

## SuperBouquet EVOLUTION

### Allergen-free Natural Antioxidants



Currently, there are several factors that make it difficult for wine to reach the consumer in the best conditions. On the one hand, the global market forces the wine to suffer long transport times during its distribution, exposing the product to great variations of temperature that accelerate the processes of oxidation. On the other hand, the increase in temperatures during the ripening of the grapes and the excess of potassium in the vineyard cause an increase in pH, which favors microbiological proliferation and affects the sulfur's effectiveness as an antioxidant.

Together with the market trend to reduce total sulfur levels, these factors have forced winemakers to look for antioxidant alternatives to offer to wine producers.

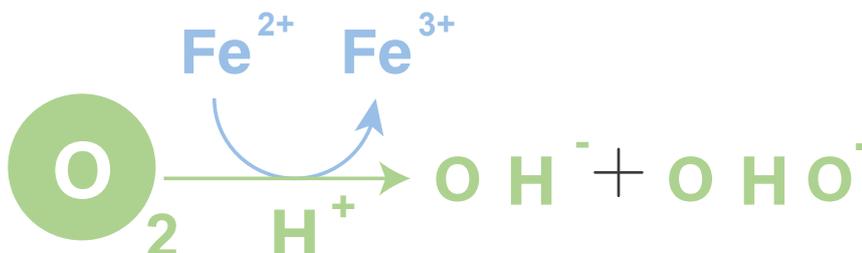
## Chemical oxidation process

The oxidation processes in wines are complex and depend on a large number of factors, but they can be summarized as follows: wine includes compounds that act as oxidation substrates and others as oxidants, in addition to the rest of the compounds and conditions they will regulate the rate of those reactions. Within this process, chain reactions occur that cause the oxidation of more compounds, in this case, without the need for oxygen to be present.

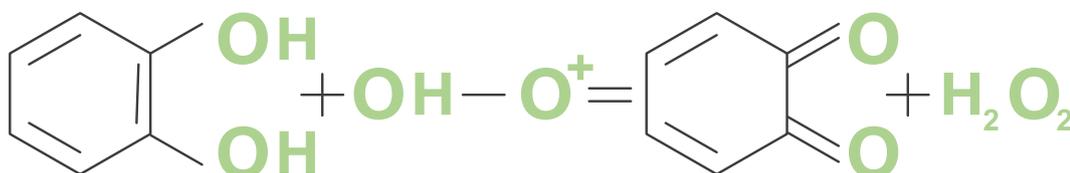
Oxygen by itself is not capable of oxidizing most of the compounds in wine. To oxidize these compounds, you need a catalyst to exchange electrons. The main catalysts are Fe and Cu, which can give electrons to molecular oxygen, generating highly oxidizing radicals.

Once these radicals are formed, the oxidation chains are produced.

In the first phase, Fe<sup>2+</sup> together with a proton (H<sup>+</sup>) react to give the hydroperoxyl radical:

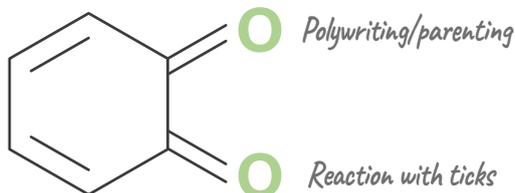


This radical can oxidize particular polyphenols to their corresponding quinone:



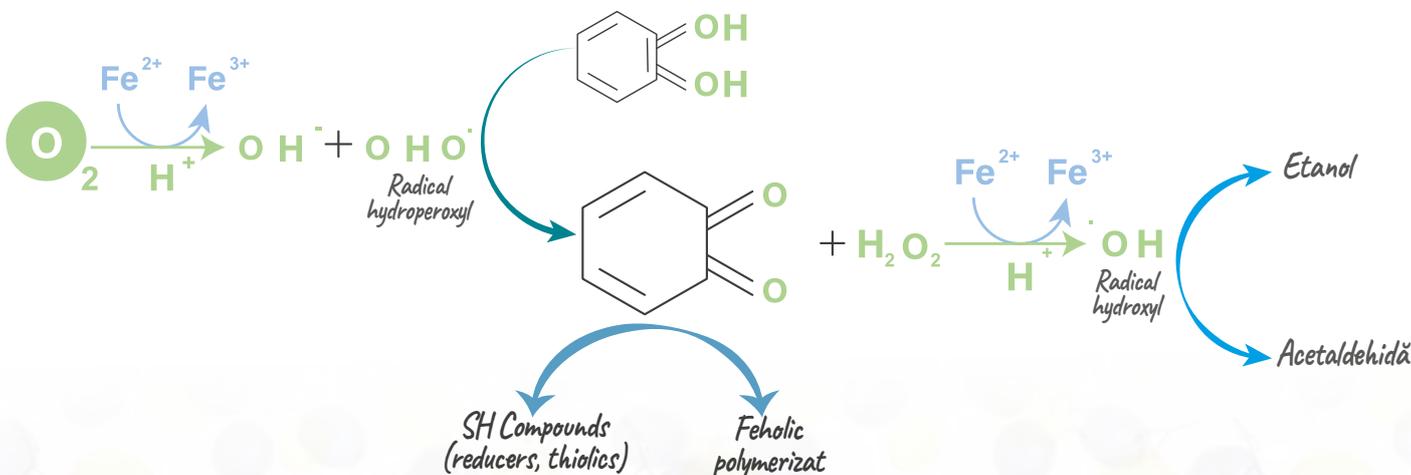
### Polyphenol oxidation to its quinone.

The products of this reaction, in turn, can generate subsequent oxidations. Quinone can, on the one hand, polymerize with other quinones and cause browning, and on the other, react with the -SH groups of thiols, considerably reducing the aromatic intensity of wines.



On the other hand, the hydrogen peroxide formed during the oxidation of quinone, again using Fe as a catalyst, generates the hydroxyl radical, a strong non-selective oxidant, which can oxidize alcohol to acetaldehyde.

In short, the chain reaction would be as follows:



As can be seen, throughout this chain of oxidations, oxygen is only present in the early stages, the reason why other types of tools are necessary to protect both the color (oxidation of polyphenols) and the aroma (thiolic oxidation and acetaldehyde generation).

## Strategies to control oxidation

As we have observed, the oxidation chain is complex, and the action should be taken to avoid such reactions in each of the phases.

Avoid contact with oxygen.

Wine can dissolve up to 8.6 mg/l of oxygen at room temperature. This ability to dissolve oxygen depends on:

1. The dissolved solids (the higher the concentration, the lower the dissolution).
2. Alcohol (the higher the concentration, the lower the dissolution).
3. Temperature (the lower the temperature, the greater the dissolution).

All actions that limit contact with oxygen will reduce the adverse effects of oxidation.

### Substrate reduction

The primary substrate for wine oxidations is polyphenols; therefore, a reduction in wines' polyphenolic content will significantly reduce the potential for oxidation or oxidation sensitivity of wines.

The techniques that can be used to reduce the polyphenolic content could be:

1. Avoid excess extraction during production, separate the different press fractions, set the musts intensively...
2. Reduce the polyphenol content in wines, mainly with clarifications. PVPP, gelatins, and vegetable proteins are usually the most effective here.

### Catalyst reduction

The role of catalysts in oxidation is decisive; it has been found that oxygen cannot react with wines completely deprived of Fe and Cu. In practice, wines with lower levels of these metals are less oxidizable.

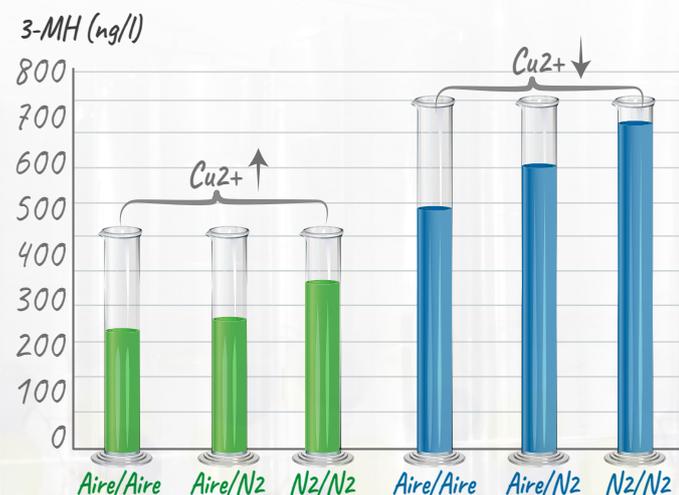


Illustration 1. The evolution of 3MH in wines as a function of contact with oxygen and the presence of copper. Source: AWRI

In an experiment carried out by AWRI, it was found that metallic catalysts in wine had a more significant influence than oxygen supply on oxidation.

Methods to reduce the presence of catalysts in wines could be:

1. Avoiding the use of containers that can yield metals to the wines.
2. Reducing the wines' contact times with bentonite since these release Fe very slowly, but if the contact times are lengthened, the Fe values can increase considerably.
3. Reducing metals with the use of PVI/PVP (Divergan HM). This clarifier can reduce the metal content in wines simply and effectively.

### Use of glutathione (GSH)

Glutathione is a naturally occurring tripeptide in cells with strong antioxidant power. Its use in enology is authorized only in the form of inactive yeast naturally enriched in glutathione.

Its antioxidant action is carried out on three levels:

1. Direct antioxidant. Due to its low potential, RedOx can react with the oxidants present in wine, protecting the desirable compounds from oxidation.
2. Protection of color, GSH can bind to oxidized polyphenols giving rise to GRP (Grape Reaction Product), a colorless compound that is not a substrate for polyphenol oxidase and paralyzes the browning of wines.
3. Aroma protection, quinones are very reactive with thiols, which causes aroma degradation. GSH, by reacting with quinones, prevents them from reacting with thiols; thus, the aroma is protected from oxidation.

# SuperBouquet EVOLUTION

## Inactive yeast rich in glutathione and its precursors.

It is the second generation of inactive yeasts specially enriched naturally in glutathione. Although the positive effects of this thiolic peptide and its precursor metabolites on the aroma and the delay in the evolution of wines are well known, the study of its impact during fermentation, the identification of the oenological practices and factors that affect its content has made it possible to develop an oenological path where its protective effect is maximized. Its protective effect acts on three levels.



1. Antioxidant effect
2. Aroma protection
3. Color conservation

### Protector:

*It competes with the O-quinone for the thiols, protecting the aromas of the wine*

### Antioxidant:

*Capable of binding the orthoquinones, responsible for darkening and loss of aromas*

### Preventive:

*Prevention of oxidation, due to the ligation of -SH with the aspartic group, generating stable and colorless GPP (Grape Peation Product)*

### Application

Its application can be carried out at any time that requires enhancing antioxidant protection.

#### Must before alcoholic fermentation:

Applied to musts before fermentation, apart from the direct protection offered by GSH, and due to the high concentration of its precursors, it helps yeast synthesize glutathione during fermentation.

In the first phase, the yeast incorporates the glutathione precursor amino acids, which will later be transformed into the production and subsequent release of glutathione to the finished wine. By doing so, antioxidant protection is provided just when the fermentation ends, and the release of carbon no longer protects against oxidation.

Furthermore, as Jackowetz and others demonstrated (2012), the addition of sulfur in fermentation causes an increase in the formation of acetaldehyde by oenological yeasts. Therefore, the addition of glutathione before fermentation allows reducing the dose of sulfur and with it the formation of acetaldehyde, which will allow having a more significant amount of free sulfur with the same amount of total sulfur, as it has less acetaldehyde with which it is combined. Wines just produced.

After fermentation, the wine is unprotected, and its sensitivity to oxidation is evident. The use of **SuperBouquet® EVOLUTION** in those first phases allows the reduction of the use of SO<sub>2</sub>.

#### Aging on lees or malolactic in barrel

In this phase of harmonization of the wines, it is not surprising that the fermentation's natural lees can carry some defects (reductions, strange aromas...) accentuated by the prolonged contact time.

On the other hand, if you want to carry out malolactic fermentation in barrels, it will not sulfite the wine. In white wines, usually kept in new barrels, oxidation of this type of wine occurs relatively frequently.

Using **SuperBouquet® EVOLUTION** during aging on lees and malolactic fermentation in barrels reduces the usual problems that arise with these practices.

#### Conservation of wines

During their conservation, wines are usually repeatedly corrected for sulfur levels, reaching in some cases values that are close to the maximum legal dose. Before bottling, these high values make it difficult to reach the desired levels of free sulfur without exceeding the legal limit.

Due to the rise of organic wines and consumer awareness, sulfur values are getting lower and lower.

**SuperBouquet® EVOLUTION** allows you to work with lower levels of sulfur without neglecting antioxidant protection.

#### Wine movements/racking/cold treatment.

As is well known, in wine movements, it is the moment when the most significant amount of oxygen dissolves. Another critical moment for oxidation is cold stabilization because the drastic reduction in the wine temperature causes an increase in the solubility of oxygen in it. Therefore, a correct addition of **SuperBouquet® EVOLUTION** in these processes will protect wines from the possible oxidation that could occur after these tart treatments.

## Origin

SuperBouquet® EVOLUTION is the result of the VINNOSO2 Research Project carried out in 2012 (INNPACTO IPT 2012-0967-060000), in which several wineries and national research centers participated, entitled:  
**"Development of an oenological path to produce high-quality wines free of sulfur dioxide."**



In this project, the influence of various products regarding their antioxidant capacity was studied, evaluating the quality of the organoleptically treated wines, the color, and other oxidation markers. The product with the most significant favorable influence on wine turned out to be the inactive yeast enriched in glutathione, both in tasting and in analytics, which is why, today, AGROVIN markets this yeast under the name **SuperBouquet® EVOLUTION**.

## Bottling

The addition of **SuperBouquet® EVOLUTION** along with correct levels of SO<sub>2</sub> before bottling has been shown to significantly decrease wine browning, keep 3-MH levels high in thiolic wines, and protect the oxidation of esters and terpenes.

## Experimental results

As previously mentioned, tasting concerning a control without sulfur and another with the addition of sulfur were favorable for the **SuperBouquet® EVOLUTION**.

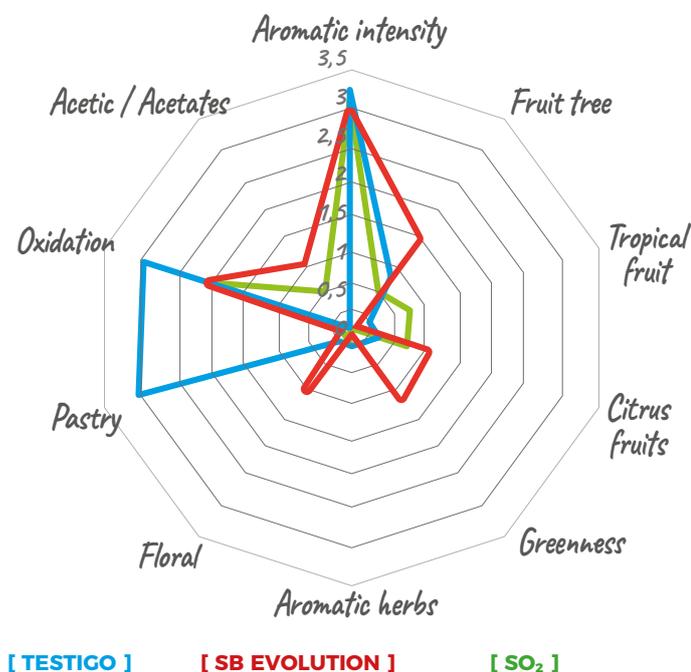


Illustration 2. Sensory evaluation of the taste and color aspect of Albariño 2013, tasting Sept 2014

Regarding color protection, in a study carried out by our technical department, the increase in coloring intensity was measured after the addition of **SuperBouquet® EVOLUTION** in wines to which no sulfur had been added:

*Increased color intensity*

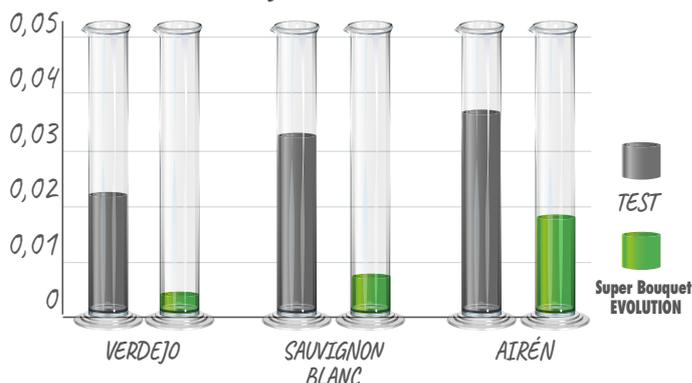


Illustration 3. Increase in coloring intensity after six months of contact. Test on Verdejo, Sauvignon Blanc, and Airén (2016 vintage) varieties without applying SO<sub>2</sub>.

In the same experiment, the browning was also analyzed by absorbance at 440nm:

*Increase of "D0440 nm" after 6 months of contact*

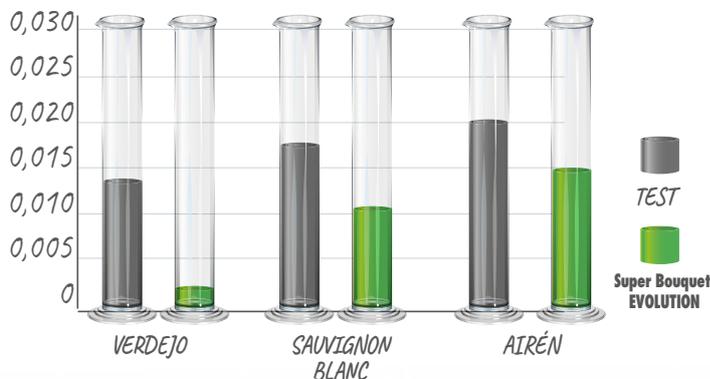


Illustration 4. Increased browning after six months of contact. Test on Verdejo, Sauvignon Blanc, and Airén variety (2016 vintage) without applying SO<sub>2</sub>.

In both graphs, the protective effect of the **SuperBouquet® EVOLUTION color is revealed**, although the influence of the polyphenolic content is also appreciated, greater in Sauvignon and Airén than in the Verdejo variety wine.