Microoxygenation of red wines

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ABSTRACT

Microoxygenation is usually applied to red wines as a cheaper alternative to oak ageing. Utilization of wood cooperage for wine storage has several advantages. Among these are extractions of flavour and aroma active components, as well as controlled oxidative polymerization, a process commonly referred to as ageing. Although stainless steel tanks are, in the long term, less costly than cooperage, stored wines do not benefit from the features offered by wood. The process of microoxygenation in steel tanks utilizes controlled exposure of wines to oxygen provided by a sparger linked via a flow meter to a cylinder of oxygen. Oxygen flow rates vary over the course of treatment. During this period, different chemical reactions take place. For example, wine phenols (tannin precursors and anthocyanins) react to form polymeric species that enhance palate structure and colour stability in the wine. Oxygen also diminishes excessively green, herbaceous characters and reductive aroma of wine.

Key words: wine, microoxygenation, microoxy-genators, oxygen, phenols, colour, stability, anthocyanins, tannins, polymerization


Kljucne besede: vino, mikrooksigenacija, mikrooxygenatorji, kisik, fenoli, barva, stabilnost, antocijan, tanini, polimerizacija

1 INTRODUCTION

High-quality red wines are traditionally stored for a long time in oak barrels to improve their sensorial attributes. Oak ageing leads to colour stabilization, lower astringency, and the disappearance of excess vegetative notes. These latter transformations seem to be associated with small quantities of oxygen that penetrate the porosity of wood, the interstices between staves, and bunghole. The process of microoxygenation aims to mimic the effects of slow barrel maturation within a shorter period and for less of the long-term cost associated with oak barrels.

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The purpose of micro-oxygenation is to bring about desirable changes in wine texture and aroma which cannot be obtained by traditional ageing techniques. The objectives of the process include improved mouthfeel (body and texture), enhanced colour stability, increased oxidative stability, and decreased vegetative aroma. As treatment proceeds, one eventually observes an augmentation of the aromatic intensity, a development of the complexity. The tannins are less hard and softer, the body of the wine is increased, and the wine's mouthfeel is rounder. The herbaceous aromas and the reduction character vanish and the length may increase.

Microoxygenation has been employed commercially in France as a wine treatment technique since 1991 when Patrick Ducournan began experimenting on the wines of Madiran in south-western France. The technique consists of continuously bubbling small amounts of oxygen in the wine, slower than the rate of consumption so that there is no accumulation of dissolved oxygen. Since its inception, the technique has commercially spread throughout the winegrowing world and is now systematically used in some wineries entire winemaking process, predominantly red wines.

2 OXYGEN IN WINE

2.1 Oxygen solubility

The dissolved oxygen concentration can be calculated by using a solubility coefficient, using Henry’s law: \( p_{O_2} = H \cdot C^* \), where \( H \) is the oxygen solubility coefficient and \( C^* \) is the gaseous oxygen concentration at equilibrium. The oxygen solubility coefficient depends on temperature, pressure and the liquid composition.

Berta et al. (1999) report that wine is saturated with oxygen at 7.7 mg/L at 20 ºC. The oxygen solubility decrease as the ethanol content increase up to 30%, but beyond that ethanol content strongly increases the oxygen solubility. The oxygen solubility also depends on wine temperature, content of total dry extract, reducing sugars and carbon dioxide (Cheynier et al., 2002).

2.2 The role of oxygen during winemaking

Microoxygenation is a controlled technique, which aims to manipulate the rate and result of the oxygen-requiring reactions in wine in order to bring desirable changes in wine texture and aroma (Castellari et al., 2000; Atanasova et al., 2002, Cagnasso et al., 2003). This can be contrasted to the well-known and widely used practice of aerated racking which adds oxygen to the wine in large, discrete doses (Figure 1).

For example, it has been estimated that ullage from proper barrel storage adds as much as 12 to 20 mL/L per year of oxygen to wines (Zoecklein, 2007). Therefore, allowing for several rackings, a total of around 30 mL/L could be added to red wine in barrel each year.

It has long been recognized that oxygen plays an important role in the numerous microbiological and biochemical events that take place during the life of a wine. These events not only facilitate the winemaking process but also ultimately affect the organoleptic characteristics of the finished wine (Morata et al., 2006).

During microoxygenation small, controlled amounts of oxygen \((O_2)\) are bubbled into wine to bring about positive changes in the wine. This is achieved by filling a known volume with gas at a high pressure. The volume is then transferred via a low-pressure circuit to the diffuser and into the wine. The latter normally

![Figure 1: Dissolving oxygen during microoxygenation and racking of wine (Cagnasso et al., 2003)](image-url)
consists of a ceramic or stainless steel sparger that produces small bubbles, which can dissolve in the wine (du Toit et al., 2006). The aim of microoxygenation is to introduce O₂ into the wine at a rate equal to or slightly less than the wine’s ability to consume that O₂ to avoid too much O₂ build up in the wine. It has to be managed in such a way that, after addition, all O₂ has been used up, while sufficient SO₂ is still left to protect the wine against excessive oxidation and microbial spoilage (du Toit et al., 2006).

• During fermentation
  Oxygen is necessary for healthy and viable yeast cells. In particular, it promotes synthesis of sterols/fatty acids in yeast cell walls. It is generally accepted that there is little risk of oxidation during fermentation. However, some aromatic and delicate white wines such as Riesling and Sauvignon Blanc may lose some volatile compounds with over-enthusiastic oxygen sparging.

• For white wines
  Oxygen can interact with lees to increase the apparent weight and mouthfeel of wines, especially those stored in barrel. Oxygen can also promote browning of colour and the loss of positive aromatics.

• For red wines
  Much research and practical experimentalations has shown the integral role of oxygen plays in the polymerization of polyphenolic compounds, especially in the early stages of maturation. Polymerizations can produce stable forms of anthocyanins that resist decolourisation by sulphur dioxide and provide better colour stability at wine pH. It can also result in coloured forms (pigment polymers) that are stable over time. On the other hand too much oxygen can help bring about the formation of large molecules with high molecular weight that are unable to stay in solution. This causes precipitation of polyphenolic material, leaving wines dry and harsh to the taste with reduced colour intensity.

• For improving aromatic profile
  Winemakers have found that repeated aerated rackings can diminish excessively green, herbaceous characters.

• For removing reductive characters
  Exposure to air, usually via racking, can help remove unpleasant reductive, sulphidic characters from wine (Parish et al., 2000; Goals …, 2001; Paul, 2002; Zoecklein, 2007)

3 EFFECT OF MICROOXYGENATION ON WINES

Microoxygenation has effect on fermentation development, ageing process, phenolic and volatile composition, colour and on the sensorial properties.

Oxygen plays an important role in the different process that take place during winemaking process and the ageing of wine. Besides, oxygen has an influence on the phenolic composition and indirectly, also has an effect on some sensorial characteristic, such as colour, aroma and astringency, all of which determine wine quality (Atanasova et al., 2002; Ortega Heras et al., 2008).
Oxidation condensation and polymerization reactions in which different compounds are involved (mainly phenolic compounds) are oxygen dependent (Rivero-Pérez et al., 2008). These reactions lead to formation of new pigments and polymeric compounds that can stabilize wine colour and reduce astringency, as pyranoanthocyanins and ethyl-bridged adducts shown in Figure 2 (Ortega Heras et al., 2008).

The oxygen dissolution, by enhancing the condensation and polymerization reactions, influence on the content of some phenolic compounds such as catechin, epicatechin, ferulic acid, p-cumaric acid, quercetin, trans-resveratrol, caffeic acid and other (Castellari et al., 2000; Llaudy et al., 2006; Cano-López et al., 2006). Oxygen has also an effect on the volatile composition of wine. The addition of oxygen showed changes in the content of some esters, short chain fatty acids, terpenic compounds, hexanol, and other volatile compounds (Ortega Heras et al., 2008).

### 3.1 Oxygen consumption in wine

Oxygen consumption is much faster in red wines than in white wines, indicating that it is largely due to the oxidation of phenolic compounds. It is also accelerated at higher temperatures (Cheynier et al., 2002). Moutonet and Mazauric (2001) report that the consumption of oxygen in red wine saturated with oxygen takes 25 days at 13 °C, 18 days at 17 °C, 4 days at 20 °C, 3 days at 30 °C and just few minutes at 70 °C. Wine lees have been shown to also contribute to oxygen uptake, thereby competing with phenolic compounds and impeding the wine ageing process (Fornairon et al., 1999). The oxygen consumption capacity varies from 80 mg/L (in whites) to 800 mg/L (in reds) and thus much exceeds the optimum oxygen supply. An increase in pH and phenolic compounds enhances the consumption of oxygen. In general, the kinetics of oxygen dissolution in wine is much higher than its consumption. The more oxygen added, the more dramatic the results will be. Nowadays, tasting is the only way to evaluate the need of oxygen for a wine, and some correlations with analytical parameters such as total polyphenols, tannins and astringency are being studied (Pérez-Mangariño et al., 2007).

The amount of oxygen added is usually indicated as mL/L or mg/L. At 15 °C 1 mg of oxygen is equal to 1.47 mL and at 20 °C 1 mg of oxygen is equal to 1.5 mL (Nel, 2001).

### 3.2 Oxygen and polyphenol reactions

Oxygen stimulates different chemical reactions, especially polymerization of anthocyanins and tannins. This has the effect of reducing the amount of free anthocyanins and increasing the amount of condensed anthocyanins. Importantly, these condensed forms are generally coloured at wine pH (Atanasova et al., 2002; Paul, 2002). Oxidation reactions involving phenolic compounds are extremely complex processes that are...
not fully elucidated. The major phenol compounds in young red wines are anthocyanins, the pigment of red grapes, and flavanols, which are encountered as monomers (catehins) and as oligomers or polymers (proanthocyanidins, also commonly called condensed tannins).

### 3.3 Chemistry of oxygen in wine

Phenolic reactions in wine generate modified tannins, degrade existing tannins, or generate new ones (Zoecklein, 2007; Ortega Heras et al., 2008). Wines are complex mixtures of grape phenolic compounds, usually grouped under the names anthocyanins and tannins, that can come from different sources: Colourful anthocyanins and less colourful procyanidins come from the grape skins, and harsh, even bitter, phenolics come from seeds, while odd-tasting, harsh phenolics come from stems. Polymerization and de-polymerization of tannins, and of tannins and anthocyanins, greatly impact their sensory characteristic. With oxygen exposure, several different structural linkages can create tannin polymerization. Polymerization reactions that occur between anthocyanins and tannins may generate stable compounds, which provide more colour intensity and are more resistant to degradation (Zoecklein, 2007).

Anthocyanins molecules have a positive charge. It increases the reactivity of the ring structure, which can lead to the destruction of the positive charge. This is countered by binding with tannin molecules, such as can occur with microoxygenation. The degree to which tannins and anthocyanins bind together is, in part, a function of the concentration of these molecules in solution. Anthocyanins and tannins bind together in two ways, depending upon the oxygen concentration (Ribéreau-Gayon et al., 2000). Under reductive conditions (low redox potential), hydrolysis may break down a tannin molecule, producing two products, one charged molecule and one neutral molecule. Depending on the concentrations of tannins and monomeric anthocyanins, the charged molecule formed will react with one or the other. If it is tannin present, a longer oligomer or polymer will be form (Ribéreau-Gayon et al., 2000; Zoecklein, 2007).

However, the process differs if an anthocyanin is involved. An anthocyanin, in the hydrated or colourless form, provides an electron-rich molecule which more readily reacts with the charged tannin. The reaction occurs between the two molecules at the carbon-4 and carbon-8 positions, and a covalent bond is formed. Once formed, the larger tannin moiety acts as an electron sink and a stabilized colour or anthocyanin-tannin adduct is produced. The terminal molecule, the anthocyanin, no longer has available electrons in excess to further react, meaning that the anthocyanin acts as a terminus for any further reactions at this end of the polymer (Ribéreau-Gayon et al., 2000; Zoecklein, 2007).

The other tannin-anthocyanin reaction method involves oxidative polymerization. As such, acetaldehyde can play an important role in the formation of phenolic polymers in a wine and, thus, in microoxygenation. Acetaldehyde-bridged molecules (Figure 3) form to bind phenolic compounds together. These compounds are relatively stable and are somewhat resistant to bleaching by bisulphite ion (Ribéreau-Gayon et al., 2000; Zoecklein, 2007). Acetaldehyde bridging can also facilitate the formation of tannin-tannin complexes. Acetaldehyde linkages usually lead to C8-C8 bonding instead of C4-C8 (Cheynier et al., 2002). This can lead to different sensorial properties.

Acetaldehyde can be produced by yeasts during fermentation, can result from the coupled oxidation of ethanol by phenolics, and can be produced by adding toasted oak wood into a fermentor. The oxidation of ethanol to acetaldehyde occurs in the presence of O₂ at an appreciable rate (Wildenradt and Singleton, 1974). This coupled reaction involves the oxidation of a simple phenol (vicinal diphenol) to produce a coloured molecule (ortho-quinone). Hydrogen peroxide (H₂O₂) is produced as an intermediary of coupled oxidation. H₂O₂, a strong oxidant, then reacts with ethanol to form acetaldehyde. The newly-formed acetaldehyde can react with phenolics in a wine (Escribano-Bailón et al., 2001). Acetaldehyde forms a polymerization product between anthocyanins and tannins through an aldehyde bridge, for example. These can further react with other procyanidins or anthocyanidins, to form more complex trimers. However, it has been shown, that the compounds formed by ethyl bridges are unstable (Escribano-Bailón et al., 2001).
Figure 3: Structure of pigments derived from the acetaldehyde-mediated condensation between anthocyanins and flavanols. $R=$flavanol units (Atanasova et al., 2002)

Acetaldehyde also participates in the formation of new pigments such as vitisin B and other pyranoanthocyanins (Fulcrand et al., 1998; Mateus et al., 2002). Pyranoanthocyanins are not present in grapes but form during wine production via various condensation reactions (Morata et al., 2006). The molecules are named from the presence of a fourth pyrane (heteroaromatic) ring which forms during the condensation reaction. Pyranoanthocyanins have common spectral characteristics with absorption maxima of 495-520 nm that are lower than those of grape anthocyanins. They contribute to the red-orange colour wines developed during ageing. The presence of this fourth ring renders these pigments more stable than grape anthocyanins to discoloration by SO$_2$, and to colour loss due to high pH and oxidative degradation during fermentation (Bakker and Timberlake, 1997, Cano-López et al., 2006).

4 PHASES OF MICROOXYGENATION

Oxygen can be supplied during different stages of the winemaking process. The total dose can range from 60 mL/L for lighter whites, to 600 mL/L for tannic reds. It can be supplied at 1-5 mg/L/day for a few days just after malolactic fermentation, especially to press wine fractions that are rich in polyphenols (du Toit et al., 2006). The stage when microoxygenation is normally applied is during the ageing period after malolactic fermentation, when between 1-6 mg/L/month is introduced into the wine, although certain researchers recommended addition even up to 10 mg/L/month (du Toit et al., 2006).

The wine’s temperature must be around 15 ºC (Figure 4) because temperatures that are too high will lead to poor solubility of O$_2$ and temperatures that are low to chemical reactions taking place too slowly.

The best time to start the microoxygenation is when the alcoholic fermentation is complete with or without lees. Lees will take up oxygen, however, so far red wines it is more effective to rack off lees when practical or at least to be sure they are well settled (Goals ..., 2001). We can divide microoxygenation into three phases:
- phase of structuration (before and after malolactic fermentation),
- phase of harmonization,
- phase of saturation – over oxygenation.
4.1 Phase of structuration

The initial phase is termed structuring and is characterised by the building of tannins or an apparent increase in the tannic structure of the wine. This phase is divided in two parts.

4.1.1 Prior to malolactic fermentation

Microoxygenation ideally begins directly after alcoholic fermentation and before malolactic fermentation, for two to six weeks, when colour stabilization occurs (Goals …, 2001; Parish et al., 2000). Getting started quickly is important because wine rapidly loses its ability to absorb O₂.

Monomeric and oligomeric anthocyanins are more unstable in the early phase of wine maturation. The early addition of oxygen is intended to stimulate polymerization and increase colour stability. At this time the tannins are more susceptible to oxidation due to the lack of SO₂ (Paul, 2002). To delay malolactic fermentation, the use of lysozyme has been suggested. During this period, it is necessary to taste three times per week to adjust treatment based on several sensory clues. For example, acetaldehyde aroma in this phase should be present only at the level of a chocolate-like aroma, more than that indicates the level of O₂ should be turned down; hydrogen sulfide (H₂S) and related reduced aromas indicate to raise the level of O₂.

The wine may also taste different because of the loss of CO₂ and green flavours when it is sparged with oxygen (Goals …, 2001; Parish et al., 2000). The most important sensory feedback concerns tannins (Figure 5). Wine tannins are classified in four ways: green, hard, soft and dry. The treatment moves tannin from green
through hard to soft, but not as far as dry (Zoecklein, 2007).

4.1.2 After malolactic fermentation and SO₂ addition
The second phase of structuration begins after malolactic fermentation and SO₂ addition. Dose rate is turned down about ten-fold, and tannin evolution continues (du Toit et al., 2006). Structuration potential depends on the initial structure of the wine. Wines high in tannins and low in anthocyanins risk dryness. High anthocyanins and low tannins indicate a low risk of dryness. High tannins and high anthocyanins make for the best situation, in which high oxygen levels may be used. Low tannins and low anthocyanins make for the most difficult winemaking (Goals …, 2001; Paul, 2002). Reactions are slower and less significant after sulphur dioxide addition because of its ability to bind with acetaldehyde and quench oxygen. SO₂ will readily bind to any free acetaldehyde, thus removing it as a reactant. In order to achieve the desired results from acetaldehyde-induced coupling, binding must occur before the wine is sulphited, or the free SO₂ level should be low (15 mg/L depending on pH) (Zoecklein, 2007). This usually means SO₂ additions are postponed until after microoxygenation is complete.

It may be difficult to differentiate between the normal hardening that occurs during this phase and a drying of the tannins. In this situation, a check on the SO₂ level, the evolution of the aromatic compounds, and the measure of the dissolved oxygen will enable you to determine if the process is occurring properly (Goals …, 2001; Parish et al., 2000). Tasting training on recognition and objective evaluation of types of tannins is a useful aid in following the wine and assessing the proper rate of addition. The temperature has a double impact on structuration. There is a direct influence on structuration. Ther e is a direct influence on structuration. On the other hand, because the notion of hard tannins and dry tannins are very close, one can be confused. If the tannins are hard, it means that the wine needs some oxygen to soften them, and that the harmonization phase must continue. If the tannins are dry, it means the contribution of oxygen must be limited (Goals …, 2001). The goals of microoxygenation are to:
- establishing desired aromatic and taste qualities of the wine,
- developing aromatic complexity,
- improving the sensory qualities of tannins,
- stabilizing the wine from reductive flavours,
- diminishing some of the herbaceous flavours and any other remaining defects.

4.2 Phase of harmonization
A harmonization phase follows the structuration phase. Once the period of tannin building has concluded, the harmonization stage is said to commence where the perceived tannic structure softens and the wine becomes more supple and approachable. The harmonization phase contrast with the structuration phase. The length of the harmonization phase is related to the structuration phase. It is the period of time going from the ageing to the bottling of the wine. The harmonization phase should generally be twice as long as the structuration phase, unless violent oxygenation (clique-age) is used to accelerate that period. We can call it the evolution phase of the wine, because the modifications occurring then are irreversible, as opposed to the first phase. Risks include the development of palate dryness, and excessive maturity accompanied by lost freshness and oxidized aromas (Goals …, 2001; Parish et al., 2000).

The dose of oxygen in this phase usually does not go over 1.0 mL/L/month (Goals …, 2001; Parish et al., 2000). The effects of the variation of the dose are more crucial than during the structuration phase. A variation in dose between 0.5 mL/L/month and 1.0 mL/L/month can produce a very different reaction in the wine. As the dose is very low during this phase, there is little risk of the accumulation of dissolved oxygen, so the temperature level is not very important. However, high temperature results in fast evolution and therefore increases the risk of oxidation (Goals …, 2001).

The ideal dose is determined by tasting, which looks for maximum aromatic benefit without causing dryness on the palate. As soon as the tannins begin to seem too dry, it is necessary to limit the oxygen or even to stop the microoxygenation. If microoxygenation is continued at this point, the result will be a wine that lacks in volume, becomes very flat, and the aromas of oxidation may appear irreversibly (Goals …, 2001; Parish et al., 2000). On the other hand, because the notion of hard tannins and dry tannins are very close, one can be confused. If the tannins are hard, it means that the wine needs some oxygen to soften them, and that the harmonization phase must continue. If the tannins are dry, it means the contribution of oxygen must be limited (Goals …, 2001).

4.3 Phase of saturation
If a wine undergoes microoxygenation for too long then the tannins tend to dry out and become more astringent. This stage is termed over-oxygenation.
Wine must be monitored during microoxygenation. This can be time-consuming, especially in the pre-malolactic phase. Monitoring of the following parameters is suggested (Paul, 2002):

a) Dissolved oxygen: There should be no discernible increase in dissolved oxygen levels if microoxygenation is conducted properly, with an appropriate oxygen flow rate.

b) Free sulphur dioxide, if present: There should be no significant decrease in free sulphur dioxide levels during microoxygenation. However, it is important to understand that this does not mean the flow rate is correct; simply that it is not too high.

c) Temperature: This is an important and often misunderstood parameter. Microoxygenation works best between 14-17 °C. If the temperature is too low, oxygen solubility is increased and reaction rates are decreased. This results in an increase of dissolved oxygen. If the temperature is too high, reactions occur more rapidly.

d) Turbidity: In general, wines should have some degree of clarification for successful microoxygenation. Wines should be below 200 NTUs (nephelometric turbidity units) and ideally below 100 (lees have a well-known affinity for oxygen). Of course, wines above these levels can be successfully treated, but more effort is required to monitor.

e) Tasting: Tasting microoxygenated wine is not intuitive. Some training and exposure to treated wines is valuable.

Acetic acid bacteria and Brettanomyces are both well-known spoilage microorganisms of wine. Both the organisms have been proven to grow in the wine when oxygen levels are increased (du Toit et al., 2006). Acetic acid bacteria can form elevated levels of acetic acid through the oxidative metabolism of ethanol. Oxygen could also stimulate the growth of Brettanomyces, but no direct link can be established with microoxygenation (Paul, 2002; du Toit et al., 2006). Brettanomyces can cause medicinal/barnyard characteristic in wine when oxygen levels are increased. If Brettanomyces grows in a microoxygenated wine, there may be other, more fundamental reasons for its proliferation, including high pH, low SO₂ and stressed fermentation amongst other. Winemakers contemplating microoxygenation need to understand the means for controlling Brettanomyces and make sure that they take the necessary precautions to minimize its impact (Paul, 2002; du Toit et al., 2006):

- If microoxygenating prior to malolactic fermentation ensure that no residual sugar is present in the wine. Brettanomyces loves sugar. Thus, a sluggish, difficult to ferment wine, may not be a good choice for microoxygenation unless the winemaker is absolutely sure of its status, both chemical and microbiological.

- Ensure prompt and stringent pH control.

- Add SO₂ at crushing (50 mg/L).

- Ensure sulphur dioxide levels in finished wines are adequate. Levels of 80 mg/L of total SO₂ have been quoted as inhibiting Brettanomyces growth.

- Do not microoxygenate at high temperatures.

- Do not microoxygenate wines made from unsound fruit. There is evidence that diseased fruit can considerably increase spoilage organism load.

6 CONCLUSIONS

Microoxygenation is a well defined process for improving wine quality. The chemistry underpinning the technique is not clearly understood at this stage, but the process is certainly developing. The effect of microoxygenation on a wine depends on the kind of wine (variety, origin, etc.) and the vintage. These facts can be related with the differences in the phenolic composition between grape varieties and therefore the doses of oxygen should be determined according to the initial phenolic composition of wines.

7 REFERENCES


Morata A., Calderón F., González M. C., Gómez-Cordovés M. C., Suárez J. A. 2006. Formation of the highly stable pyranoanthocyanins (vitisin A and B) in red wines by the addition of pyruvic acid and acetaldehyde. Food Chemistry, 100: 1144-1152.


