OXYGEN AND WINE

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Extract from Technical Notes of Code of Best Practice for Organic Winemaking, produced under the EU FP6 STRIP project ORWINE

General Principles

Oxygen represents about 20 % of the air that we breathe thus it is everywhere. Thus wine-makers must be aware of it's important role in affecting different technological operations.
There are different wine-making theories with regards oxygen management in oenology.
Some producers are convinced that O_2 is an "enemy" for the wine (oxidations, browning) whilst others think that a limited and controlled oxygenation is fundamental for correct wine development.
These opposite beliefs lead to the definition of two different strategies in the management of the interactions between oxygen and wine.
Firstly there is the total protection of the wine itself from contact with air (e.g. in hyper-reductive technologies) or conversely the controlled oxygenation of the wine (such as in micro- or hyper-oxygenation).
Both these approaches are utilized in wine-making nowadays with different technological implications and different impacts on the characteristics of the products obtained.

Effects of oxygen dissolution in wine

Oxygen can play a double role in wine, affecting sometimes positively, sometimes negatively the wine characteristic. The equilibrium between these effects depends on the amount of the dissolved concentration of oxygen, on the moment of the dissolution, and on the characteristics of the wine itself (e.g. red wines are less sensitive to oxidation).
In particular, the effects of oxygen are related to the following aspects:

1. Modification of phenolic compounds:
   - Browning and modification of colour for both musts and wines, as a consequence of the oxidation of polyphenols.
   - Positive effects on wine evolution and aging (e.g. reduction of astringency, stabilisation of the phenolic fraction).

2. Modification of aromatic fraction:
   - Evolution of wine aroma and formation of aging related compounds.
   - Decrease in varietal notes and development of oxidation characters.

3. Effects on the multiplication and growth of micro-organisms.

As mentioned above the equilibrium between these positive and negative effects of O_2, depends on different factors:

- Variety
  Some varieties (e.g. Sauvignon blanc, all Muscat varieties) are very sensitive to air contact. The resistance of a substrate to oxidation is related to its composition: a higher content of natural antioxidant compounds in the juice (polyphenols, glutathione, ascorbic acid.) can improve such resistance, reducing the susceptibility to O_2.

- Temperature
  This variable affects both the dissolution and the activity of O_2 in musts and wines. At 20-25 °C, the maximum possible amount for the dissolution of oxygen is approximately 6-7 mg/L (concept of "saturation") but this level can increase at lower temperatures: approx. 10 mg/L

at 5 °C. On the contrary, the rate of oxidation reactions increases at higher temperature. For example the oxidation of the red wine colour compounds such as anthocyanins occurs faster at 30 °C than at 20 °C.

- Step of the wine-making process
  The oxidation rates of musts are usually higher than those detectable in wine, because in musts oxidations are enzymatically catalysed by polyphenoloxydases (PPO). These enzymes are derived from the grape (tyrosinase) or from moulds (laccase from *Botrytis cinerea*) and they are able to dramatically increase the oxidation reaction. Laccase, in particular, can induce damage on the composition of the must itself. This is why the vinification of grapes infected by *Botrytis* is often problematic from the point of view of O_2 management, and higher levels of sulphites are needed.

- Time of exposure to air
  Oxygen is quickly consumed after its dissolution, and the effect of this utilisation is dependent on the composition of the wine. The uptake of O_2 entails the development of certain reactions. If the air contact is limited in time, the effects of oxygenation will remain limited to the consumption of the dissolved amount, but if the dissolution is prolonged in time, we will observe a continuous sequence of dissolution - consumption. The final effects of this sequence will depend on the ability of the must - wine to resist oxidation. If the content of antioxidants is low, the wine will not be able to effectively resist the effects of O_2 consumption.

Redox equilibrium of the wine and antioxidant compounds

Many compounds in the must and wine coexist as mixtures of their oxidised and reduced forms, the so called “redox pairs”. The reduction of one compound always causes automatically the oxidation of another one. In chemical terms these oxidation-reduction (redox) reactions continue until the “equilibrium point” is reached and neither reduction nor oxidation compound dominate.
In the wine-making-related reactions, this “redox” equilibrium reflects two groups of compounds: some of them can act as oxidising agents whilst others are reducing agents.
The most important oxidising agent in musts and wines is oxygen. Other chemicals can increase its action in wine by acting as powerful oxidants themselves. An important example of this is related to the heavy metals such as iron and copper. These compounds are normally present in wine and are powerful catalysts. They can strongly increase the action of oxygen and the rate of oxidation reactions. In addition some free radicals and peroxides (e.g. hydrogen peroxide – H_2O_2) are produced from the oxidation of phenolic compounds and can also be involved as oxidising compounds.

The most important reducing agents found in wine are sulphur dioxide (SO_2), ascorbic acid, phenolic compounds and glutathione.
Ascorbic acid (AA), known also as vitamin C, can be found in a wide range of concentrations in different fruits. This compound plays an important role in limiting enzymatic browning in musts, but in its action in wines it has been demonstrated that it can react with oxygen generating hydrogen peroxide (a powerful oxidising compound). Ascorbic acid is normally used in wine in combination with SO_2 in order to scavenge H_2O_2 reducing the risk of “oxidative damage”.
Glutathione (GSH) is a tripeptide (made by glutammic acid, glycine and cysteine) widely occurring in nature in plants and micro-organisms. It is active against free radicals and other oxygen reactive compounds. GSH can strongly reduce the process of must oxidation, reacting with some products of the enzymatic transformation (PPO) of caffeoyltartaric acid (one of the most oxidisable substances present in the grape juice). The result of this reaction is named 2-*S*-glutathionyl-*trans*-caffeoyltartaric acid, also known as “Grape Reaction Product” (GRP). In normal conditions (with healthy grapes) this compound is stable in successive oxidations and for this reason glutathione is able to stop the oxidation chain which can lead to must oxidation and browning.
The problem remains in the musts affected by *Botrytis*, because GRP can be a substrate for laccase enzyme; for this reason the vinification of botrytised grapes always has more browning reaction problems.

It is well known that polyphenols and tannins are powerful antioxidants. These compounds are one of the main oxygen reactive chemicals present in musts and wines. The results of their oxidation are browning and the loss of colour as well as the formation of polymers with their subsequent precipitation. The presence of polyphenols in greater quantities in red wines explains the higher resistance of such products to oxidation.

Oxygen reactions in the musts

The oxidation reactions in musts are mainly related to enzymatic activities (PPO), on phenolic acids (e.g. caffeoyltartaric acid).

In the case of healthy grapes, tyrosinase (from the grape itself) is the major enzyme involved in the browning reactions. The activity of this macromolecule is easy to reduce in the juice, because it is quite sensitive to SO_2 and it is easily removable by some fining agents such as bentonite. On the contrary, laccase from *Botrytis cinerea* is poorly affected by bentonite treatments as well as by sulphites this being a greater problem for wine-makers.

The strong reactivity of the musts to oxidation can be used to stabilise the must itself. The concept of hyper-oxygenation is based on the saturising O_2 addition to the juice in such a way that all the oxidisable substances are eliminated by polymerisation and precipitation with a simple racking .

Oxygen reactions in the wines

As opposed to the reactions in musts, wine oxidation is mainly related to chemical or non enzymatic reactions.

It is important to remember that O_2 is not always negative for wine evolution. Pasteur himself during his studies observed that suitable aeration was important in the development of alcoholic fermentation.

A well managed oxygen supply can determine certain advantages to wine especially red ones viz:

- evolution and stabilisation of the colour by the reaction between anthocyanins and tannins;
- reduction of astringency by the evolution of tannins;
- Better development of alcoholic fermentation by the production of basic growth nutrients for the yeast.

These advantages (particularly the first two points) have been rercognised since the dawn of the wine-making with the technique of wood aging (limited and controlled O_2 dissolution throughout the wood) and nowadays through the modern application in micro oxygenation technology (microox). It is also well known that the passage of a limited flow of oxygen through the bottle closures is beneficial for the correct development of a wine as well as for its conservation.

When the oxygen supply is too high respect to the ability of the wine itself to resist to the consequences of its consumption, oxidations automatically occur.

As reported for the musts, it is the phenolic compounds that react with the oxygen which results in the browning and loss of the colour, together with the precipitation of the colouring matter.

These oxidation reactions may also cause the formation of different kinds of volatile compounds which are sometimes responsible for aromatic changes. Acetaldehyde (MeCHO) is the main volatile compound involved with oxygen consumption. In this case, it does not derive from microbial metabolism but from the oxidation of ethanol which has been catalysed by some heavy metals (iron and copper).

In wood aging or micro-ox, it is this acetaldehyde that is involved in some reactions related to colour and phenolic stabilization. If O_2 dissolution is concentrated or prolonged, the higher amounts of MeCHO formed can induce the production of other aroma compounds (acetals) which are responsible of the typical sensory notes of the oxidised wines.

Important note

When speaking about the effects of aeration on aromatic compounds, it must be stated that in the early steps of the vinification process, volatile compounds are relatively protected against O_2 as they are present in the form of “precursors”. For example, terpenes, an important family of compounds which characterize the aroma of Muscat grapes (but are practically present in all the fruits) are mainly present in the must as glycosides (bound to sugars). In this form such molecules are less sensitive to oxidation than in the free form.
On the basis of this concept, the practice of hyperoxygenation, which is based on a heavy oxygen supply just after the obtainment of the juice, will poorly affect the aroma composition of the final wine, because of the protection of aroma in the combined form of these precursors.
On the contrary, due to the fact that glycosides are broken during the vinification with the subsequent release of the volatile compounds in free form, the effects of O2 on the wine aromatic fractions will negatively affect the varietal characters of the product: in fact, the aromas in free form will be more sensitive to oxidations.
This is particularly true for some compounds produced from specific aromatic varieties, such as Sauvignon blanc or Muscat varieties. The varietal aroma of Sauvignon blanc wines is related to the presence of certain sulphur containing compounds which are very sensitive to air. In the must these molecules are relatively protected as precursors (bound to the amino acid cysteine), but in the wine the free form is very sensitive to O_2.
Some evidences confirm that, due to their extreme susceptibility to oxidation, these volatile sulfur compounds can be partially lost even in must, if oxygen contact is not managed as well; some specific technologies (hyper-reduction practices), based on the use of ascorbic acid and sulfur dioxide during crushing, were suggested to preserve as well these O_2 sensitive varietal notes

Effects of oxygen on yeasts growth

It is generally accepted that in must, yeasts are able to respire sugars in aerobic conditions whilst they perform alcoholic fermentation (AF) in anaerobiosis.
As a matter of fact, the ability of wine yeasts to use the glucose through respiration is dependant on the sugar content of the must. If the sugar concentration is higher than 9 g/L, *Saccharomyces cerevisiae*, the main micro-organism involved in alcoholic fermentation, is unable to bring about the aerobic transformation of sugars. This means that under normal conditions in the must (sugar content approx. 180-220 g/L), the yeasts can only perform alcoholic fermentation. This phenomenon is known as “Crabtree effect”.
Anyway, it is well known that the aeration of the must after the inoculation of selected yeasts (or the oxygenation of the *pied de cuvee* before addition), benefits the development of the fermentation process. These benefits are not related to increased yeast populations obtained by the respiration process[1], but instead they are mainly related to the fact that the oxygenation itself leads to the production of fundamental growing factors for the yeasts, such as some fatty acids and sterols. Similarly a slight air supply (e.g. by pumping over) at the middle of AF, is also useful in obtaining satisfactory development of the final steps of the fermentation process.

Important note

As well as its action on yeasts, oxygen can also affect the metabolism of other micro-organisms. For example acetic bacteria are responsible of the oxidation of sugars which occur in aerobic conditions. In extreme conditions glucose is completely oxidised by these micro-organisms to water and carbon dioxide.
Ethanol is also a potential substrate of these bacteria. It is transformed in acetic acid and

[1] The utilization of sugars by respiration produces more energy than the fermentation process. Thus respiration is encouraged in order to obtain a rapid multiplication of yeast populations during the industrial production of selected yeasts

then ethyl acetate, compounds that are responsible for the increase of volatile acidity and for the formation of the typical odour which occurs in wines affected by acescence.

Thus the reduction of oxygen during wine storage is essential for the prevention of both chemically and microbiologically-related oxidations. Therefore producers should take care to completely fill all the containers avoiding extended exposure of the wine to the oxygen present in the headspace (e.g. leaving the tanks empty after a racking).The use of inert gases such as nitrogen or carbon dioxide, and the control and eventual reintegration of sulfur dioxide levels, could be useful strategies to protect the wine during transfers and storage.

ACKNOWLEDGEMENT

The authors gratefully acknowledge from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Specific Targeted Research Project “ORWINE” SSPE-CT-2006-022769.