Wine oxidation

Good practices

for the production of wines with low oxidisation

from Sangiovese grapes

Managing the risk of oxidation in wines made from Sangiovese

Project financed within the framework of the **Integrated Supply Chain Project of the Region of Tuscany "Sangiovese, the "territory"**, sustainability: an innovative vision of cultivation for a sparkling production", under sub-measure 16.2 - European Partnership for Innovation

Wine oxidation

Oxidation is one of the main phenomena that affect the quality of wine.

It can occur in all wines (white, rosé, red and sparkling) and concerns all sensory characteristics (colour, smell, taste).

With oxidation, the **colour** of white wines initially loses the greenish hues typical of a young wine, then increases the intensity of the yellow colour, which first turns golden and then gradually tends to brown. In red wines, oxidation causes the wine to lose its violet hues first, then reduces the intensity of the red colour, which is gradually replaced by orange tones.

Aroma is the component that is most altered by oxidation: at an early stage in the process, well before the smell of 'oxidised' is perceived, there is a drastic reduction in the fresh aromas of fruit and flowers, and - very prematurely - the complete disappearance of those of tropical fruit and boxwood. Even slight oxidation can practically cancel out the varietal component of a wine, leaving only the 'vinous' aroma produced by fermentation. As oxidation proceeds, other volatile compounds begin to appear, giving rise to hints of cut apple, honey, potato skin and sultanas, which can completely mask the wine's original characteristics.

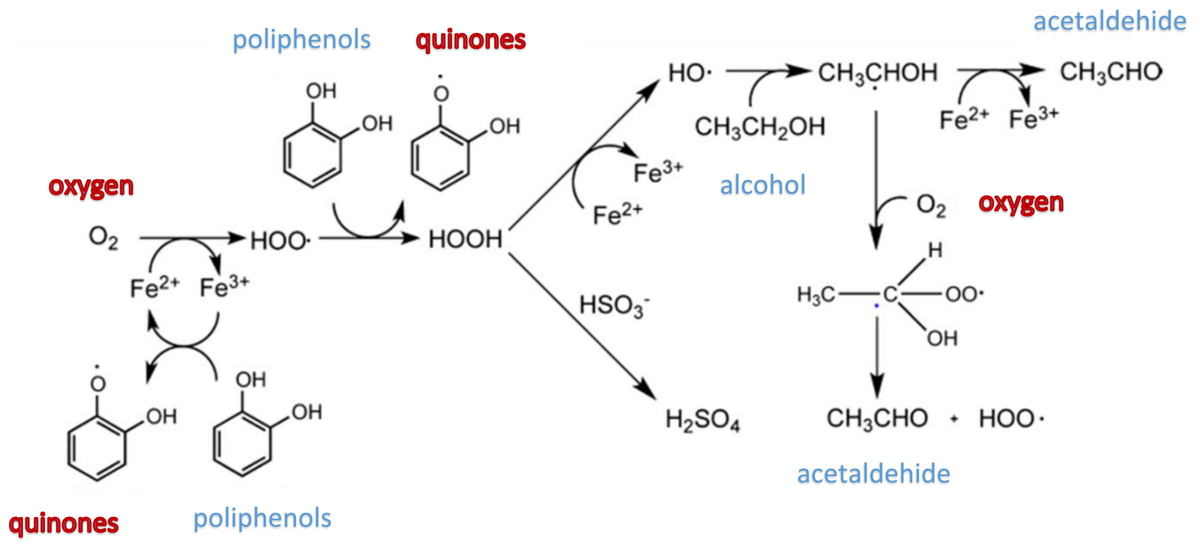
Taste is also affected by oxidation, which seems to increase sensations of 'dryness' probably due to the role it plays in the chemical reactions of arrangement and polymerisation of phenolic compounds.

Why does wine oxidise?

Oxygen is certainly needed to oxidise a wine, but there are many factors that come into play and make a wine more or less oxidisable.

In order to understand the complexity of wine oxidation mechanisms, the diagram below illustrates the **chain of chemical reactions** which, starting with oxygen, lead to the formation of certain compounds and the transformation of others. The diagram shows an important concept for understanding the oenological practices considered in this document: it is not oxygen that reacts with the compounds in wine leading to oxidation, but the **radicals** and **quinones** formed by the **iron** present in the wine that trigger chain reactions affecting polyphenols, alcohols and aroma compounds. The greater the quantities of polyphenols and iron present in the wine, the greater the extent of these reactions with the same amount of dissolved oxygen.

The diagram below also shows the mechanism of aldehyde formation, which originates from the abundant alcohols in wine that react with radicals and oxygen. In the diagram, the formation of acetaldehyde from ethyl alcohol, the quantitatively most important Strecker reaction, is shown. Acetaldehyde smells like oxidised apples and contributes to the "oxidised" note of the wine, but even more relevant from an organoleptic point of view are other aldehydes such as phenylacetaldehyde and methional, compounds with a much lower perception threshold than acetaldehyde and which are formed by the same chemical mechanism.



Is oxygen always an enemy of wine?

Controlled oxygen intervention can also have **positive effects** in certain process situations:

- ✓ In the first half of **alcoholic fermentation**, the oxygen that enters the system is immediately used by the yeasts before reacting chemically with the wine compounds, and a controlled supply is very useful in making the yeasts more active in the latter stages of fermentation. In some cases, racking in contact with air or **macro-oxygenation** are used to ensure a certain supply of oxygen to the must-wine;
- ✓ Moderate oxygenation of the wine can **prevent the onset of reduced odours**, caused by the formation - in a strongly reducing environment - of sulphur compounds which can mask the secondary aromas of the wine or even cause the appearance of aromas of rotten eggs, onions, and cauliflower. However, this practice must be carried out with great care because a) the hydrogen sulphide must be oxidised before it is transformed into sulphides, so the reaction must take place very early, b) together with the negative sulphur compounds, the thiols - responsible for certain varietal aromas - are also oxidised and the resulting wine may be less characteristic and complex;
- ✓ In red wines, acetaldehyde promotes the **stabilisation of anthocyanins and the polymerisation of tannins**, making the wine softer on the palate and more stable in colour. This principle is the basis of **micro-oxygenation**, the aim of which is to solubilise small quantities of oxygen in the wine, just enough to cause positive reactions without leading to the negative effects mentioned above.

Thus, a process in total absence of oxygen - from processing to final consumption, the so-called "vinification in reduction" - is usefully applicable in the production of certain wines (e.g., varietal white wines with a short shelf-life) but cannot be generalised to all product types.

Oxidation is irreversible

All oxidation reactions in wine are irreversible. The compounds that are transformed and degraded will not return to their original state even if the wine is kept in the total absence of oxygen and with the addition of antioxidants for a long time. It is therefore not possible to intervene on an oxidised wine using physical processes or chemical methods to restore the wine to its original organoleptic characteristics. The only way to preserve the quality of a wine is to **prevent** oxidation.

Preventing oxidation

Fortunately, oenological technology has made available numerous tools and methods for limiting oxidative phenomena in wine that alter its quality.

Several strategies can be adopted to achieve this objective:

LIMITING THE EXTRACTION OF OXIDISABLE COMPOUNDS FROM THE GRAPES

Soft pressing

In order to have a low quantity of oxidisable polyphenols (phenolic acids, catechins, etc.) in white wines, it is a good practice to minimise their extraction from the skins during pressing. Therefore, the fractions of must obtained by more vigorous pressing, with destemmed and/or macerated grapes, are the most sensitive to oxidation; vice versa, the fractions obtained by soft pressing whole grapes are those that have the lowest content of polyphenols, but also of aromas and other compounds that give body and volume to the palate. It is therefore necessary to find the right compromise every time.

REMOVE POLYPHENOLS AND METALS FROM THE MUST

Hyperoxygenation

Another strategy to reduce the presence of oxidisable polyphenols in white wine is to oxidise them entirely in the must before alcoholic fermentation. This is achieved by insufflating large quantities of air or pure oxygen into the freshly obtained must, causing the polyphenols to polymerise and be eliminated in the clarification phase. This technique can be combined with static or continuous flotation. Hyper-oxygenation is more difficult to apply in the processing of grape varieties with good primary aromas (terpenes, nor-isoprenoids, thiols, etc.), which are largely oxidised and therefore lost through forced oxidation.

Treatment with oenological charcoal

Charcoal has a strong absorbent power. Its use is only permitted in white wines before the end of alcoholic fermentation. It removes from the system the phenolic compounds that are substrates for oxidation, thus making the wine less susceptible to oxidation. However, the removal of compounds is not at all selective, and together with the oxidisable compounds charcoal removes positive aromas

Treatment with PVPP-PVI

Polyvinylimidazole/polyvinylpyrolidone is a synthetic polymer that absorbs iron and other metals naturally present in wine, making it less susceptible to oxidation. It has a limit of use of 500 mg/l.

Casein treatment

Casein is a milk-derived adjuvant capable of absorbing polyphenols and metals. It is used in both fermentation and clarification, in combination with bentonite to facilitate settling.

Treatment with vegetable proteins

Proteins obtained from peas or potatoes - non-allergenic substances - have the capacity to absorb polyphenols and iron and reduce the wine's sensitivity to oxidation.

Treatment with chitosan

In addition to its antimicrobial function, chitosan has demetallizing properties and can remove a certain amount of metals from the wine.

Treatment with potassium ferrocyanide

A historic practice for the selective elimination of iron from wine, this treatment has now fallen into disuse due to the complications of its implementation and potential toxicological risks.

AVOIDING THE PRESENCE OF OXYGEN IN WINE

Contact with yeast lees

Saccharomyces cerevisiae is a strong consumer of oxygen, not only during alcoholic fermentation, but also afterwards when the yeast is no longer vital. A common practice to protect wine from oxidation is therefore to keep a certain amount of fine lees in suspension in the wine, even for several months, to naturally consume the oxygen that enters the solution, while having a positive effect on the taste of the wine and its tartaric stability. The risks associated with this practice are linked to the possible emergence of negative aromatic notes, of reduction if the lees are not kept in suspension, of vegetal if the particles of grape tissue have not been removed from the lees.

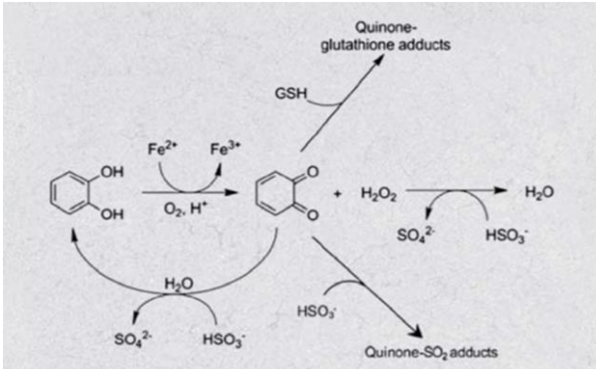
Use of inert gas

In order to prevent the wine from coming into contact with air and thus the 21% of oxygen it contains from entering into the solution, it is common practice to saturate the space in contact with the liquid with an inert gas such as carbon dioxide, nitrogen or argon. t is worth recalling here the principle of the law of gases, according to which each gaseous species behaves independently of the others present in the same space: in other words, a volume saturated with an inert gas can also be penetrated by oxygen, until the latter becomes saturated, if a contact with air remains open. Therefore, the inertisation of the volume of gas in contact with the liquid is achieved by removing the air with a flow of inert gas and immediately afterwards limiting the exchange with the external air.

In general, carbon dioxide - from cylinders or dry ice, in some cases from alcoholic fermentation - is used on grapes, must and must-wine, while after the end of alcoholic fermentation, flows of argon or nitrogen (which do not solubilise in the wine) are used to remove air from the head spaces of tanks and bottles before they are closed.

BLOCKING THE CHAIN OF OXIDATIVE REACTIONS

Addition of sulphur dioxide



This is the most widely used additive in winemaking. It came into use in the second half of the 19th century and marked the transition to modern oenology, where the aim is to know and control the chemical and biological phenomena occurring in wine. As well as having an antioxidant effect, sulphur dioxide inhibits all species of bacteria and slows down many yeasts, allowing *Saccharomyces cerevisiae* – the main agent of alcoholic fermentation - to dominate the transformation of must into wine.

Sulphur dioxide does not react directly with oxygen, but with quinones - formed instantaneously by the iron-catalysed Fenton reaction - which oxidise sulphite to sulphate and thus become no longer reactive phenols.

So, the amount of sulphur dioxide needed to prevent oxidation in a wine will be all the greater the more oxygen that passes into solution, but it will also depend on the content of phenols and iron in the wine, which trigger the production of radicals and chain reactions affecting the components of the wine.

It should be remembered here that the only fraction of sulphur dioxide active against oxidation is the **molecular** fraction, which is a part of the free portion, and the more acid the wine, the greater the proportion. The fraction of free sulphur dioxide is the one that does not bind with aldehydes and ketonic acids produced during fermentation which vary greatly from yeast to yeast.

In short, it is good to remember that the same amount of sulphur dioxide added to different wines gives very different protection from oxidation, varying according to the time of addition, the iron and phenol content, the pH of the wine and other parameters: **a standard addition of a certain amount of sulphur dioxide** to all the wines in a cellar, even if precise and accurate, is **a very rough practice** with regard to protection from oxygen.

Sulphur dioxide can have a mild curative effect: when added to a 'tired' and slightly oxidised wine, sulphur dioxide can make the wine 'fresher' simply by binding to the volatile aldehydes and eliminating the hint or masking of more pleasant aromas.

Addition of ascorbic acid

Ascorbic acid, also known as vitamin C, is an easily oxidised compound, even faster than sulphur dioxide. Its use is permitted in oenology (limit 250 mg/L) precisely because of its characteristic of immediate reactivity with quinones and radicals formed by oxygen, which discharge their oxidative potential onto ascorbic acid before reacting with other phenols or aroma compounds. Ascorbic acid anticipates sulphur dioxide and - especially in more sensitive white wines - minimises the organoleptic effects of oxygen solubilisation in the wine. But beware: ascorbic acid does not disappear but is transformed into dehydroascorbic acid, which can in turn oxidise wine compounds if there is not enough sulphur dioxide to make it harmless. Ascorbic acid should therefore **never be used on its own**, but always together with adequate doses of sulphur dioxide.

Addition of glutathione

Nature has already provided grapes with a reserve of antioxidant substances, the most important of which is glutathione, a tripeptide found in all living organisms that neutralises free radicals. It is found in high quantities in grape must and protects it at least partially from oxidation during mashing. In addition to the amount that remains in the grapes after initial oxidation, we also find significant amounts of glutathione in the wine, produced by the yeast during fermentation and when it stays on the lees. Today, it is possible to reinforce the natural glutathione content in wine by adding the compound as it is (limit 20 mg/l) or inactive yeast produced in such a way as to maximise the glutathione content.

Addition of tannin

Tannin is another natural antioxidant found in abundance in grapes. It consists of polyphenols, which have the ability to react with quinones produced by oxidation to create compounds of greater complexity, thus removing radicals from the system before they react with more degradable substances such as volatile compounds. While tannin alone cannot prevent oxidative phenomena, it can help contain oxidation and limit the addition of sulphur dioxide.

THE REASONED OXYLESS APPROACH

All the practices described above have an **impact on the quality and integrity of the wine:**

- subtractive techniques, in reducing the presence of oxidisable substances, also remove from the wine part of the aromas or other compounds that are positive for its organoleptic profile.
- additive techniques add substances to the wine that alter its natural composition.

The aim of the **OXYLESS project** was to identify winemaking strategies for the production of sparkling wines with character and originality from Sangiovese red grapes, i.e. wines that are as little as possible stripped of the intrinsic properties of the grapes of origin, but at the same time not very oxidisable and therefore with an adequate shelf-life for a sparkling wine.

The technological approach adopted by OXYLESS was therefore to:

- ✓ objectively **assess the sensitivity of the wine to oxidation**
- ✓ **intervene only when necessary** and with the least invasive techniques
- ✓ **monitor the evolution of the wine** to improve the process with each vintage

OXYLESS test to assess the oxidability of a wine

The performance of the test involves, after the addition of hydrogen peroxide to the samples, the evaluation of:

Variation of the voltammetric profile

By comparing the anodic part of the voltammograms, it is possible to obtain information on how the phenolic component changes over time, on the effects of sulphitation or SO2 consumption, on the course of oxidative phenomena or on the effects of clarification or stabilisation treatments.
In this application context, however, voltammetry does not allow quantitative information to be obtained; it will therefore be necessary to integrate the data obtained with information provided by other analytical variables indicative of the state of the phenolic fraction.
Voltammetry made it possible to highlight oxidation by the addition of H2O2, differentiating the oxidised samples from the control theses; in Figure 1, oxidation is made evident by the decrease in the anodic trace of the voltammogram, presumably linked to the changes brought about by the hydrogen peroxide on the phenolic fraction of the wines themselves.

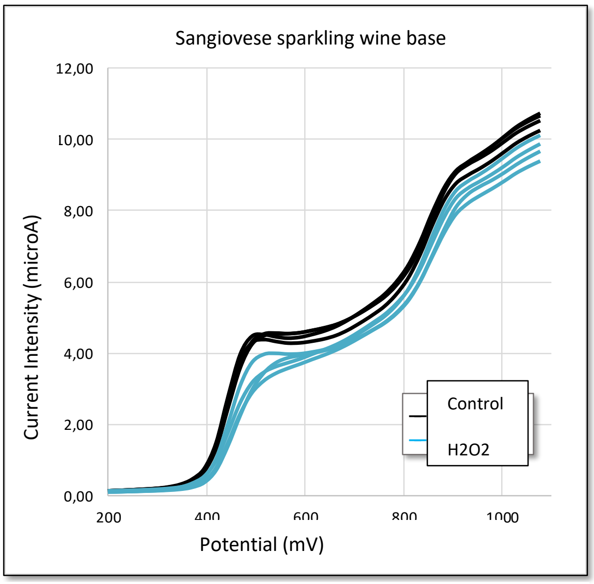


Figure 1. Changes in voltammograms of sparkling base wines following the addition of hydrogen peroxide (H_2O_2)

ΔE derived **from CIELab parameters**

If this parameter is <2, the risk of oxidisability is low, a medium risk for values between 2 and 4, and a high risk if >4. Figure 2 shows the significant correlation between ΔE and factorial distances.

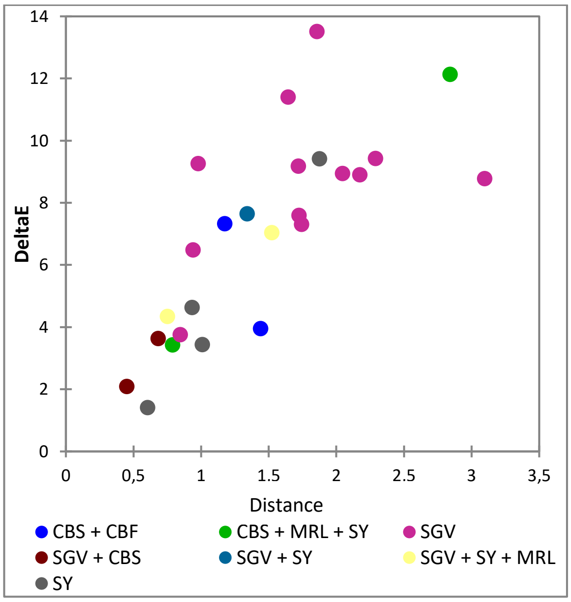


Figure 2. Correlation in the different varieties of wines between ΔE calculated from CIElab parameters and factorial distances calculated from the PCA of the chemical data.

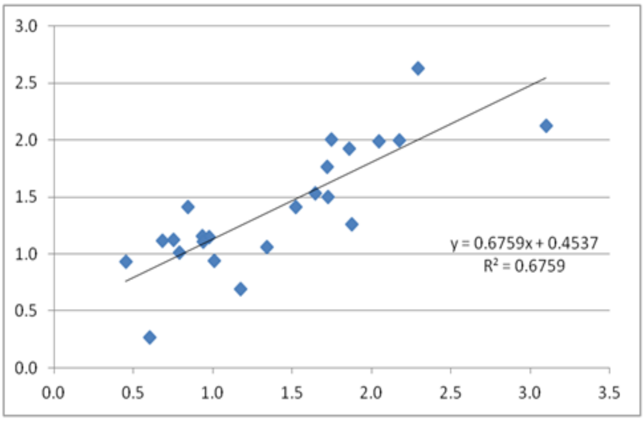
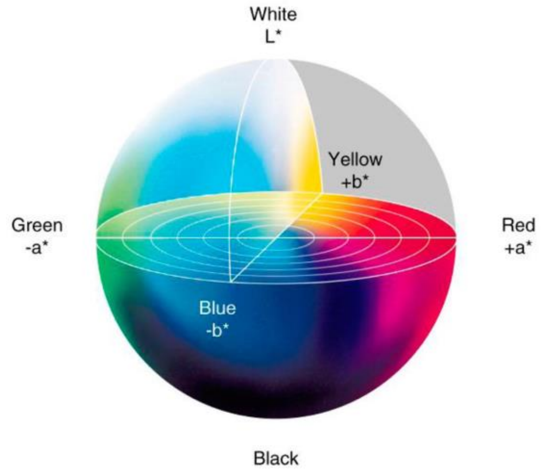


Figure 3. Correlation between predicted Δ_{CHIM} and that calculated using an MLR model.



In addition:

<u>for WHITE wines</u>

Catechin index

if it is <10 the wine presents a low risk of oxidisation, if >20 the risk is high.

<u>For RED wines</u>

Δ_{CHIM} index

derived from the variation of certain parameters (free anthocyanins, sulphur dioxide and colour) before and after treatment with hydrogen peroxide. Therefore, if the ΔCHIM s less than 1, the sample can be considered to be at low risk of oxidisation, if it is greater than 2, it is at high risk. Figure 3 shows the correlation between the predicted ΔCHIM and that calculated using an MLR (Multiple Linear Regression) model.

In the event of an adverse outcome (medium or high risk), we recommend specific treatment and, if necessary, an in-depth analysis aimed at a more detailed characterisation of the problem (Aroma Analysis, Polyphenols, etc.).

STANDARD MONITORING:

1. Systematic screening at regular intervals:
   - Free and total sulphur dioxide, Volatile acidity
     - White wines: Colour
     - Red wines: Anthocyanin/tannin profile
2. Post-bottling monitoring:
     - Newly bottled wine: Measurement of dissolved O_2 + sulphur content
     - Systematic monitoring at regular intervals: Sulphur content, volatile acidity, CieLab and Aldehydes

MONITORING OF THE CRITICAL PROCESS:

1. Grapes affected by rot or grape moth, overripe:

   - Acetic acid, Gluconic acid
   - Bacterial contamination
   - Laccase activity

2. Particular conditions, such as vinification of:
   > Grapes highly concentrated in metals (ex.: Cu)
   > Whites made with maceration on the skins
   > Fermentations carried out by oxidative strains (ex-spontaneous)
   > Prolonged fermentations (stalled/arrested)
   > Excessive fermentation temperatures
   > Excessive oxygenation, storage in tanks that are not completely filled or always-full tanks

   in any case in the presence of deterioration of colour or aromatic freshness

WINEMAKING STRATEGIES TO AVOID OXIDATION

ROSÉ SPARKLING WINES MADE FROM RED GRAPES

In general, the production of rosé wines is particularly delicate because there is a sufficient quantity of polyphenols in wine to give an evident pink colour, but not enough to "unload" the chain of oxidative reactions. On the other hand, in rosé wines, even more than in white wines, it is necessary to extract a certain quantity of components from the grapes; the strategy cannot be that of classic method sparkling base wines, where there is a tendency to have a very neutral base to be completed with re-fermentation.

In rosé wines, oxidation leads to a rapid change in colour towards orange tones and a clear reduction in colour intensity, as well as the sudden disappearance of fresh, floral and fruity aromas.

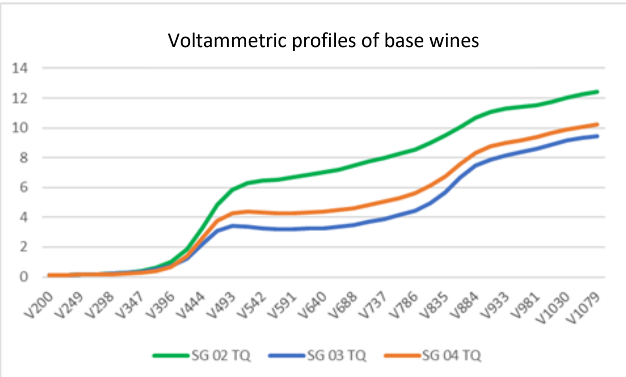
In the specific case of the Oxyless project, the aim was to identify a strategy for the production of a sparkling wine made from Sangiovese red grapes, which would have an original character and express the territory and the grape variety. Tests were carried out with different pressing and clarification techniques, and the conclusion was reached that, in order to have the right balance between character and low oxidability, it was advisable to produce three different base wines, to be blended in varying proportions according to the vintage into a cuvée with the right profile.

Specifically, the three masses can be obtained as follows:

Non-macerated: the grapes are macerated with a minimum extraction from the skins, whole in the press or - if large quantities are processed - only de-stemmed, not crushed, and in a low-pressure drainer. Dynamic clarification with flotation and gelatine allows the wine to go quickly through fermentation and obtain a wine with good colour intensity and medium acid structure. This base wine can represent up to 60-70% of the final blend.

Light maceration: Light maceration: a portion of the grapes must have a light skin maceration, which can be obtained by pressing crushed destemmed grapes in the press for a few hours. In this way excessive colour is extracted from the skins, but also minerals, acidity and polysaccharides that give a saline and structured wine. After continuous flotation with gelatine - to avoid uncontrolled microbial development - and fermentation with selected yeasts, this base wine is used in the assembly up to 25% depending on the vintage.

Neutral portion: In order to balance out the variability of the vintage, which can produce more or less colourful and/or fragrant grapes, it is advisable to produce a quantity of neutral base wine, practically without colour and primary aromas, but with intense, fresh fermentation aromas and good acidity. This share can be obtained from grapes brought to the cellar fairly early, destemmed, pressed, then almost completely de-coloured with charcoal, then flocculated and fermented with suitable yeast strains and nutrients. A proportion of up to 30% in the cuvée serves to reduce colour and balance the excessive structure of the other two shares.



WHITE WINES

Oxygen: the #1 enemy

In white wine making, the solubilisation of oxygen in the wine is usually limited or even avoided.

White wines are, in fact, much poorer in polyphenols that can discharge the chain of oxidative reactions, and they suffer more from the negative effects of oxidation: the darkening of colour, the loss of fresh fruity aromas and the appearance of oxidised notes are phenomena perceived much more easily in white wines than in reds. In the last few decades, oenological techniques have therefore developed a series of solutions to prevent contact between wine and oxygen which, together with the use of antioxidant substances, make it possible to carry out the so-called **vinification in reduction**, aimed at maintaining the primary and secondary aromas in the wine at the highest possible levels until the moment of consumption.

It must be said that not all white wines need a total lack of contact with oxygen to express themselves at their highest level, and also that vinification by reduction can lead to very "reduced" organoleptic profiles, with smells of flint, burnt matchstick, garlic and onion that compromise quality. However, in this guide - for educational purposes - we will briefly describe the various tricks to avoid oxygenation of the wine in each of the stages of white wine production from grape to glass, knowing that it is the winemaker who knows when and how much to apply these techniques to get the best from the raw material available.

Destemming, crushing and pressing

Many white grapes are macerated after a step of removing the stems and cracking the berries, to make pressing faster and increase the must yield.
This is one of the most delicate steps in winemaking by reduction because:

- the surface area of grapes and must exposed to air is very large
- the most oxygen-reactive compounds (cinnamic acids, grape glutathione, thiol primary aromas, etc.) can already be completely lost in a few seconds of exposure to air
- the greater the mechanical action on the grapes (rubbing, pressure), the greater the extraction of substances that react with oxygen with detrimental effects on the quality of the wine: lipids are oxidised into aldehydes with a grassy smell, polyphenols are transformed into quinones and trigger oxidation cascades

For these reasons, in some productions it is preferred to work with **whole bunches**, thus avoiding all mechanical stresses and obtaining from the first pressing fractions musts that are very low in polyphenols and metals (but also in aromas, flavour and smoothness on the palate).
If you want to reduce or avoid the contact of the must with oxygen, it is necessary to saturate all spaces with an inert gas by removing the air. There are **pneumatic presses** on the market that can be filled with carbon dioxide before receiving the grapes or the crushed grapes, with the possibility of recovering the inert gas in a lung to reduce additional costs.

Another solution is the use of **dry ice** or carbonic snow, i.e., carbon dioxide in a solid state which at room temperature sublimates, creating a flow of inert gas that displaces the air from the hopper, the plate and the body of the press and occupies the cavities due to its weight, which is greater than that of air. A secondary but important effect of dry ice is the cooling of the grapes or crushed grapes, which slows down all the chemical reactions in the must.

During harvest, it is possible to use **carbon dioxide from fermentation**, which is produced by the yeasts as they transform the sugars into alcohol: there is ample and free availability of this gas in the winery, if it can be channelled from the tanks that are already fermenting into the pipes, pumps, presses and hoppers, and into the must collection tanks, thus rendering inert the entire path that the must has to take.

In order to obtain a white wine with good body and intense aromas, it is necessary to extract components from the skins, with relatively strong pressing and in some cases even a short skin maceration. It should be remembered here that the greater the extraction from the skins, the more the must will be loaded with oxidisable polyphenols and therefore the greater the need to protect it from oxygen.

Clarification

Clarification of the must prior to fermentation is typically carried out by adding adjuvants to the freshly pressed must, allowing the turbidity to settle for 12-24 hours, and then transferring the clear must to another fermentation tank. To avoid a premature start of indigenous yeast activity, the must is normally cooled to 10-12°C, often by passing it through an external heat exchanger. This phase is therefore very critical, because the must is moved several times and receives the contribution of adjuvants suspended in water under aerobic conditions; therefore, it can easily come into contact with air when the low temperature favours the solubilisation of oxygen. If oxidation is to be avoided during static clarification, various measures must be taken: **filling the pipes and tanks receiving** the liquid **with carbon dioxide**, dissolving the adjuvants in an inert environment and adding them in the circuit.

A practice that greatly helps to reduce the risk of oxidation is **static flotation**, where inert gas is injected into a tank containing must with specific adjuvants added: in this way, clarification is achieved more quickly, without reducing the temperature of the must, and with less movement of the must.

Fermentation

From the moment the yeast starts its fermentation activity, we no longer have to worry about oxidation. In fact, an important flow of carbon dioxide is produced, which physically prevents air from coming into contact with the surface of the must-wine. In addition, what little oxygen there is is immediately used by the yeast metabolism well before it can react with the chemical compounds in the must.

So the sooner alcoholic fermentation starts, the lower the risk of oxidation.

Good practice at this stage is the **early inoculation of the yeast**: on the day of processing the grapes, a small portion (5%) of the free-run must (the least loaded with vegetable lees) is sent directly from the press to the tank in which the mass is to be fermented, which is inoculated with all the selected yeast required for the entire mass. This portion of must, with a high yeast load and at room temperature, immediately starts pure fermentation. At the end of clarification, the receiving tank will already be saturated with carbon dioxide, which can also be used to render the process inert, and the clear must will soon start fermenting, even if it is still at a low temperature.

Once fermentation has begun, however, we must worry about the health of the yeast, which must be able to complete the transformation of sugars into alcohol without any problems. In order to become resistant to the alcohol it will find at the end of fermentation - which in recent years can be at a high concentration even in white wines - the yeast must have oxygen available to it during the cellular growth phase. The paradox is therefore that, if before and after alcoholic fermentation we must absolutely avoid oxygen dissolution, **during alcoholic fermentation** - and more precisely between one third and one half of the sugars consumed - we must **add oxygen** in significant quantities (8-10 mg/lm, equivalent to saturation). This operation is safe because at this stage the yeast absorbs and metabolises oxygen much faster than any chemical oxidation reaction.

Storage

The storage phase technically begins at the first racking after the end of alcoholic fermentation, when many yeasts are still in suspension, progressively dying but still very active in using oxygen for months. Therefore, as long as the wine is kept on the 'fine lees', with frequent agitation, it is well protected from oxidation that can come from opening the container or decanting under non-inert conditions. As long as there are **yeast lees in suspension**, the addition of sulphur dioxide as an antioxidant can also be avoided, as this could lead to the appearance of reduced aromas in the presence of still-living yeasts.

However, the yeast undergoes autolysis and releases substances into the wine which give complexity, smoothness and volume to the wine, but also nutrients for the bacteria which - in the total absence of sulphur dioxide - could initiate malolactic fermentation or even dangerous contamination. The usual practice is to proceed with **sulphurisation** several weeks after the end of alcoholic fermentation, in the meantime carrying out 2-3 rackings to remove all the vegetable lees and keeping the yeasts in suspension, trying to keep the temperature below 15°C.

From the moment that the wine no longer has yeast in suspension, it is totally defenceless against oxidation. Indeed, having so far avoided any reaction, all the potential for oxidation is intact. It is therefore essential, if we do not want to ruin all the work done so far, to protect the wine with almost obsessive inertisation practices, keeping **storage temperatures low**, and maintaining the level of antioxidants in sufficient quantity to block cascade reactions should air come into contact with the wine.

Bottling

Bottling a wine produced in reduction is a delicate operation that requires great care and skill. In fact, it is the last opportunity to prepare the wine for transport and storage outside the cellar, over several months and in conditions that cannot be controlled, handling a wine that is easily oxidised. Protein stabilisation, tartaric stabilisation and filtration must be carried out with completely inert **pipes and containers**, taking care not to have any air infiltration from pumps and fittings. The same applies to the **bottling plant**, which, if it is not previously saturated with an **inert gas**, can cause severe oxidation, especially in the first few hundred bottles that pass through it. It is also useful to remember that the wine must have good "internal" protection, with a **sulphur** content such that it is always present in free and molecular form until the moment of consumption, often accompanied by **ascorbic acid** and/or other antioxidants.

Wine prepared at bottling with the care described above should therefore have very little or no dissolved oxygen. But there are at least 4 other sources from which oxygen can reach the bottled wine.

First: the air in the bottle that comes into contact with the wine, and must be removed with a **flow of nitrogen** before filling begins

Second: the **headspace** remaining between the level of the wine and the base of the closure, if it is composed of air it can bring several mg/l of dissolved oxygen to the wine, especially with short stoppers or screw caps. It is therefore essential to flush the headspace with inert gas before inserting the closure.

Third: the air contained in the pores of the closure, whether it is made of cork or synthetic material, which, as a result of the compression applied during insertion, diffuses into the headspace and thus into the wine over the following weeks (**degassing**)

Fourthly: the air that penetrates **through the closure** and between the cork and the edge of the bottle, which can be in very variable quantities depending on the type of closure (practically zero for screw caps with tin layer, maximum for synthetic moulded corks) and within the same type of closure (maximum variance around the average for natural corks).

RED WINES

Oxygen? A friend, but not so much

For red wines, oxygen - in the right quantities and at the right times - is more of an ally than an enemy. Polyphenols, which are present here in much greater quantities than in white wines, react with oxygen and slow down the chemical changes of other wine compounds, including those of the aroma. Thus, red wine can receive significant amounts of oxygen in solution without developing the typical oxidised notes.
On the contrary, some types of polyphenols become more stable with oxidation: the best-known phenomenon is the combination of anthocyanins with tannins, with the formation of compounds with an intense red colour and much more stable than free anthocyanins over time. Be careful, however: if more oxygen is present in the system than is used in the tannin-anthocyanin reaction, the latter will oxidise and colour will be lost.
Balance is therefore the key word in red wine making: the wine must have at its disposal quantities of oxygen lower than its "absorption" capacity, so that it is fully consumed in chemical reactions favourable to the quality of the wine, without ever being in excess of oxygen and thus triggering degenerative processes affecting the colour or aroma compounds.

Destemming and crushing

As with white wines, it is necessary to **avoid mechanical action** on the green parts, so as not to release compounds into the must which oxidise into aldehydes (hexanal, hexenal) giving herbaceous notes to the wine.

Fermentation and Maceration

During alcoholic fermentation, the yeasts consume oxygen at a much faster rate than the chemical reactions between compounds in the must. Our quality assets have two sources of protection here: yeasts and polyphenols. Since oxygen is used by the yeasts to make their cell membranes more resistant to alcohol, it is very important to introduce oxygen into the system in an amount proportional to the potential alcohol content, but in the first half of alcoholic fermentation. The most common method used in small wineries to **solubilise oxygen** is an open circuit pump over, where the must-wine is jetted out of the tank into a vessel, from where the pump draws in liquid to wet the cap at the top of the tank. However, it is not easy to solubilize an effective quantity of oxygen (e.g. a saturation of 8 mg/l of O_2), because there is a large quantity of CO_2 which is released from the must-wine and creates a flow of gas which takes the air away from the contact with the surface of the liquid; it is therefore necessary to use a large vessel, drop the

liquid from a sufficient height (> 40-50 cm) and pump-over at least 1/3 of the volume of the tank.
There are more effective and controllable systems, which have become more widespread, particularly in large cellars.
The use of a Venturi tube in the system makes it possible to add a constant quantity of air to the must-wine used for pump-overs, which is much simpler and does not require as much labour as an open circuit pump-over.
The limitation of these two techniques is that the amount of oxygen introduced into the system depends on the volume used for the pump-overs and the frequency of the pump-overs, which depends on the desired extraction dynamics and not on the fermentation progress. In order to have more control over the operation, the practice of macro-oxygenation has become widespread, i.e. the injection of compressed air into the tank via a diffuser that creates bubbles of such a small size that all the oxygen can enter the solution before reaching the top of the liquid: in this way it is possible to calculate the exact quantity of oxygen introduced into the system and carry out the operation at the most opportune moment.

Racking and malolactic fermentation

At racking, the yeasts are not multiplying but are still consuming oxygen even after their death. The yeast lees, therefore, together with the polyphenols, represent an important barrier to oxidation.
In many cases, even too much so.
At this stage, in fact, red wine can be improved by **controlled oxygenation**, which introduces enough oxygen into the system to allow the polymerisation of the tannins and the fixation of the anthocyanins and to avoid the formation of sulphur compounds in such quantity and nature as to cause organoleptic defects in the finished wine.
Malolactic bacteria are micro-aerophiles, so they are not inhibited by a small amount of oxygen in solution. The most commonly used aeration methods are air racking and **micro-oxygenation**; the latter technique is certainly the one that allows greater control over the quantities of oxygen involved, but it requires a certain investment to equip a number of tanks in the cellar.

This is the most delicate phase, where the winemaker's skill and experience play an essential role in identifying the right amount and frequency of oxygenation, which is almost always defined on the basis of organoleptic judgement that must, however, consider all the journey the wine still has to make before it is finished.

Another important element of criticality in this phase is the microbial system: normally in red wines it is desired to induce malolactic fermentation, and at racking the addition of sulphur dioxide is delayed and milder temperatures are sought to encourage the development of bacteria. These conditions are also favourable for polyphenol polymerisation, so it is the best time for micro-oxygenation. However, these are also the best conditions for negative microbial contamination (*Brettanomyces* or harmful lactic acid bacteria). In order to avoid having to end this delicate phase hastily, the **co-inoculation of bacteria** during fermentation is increasingly used, so that only the oxidative balance is managed at racking and not the microbial balance at the same time.

Storage and ageing

Throughout the period that the red wine remains in the cellar, before bottling, it is necessary to maintain the oxidative balance, allowing enough oxygen to enter into solution to allow the phenolic and aromatic characteristics to evolve positively, without there ever being more oxygen than these reactions can consume. This balance is not at all easy to determine, as each individual wine has its own specific behaviour. Storage in full vats does not allow oxygen to enter, whereas wooden containers allow a certain amount of air to pass through, especially from the closures.

But the most important contribution occurs when the **wine is being moved** from one tank to another for racking or when the barrels are uncorked for batônage (where negative pressure is formed and sucks in air when the barrels are being opened). It is therefore important to pay attention to the timing and method of these operations, bearing in mind that the lower the temperature of the wine, the greater the quantity of oxygen that passes into solution, with the same exposure to air.

Bottling

The same balance sought in vinification and storage must be considered at the bottling stage. The strategy to be adopted with regard to oxygen depends very much on the composition of the wine and its expected lifespan: a wine that is very rich in polyphenols and destined for a long life in the bottle will need a slow but significant **supply of oxygen through the closure** in order to evolve a complex but not reduced bouquet; conversely, for a less important wine, to be drunk young, it is best to avoid oxygen entering through the closure.

Especially in the case of less full-bodied wine, it is essential to control the oxygen present in the **headspace** of the bottle, which must not be more than the wine can consume in the days following bottling. Attention should also be paid to the operations involved in preparing the wine for bottling: handling with pumps for final stabilisation, cold stabilisation, filtration, filling the bell of the bottling machine are all steps that can bring in significant quantities of oxygen.