Oxygen in wine and its role in phenolic reactions during ageing

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Introduction

Oxidation reactions that arise from the presence of dissolved oxygen induce various changes in the organoleptic properties of beverages. As long ago as 1873, Pasteur stated: 'l'Oxygène est le pire ennemi du vin,' (oxygen is the greatest enemy of wine) but also, 'C'est l'oxygène qui fait le vin, c'est par son influence qu'il vieillit.' (oxygen makes the wine, which ages under its influence).

Since that time, various researchers have studied the relationship between oxygen and wine. It is commonly admitted that extensive oxidation is unfavourable to wine quality, but slow and continuous oxygen dissolution may play a positive role in wine ageing. To promote the beneficial effects of oxygen exposure while avoiding spoilage risks, it is essential to understand the mechanisms governing oxygen dissolution and consumption in wine. The purpose of this talk is to review current knowledge on these matters, with special focus on oxidative reactions involving phenolic compounds. Some of our recent results on the impact of micro-oxygenation on phenolic composition and subsequent colour changes will also be presented.

Oxygen solubility

In a gas mixture, a gas exerts a partial pressure that corresponds to the pressure it would have if it occupied the entire volume alone. The proportion of oxygen in dry air is 20.9%, so its partial pressure (PO2) is 1.013 x 10³ x 20.9 = 21.2 x 10³ Pascal (Pa), at 20°C and atmospheric pressure. In air saturated with water, the partial pressure of oxygen is 18.8 x 10³ Pa. At equilibrium, the partial pressure of a gas dissolved in a liquid is identical to the partial pressure of the gas in the gas phase. Thus, at equilibrium, the partial pressure of oxygen in air saturated wine or water at 20°C is 18.8 kPa. If the liquid phase were saturated with oxygen instead of air, the partial pressure would be around five times more.

Methods that measure dissolved oxygen using electrolytic cells give access to partial pressure and can be calibrated in % saturation. The liquid medium is saturated with oxygen when the measured value (100% saturation) is the same in the liquid phase as in the vapour phase. Partial pressure and % saturation depend on pressure and temperature, but are independent of the liquid composition.

The dissolved oxygen concentration can be calculated by using a solubility coefficient, using Henry's law: PO2 = H x C*, where H is the oxygen solubility coefficient and C* is the gaseous oxygen concentration at equilibrium. The oxygen solubility coefficient (H) depends on temperature, pressure and the liquid composition.

Table 1. Dissolved oxygen concentration in red wine stored in a 2,700L tank.

<table>
<thead>
<tr>
<th>Distance to wine surface (cm)</th>
<th>Dissolved oxygen concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.4</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The oxygen concentration decreases as the temperature increases: in water, solubility is 14.67 mg/L at 0°C, 9.2 mg/L at 20°C, and only 5.6 mg/L at 50°C. Oxygen solubility decreases as the ethanol content increases up to 30%, but beyond that ethanol content the oxygen solubility strongly increases. The presence of solutes in the liquid phase also decreases oxygen solubility. Thus, the oxygen concentration in wine that is saturated with air is 6 mL/L or 8.4 mg/L whereas, in water, 100% saturation corresponds to 9.2 mg/L oxygen at 20°C and atmospheric pressure.

Oxygen dissolution and consumption in wine

Wine that is stirred with air becomes saturated in approximately 30 seconds (Ribéreau-Gayon and Peynaud, 1961). So any operation involving air contact, such as wine transfer or stirring, rapidly induces oxygen dissolution. After racking, wine usually contains about 3mg/L dissolved oxygen but this may increase, up to saturation, if there is a pumping system fault. Similar amounts of oxygen may be incorporated in stabilisation or bottling, although modern bottling lines usually limit oxygen enrichment to 0.3 mg/L.

In storage tanks or barrels, the level of dissolved oxygen is extremely low and usually between 0.02 and 0.05 mg/L. Measured levels of dissolved oxygen decrease in the first 10 centimetres below the wine surface (Table 1), indicating that the oxygen consumption rate exceeds the oxygen dissolution rate.

The extent of oxidation thus depends on the ratio between the wine surface area in contact with air and the wine's volume. In barrels, gas exchange is more intense as the wine volume decreases. Furthermore, wood micro-porosity ensures that there is an air-liquid interface in the barrel wall. Evaporation through the barrel further increases the ullage volume and increases the wine surface area in contact with the headspace gas, which can contain variable concentrations of oxygen, depending on the type of bunging. Wine oxygen exposure is affected also by the frequency of toppings.

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OXYGEN IN WINE AND ITS ROLE IN PHENOLIC REACTIONS DURING AGEING

[23] ASVO PROCEEDINGS • USE OF GASES
However, oxygenation of wines stored in barrel is highly variable from one barrel to another and thus is very difficult to control.

This has led to the development of a new process, referred to as micro-oxygenation, that allows continuous oxygen supply in quantities small enough to ensure that no oxygen accumulation takes place and that the exchange occurs in a very small volume zone so as to mimic conditions encountered in barrel ageing. Oxygen is usually provided in the range 2-4 mg/L/month. Changes in the red wine organoleptic characters have been described to involve, first, a loss of young wine aroma, with increase of coarseness and colour intensity (a “structuring” phase), then an increase of aroma complexity, with a loss of veggie and reduced characters, along with tannin softening (a “harmonising” phase). Excessive oxygenation may then lead to the development of oxidised characters.

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. Wine lees has recently 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.

**Oxygen and polyphenol reactions**

Oxidation reactions involving phenolic compounds are extremely complex processes that are not fully elucidated. The major phenolic compounds in young red wines are anthocyanins, the pigments of red grapes, and flavonols, which are encountered as monomers (catechins) and as oligomers or polymers (proanthocyanidins, also commonly called condensed tannins).

Oxidation (i.e. removal of an electron) of phenolic compounds is much easier from the phenolate anion form than from the unionised phenol, and is thus much faster at higher pH values (Singleton, 1987). Single electron oxidation of the phenolate anion yields a semi-quinone radical which, if the phenol contains a second ortho or para hydroxyl group, spontaneously proceeds to the corresponding quinone by losing another electron and a proton (Figure 1).

Molecular oxygen (O$_2$) in its lowest energy triplet state, has two unpaired electrons. Consequently, it is unable to react with most organic compounds, which are singlet compounds (with no unpaired electron). Triplet oxygen can be activated to a singlet species which then spontaneously decays to various activated species (Figure 2).

It can also be activated by reaction with free radicals or metal ions (Figure 3). Reaction of oxygen with phenolic compounds requires that one or the other is activated. The resulting reaction cascade can be summarised by the following equation: O$_2$ + ortho-diphenol $\rightarrow$ H$_2$O$_2$ + ortho-quinone. Radical reactions and hydrogen peroxide, formed through autoxidation of phenolic compounds, also oxidise ethanol to acetaldehyde (Wildenrath and Singleton, 1974). Although acetaldehyde is also a major yeast metabolite, it can be considered as an oxidation marker. Consequently, major oxidation-induced reactions of polyphenols include, on the one hand, reactions of quinones and, on the other hand, reactions of polyphenols with acetaldehyde and other aldehydes.

Ortho-quinones are extremely unstable, being both powerful oxidants and electrophilic species (i.e. they are molecules showing an electron deficiency and thus attract species with an electron excess, called nucleophiles). They are readily reduced, through oxidation of lower redox potential molecules, including sulfites, ascorbate, and other ortho-diphenols (o-diphenols), and they can also suffer nucleophilic addition with nucleophiles such as thiols, amines, and phenolic compounds (Figure 4). Both types of reaction regenerate the o-diphenol moiety. Besides, some of the adducts resulting from the second pathway (nucleophilic addition) have lower redox potential than the original phenol and are thus more easily oxidised (Singleton, 1987).

For instance, reaction of the tannin monomer unit, catechin, with its o-quinone yields dimers in which the original catechin units are linked by biphenyl or ether bonds (Guyot et al. 1996). These molecules are isomers of grape proanthocyanidins, so they may be similarly astringent, but they are much more resistant to acid-catalysed cleavage. Furthermore, they oxidise to yellow pigments.

Phenolic compounds also react readily with acetaldehyde (Figure 5). The first and limiting step of this process is the

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O_2 + HQ \rightarrow O_2^- + Q + H^+ \\
Fe^{II} + O_2 \rightarrow Fe^{III} + O_2^-. 
\]

**Figure 1. Oxidation of phenolic compounds.**

**Figure 2. Reaction of oxygen leading to reactive oxygen species.**

**Figure 3. Oxygen activation through reaction with radical species and metal ions.**
similar synthetic pigments are greatly stabilised by sandwich-precursors, presumably because of self-association, since 540 nm). They are more stable than their anthocyanin
(2002). Anthocyanin-ethyl adducts are purple pigments (Cheynier et al. 1997, Saucier et al. 1997, Atanasova et al. 2002), which have all been found in wines including ethyl-linked flavanol oligomers (Fulcrand et al. 1996), as well as direct tannin-anthocyanin adducts (Somers, 1971, Remy et al. 2000) are also known to form in wine.

Micro-oxygenation experiments were carried out on a red Vitis vinifera wine, made from var. Cabernet Sauvignon (60%) and Tannat (40%) grapes of the 1999 vintage (Atanasova et al. 2002b). The wine was distributed into six tanks; three of the tanks were saturated with N₂ (control) and three tanks were provided monthly with 5 mL/L pure oxygen through a microdiffuser (oxygenated). Thus, the experiment (including storage in tanks, fractionation and analyses) was performed in triplicate.

The influence of controlled oxygenation on the colour characteristics and the phenolic composition of the red wine was studied by high performance liquid chromatography with diode-array detection (HPLC-DAD), UV-visible spectrophotometry, mass spectrometry and thiolysis. The latter method enabled determination of the tannin content and the average tannin chain length (mean degree of polymerisation or mDP). Colour parameters included the following: wine colour (absorbance at 520 nm), total colour of pigments (absorbance at 520 nm after dilution in 2% HCl to ensure that all anthocyanins and derived pigments are in the coloured flavylium form), copigmentation (wine colour—wine colour measured after dilution at wine pH value, to dissociate pigment-copigment complexes), colour resistant to sulfite bleaching, colour not resistant to sulfite bleaching, chemical age (ratio of colour of pigments resistant to sulfite bleaching to wine colour), colour intensity (sum of absorbances at 420, 520 and 620 nm, that is in the yellow, red and blue regions), and tint (ratio of absorbances at 420 and 520 nm). Derived pigments were estimated as the difference between the wine pigments (calculated from the total colour of pigments) and the free anthocyanins (measured by HPLC).

Figure 4. Reactions of quinones.

protonation of the aldehyde, a strongly pH-dependent process. The resulting carbocation then suffers nucleophilic addition to yield an intermediate polyphenol-ethanol adduct.

This then adds a proton and loses a water molecule to give a new carbocation intermediate which is in turn attacked by another nucleophile to form an ethyl-linked dimeric compound.

Both anthocyanins and tannins can act as nucleophilic species in this process. The presence of phenolic hydroxyl groups induces partial negative charges (δ-) in the ortho and para positions, thus conferring a nucleophilic character on carbons C-6 and C-8 (Figure 6). Anthocyanins, in their coloured flavylium cationic form, react as electrophiles. However, the cationic form is predominant only in very acidic media and, as the pH increases, it readily undergoes either deprotonation, leading to a quinonoidal base, or hydration, leading to hemiketal and chalcone forms. Consequently, at wine pH, anthocyanins are mostly present as the colourless uncharged hemiketal water adduct which reacts as a nucleophile.

Thus, products of acetaldehyde-induced condensation include ethyl-linked flavanol oligomers (Fulcrand et al. 1996), ethyl-linked anthocyanin-flavanol adducts (Timberlake and Bridle, 1976), and also ethyl-linked anthocyanin oligomers (A tanasova et al. 2002), which have all been found in wines (Cheynier et al. 1997, Saucier et al. 1997, A tanasova et al. 2002). Anthocyanin-ethyl adducts are purple pigments (λmax 540 nm). They are more stable than their anthocyanin precursors, presumably because of self-association, since similar synthetic pigments are greatly stabilised by sandwich-type stacking (Escribano-Bailon et al, 1996).

Figure 5. Mechanism of acetaldehyde-induced condensation.

Figure 6. Nucleophilic and electrophilic forms of flavanols and anthocyanins.

Furthermore, in the wine pH range, the ethyl-linked anthocyanin dimer exists mostly in the coloured monoflavilium form, as the pK of its first hydration is very low.

Other reactions involving acetaldehyde lead to a different group of pigments based on a pyranoanthocyanin structure (Cheynier et al. 1997; Benabdelljalil et al. 2000; Francia-A richa et al. 1997, Mateus et al. 2002). These orange pigments are exceptionally stable towards sulfite bleaching and pH variation due to substitution at the flavilium C-4 position.

Other pyranoanthocyanins derived from various yeast metabolites, such as pyruvic acid (Fulcrand et al. 1998), as well as direct tannin-anthocyanin adducts (Somers, 1971, Remy et al. 2000) are also known to form in wine.
The concentration of grape anthocyanins decreased during ageing and the decrease was significantly more in oxygenated wines.

The tannin concentration also decreased during ageing, but it was significantly higher in the oxygenated wines than in the control wines. Derived pigments, including carboxy-pyrananthocyanins resulting from reaction of pyruvic acid with anthocyanins and anthocyanin-tannin adducts, accumulated during ageing and were significantly more abundant in the oxygenated wines. The concentration of ethyl-linked anthocyanin-tannin adducts gradually decreased in the control wines, confirming the lability of these pigments (Escribano-Bailon et al., 2001), whereas it increased in the oxygenated wines as a result of acetaldehyde accumulation. Wine colour and total colour of pigments decreased throughout ageing, indicating that formation of derived pigments did not compensate for anthocyanin degradation.

Colour intensity also decreased, but the decrease was less in the oxygenated wines, whereas tint increased similarly in both types of wines. This confirms that pigments formed during ageing are less red but more yellow than their anthocyanin precursors, as described earlier (Somers, 1971), and it indicates that those resulting from oxygenation are more purple. Finally, the proportion of pigments resistant to sulfite bleaching also increased during ageing, but significantly more so in the oxygenated wines. All the derived pigments mentioned above are resistant to sulfite bleaching. Moreover, acetaldehyde accumulation following oxidation is expected to enhance the formation of both the orange acetaldehyde-derived pyrananthocyanins and the purple ethyl-linked flavanol-anthocyanin adducts.

Principal component analysis was applied to all data obtained from the control and oxygenated wines over a seven-month storage period. Distribution of the wine samples along the first and second principal components showed that the former, accounting for 69% of the variance, corresponds to an "ageing" axis whereas the latter, which represents 16% of the variance, can be interpreted as an "oxygenation" axis. Examination of the contribution of the analytical variables to these axes indicated that young wines were characterised by high levels of free anthocyanins and native tannins (both originating from the grapes), wine colour, colour due to co-pigmentation, colour due to derived pigments and colour not resistant to sulfite bleaching. All these parameters were highly correlated. Thus it seems that wine colour at earlier stages is due to free anthocyanins and to derived pigments showing the same characteristics as free anthocyanins, in particular with respect to susceptibility to sulfite bleaching and involvement in copigmentation or self-association, as shown in Figure 7.

In contrast, wine ageing was associated with larger amounts of pyrananthocyanins, colour due to derivatives resistant to sulfite bleaching, increased chemical age (ratio of pigments resistant to sulfite bleaching to total pigments) and tint (ratio of yellow to red pigments), which were also correlated. This confirms that the wine colour gradually changes from red to tawny during ageing, and that it becomes more resistant to sulfite bleaching, as described earlier (Somers, 1971). Among the pyrananthocyanins, only carboxy-pyrananthocyanins resulting from the reaction of pyruvic acid with anthocyanins were assayed and their concentration is too low to account for all pigment that is resistant to sulfite bleaching.

Conclusion

In wine-like model systems, similar reactions involving glyoxylic acid, derived from tartaric acid oxidation, have also been shown to occur. This leads to analogous derived tannin and pigment structures, including yellow xanthylum salts. Thus, oxidation reactions involving polyphenols in wine seem to occur mostly through the formation of aldehydes followed by their reaction with anthocyanins and/or tannins.

The colour properties of the pigments resulting from these reactions are different from those of grape anthocyanin pigments and they are usually more resistant to sulfite bleaching. Determination of their taste and colloidal properties remains a major challenge in oenology. Better understanding of the relationships existing between the structure and the taste of these various molecules and of the influence of wine-making practices on the reaction mechanisms controlling their formation is required to predict and master the effect of processes such as micro-oxygenation on wine quality.

References


