- **High pH.** Antimicrobial protection where sulfur dioxide is less effective.
- **Wines without SO_2.** Complete alternative to sulfur dioxide usage.
- **Reduce SO_2**. It can be combined with the sulfur dioxide to improve conservation by keeping the levels of sulfur dioxide low.
- ***Brett* control** , reducing *Brett* populations at critical times, filling and homogenizations of barrels.
- **Increasing antioxidant protection.**
- In white wines:
  - ·Wines without SO_2
  - ·Inhibit MLF
  - ·Microbiological control
  - ·Oxidation-reduction.